DOI: 10.1002/ejoc.201403145

Triphosgene-Mediated Couplings in the Solid Phase: Total Synthesis of Brachystemin A

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Keywords: Synthetic methods / Solid-phase synthesis / Amidation / Peptides / Total synthesis

A reproducible, highly efficient, and racemization-free protocol for the triphosgene-mediated solid-phase coupling of proteinogenic amino acids was developed. The efficiency of the reaction was demonstrated by the first total synthesis of the bioactive natural octacyclopeptide brachystemin A. This protocol will broaden the application of triphosgene in the synthesis of a wide range of peptides, including aromatic amino acids, *N*-alkylated amino acids, as well as proteinogenic amino acids.

Introduction

The activation of amino acids for the formation of amide bonds is a key step in solid-phase peptide synthesis (SPPS). Following the growing interest in peptides, numerous coupling reagents have been developed and have become commercially available, mainly including carbodiimides {*N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*,*N*′-diisopropylcarbodiimide (DIC), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl)} alone or plus additives [hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt)], phosphonium salts [(benzotriazol-1-yloxy)-tris(dimethylamino)phosphonium hexafluorophosphate (BOP), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP-Cl), pentafluorophenyl diphenyl phosphate (FDP), pentafluorophenyl diphenylphosphinate (FDPP), etc.], and uronium (iminium) salts [*O*-(7-aza-1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), etc.].[1] Triphosgene (BTC) as a reagent putatively forms acid chlorides in situ;[2] acid chlorides are highly electrophilic, which allows rapid amidation of sterically and electronically less-reactive amines. In 2002, Jung et al. reported an fluorenylmethyloxycarbonyl (Fmoc)-based synthesis of cyclic peptides containing sterically hindered secondary amines on a solid support in which BTC was used as the coupling agent.[3] A highly efficient strategy was developed for the synthesis of DNA-binding hairpin polyamides by using BTC-mediated coupling of *tert*-butoxycarbonyl (Boc) protected aromatic amino acids.[4] BTC has also shown application in the synthesis of aromatic oligoamides by using automated peptide synthesizers.[5] Although far superior to benzotriazole-based protocols for couplings of *N*-methylated or aromatic amino acids in both Fmoc-based and Boc-based SPPS, Jung et al. found in the total synthesis of cyclosporine O and omphalotin A that for unmethylated amino acids such as Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Ile-OH, and Fmoc-Trp-OH, the BTC-mediated coupling failed to drive the reaction to completion. In these cases, the coupling reaction had to be done by additional coupling cycles with the use of HATU or HOAt/DIC as the reagents with extended reaction times (16 h).[3] It was assumed that proteinogenic amino acids bearing an NH proton of the urethane function were susceptible to side reactions during strong activation with the BTC reagent.[3] Owing to the low efficiency of BTC-mediated proteinogenic amino acid couplings, the BTC strategy has not been generally used in the synthesis of peptides composed of proteinogenic amino acids since the first report of this method by Falb et al. in 1999.[2b]

The growing need for new and more complex peptide structures, particularly for biomedical studies and for the large-scale production of peptides as drugs, demands the manufacture of peptide products by efficient synthetic strategies, at reasonably low prices. It is highly desirable if one

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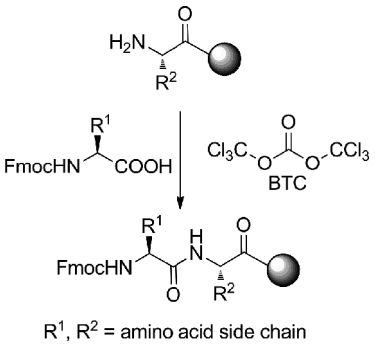
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201403145.

versatile coupling reagent could be applicable for the coupling of both proteinogenic amino acids and non-proteinogenic amino acids including *N*-alkylated amino acids and aromatic amino acids. For this purpose, the use of BTC in the activation of amino acids is quite attractive, because BTC is inexpensive, unlike many condensation agents, and has proved to be very efficient in the synthesis of difficult sequences such as *N*-methylated peptides and aromatic polyamides. In view of the limitations in activating proteinogenic amino acids, the development of an efficient activation method for this type of amino acids is crucial for the generality of the BTC strategy. Recently, Fuse and Takahashi reported efficient amide bond formation through rapid and strong activation of various carboxylic acids with BTC in a microflow reactor.[6] Side reactions including epimerization were suppressed by reducing the residence time of the highly active species generated through microflow synthesis. Although this method performs well in solution phase, it is not applicable for solid-phase synthesis, which needs a longer time to accomplish the coupling. Therefore, along with our continuous interest in the search for efficient strategies in SPPS, we examined the problematic activation of proteinogenic amino acids and describe herein a highly efficient procedure for the BTC-mediated solid-phase synthesis of proteinogenic peptides (Scheme 1).



Scheme 1. BTC-mediated solid-phase coupling of proteinogenic amino acids.

Results and Discussion

We first chose Fmoc-Val-OH to evaluate the activation efficiency of the BTC method. A series of experiments was performed by using different ratios of BTC and Fmoc-Val-OH. According to Jung's procedure, Fmoc-Val-OH and BTC were dissolved in dry THF, collidine was added slowly, upon which a white precipitate formed. This suspension was gently shaken for about 2 min before an excess amount of dry MeOH was added to generate Fmoc-Val-OMe to facilitate the HPLC analysis. As shown in Figure 1, we found that 0.33 equiv. of BTC, typically used in the activation step to release an equal molar amount of phosgene, was not sufficient to completely convert the amino acid into the corresponding methyl ester (Figure 1, trace a). Considering the NH proton of the Fmoc-protected amino group might consume some phosgene through a competitive side reaction during BTC activation, we surmised that increasing the ratio of BTC might produce Fmoc-Val-OMe with better conversion. As a matter of fact, the activation efficiency was significantly improved if 0.41 equiv. of BTC was used (Figure 1, trace b) without any new byproduct detected. Possibly because of the moisture in the reaction system, a small amount of Fmoc-Val-OH was left unreacted, which could not be avoided by a further increase in the BTC amount to 0.50 equiv. (Figure 1, trace c). Therefore, we believe that 0.41 to 0.50 equiv. of BTC is appropriate to activate proteinogenic amino acids efficiently and will lead to a successful coupling in the SPPS.

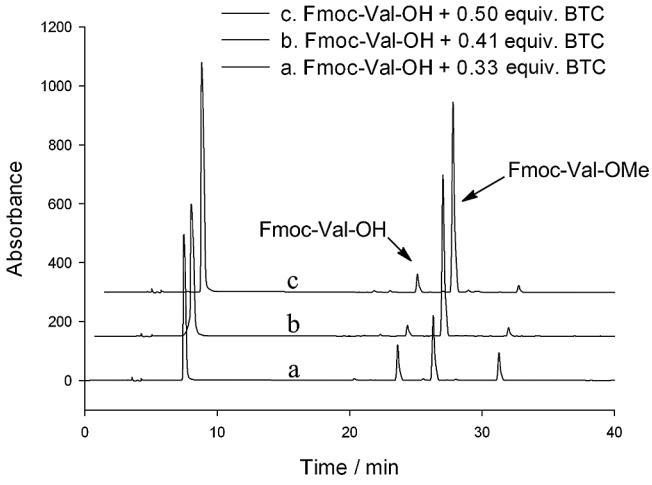


Figure 1. Activation of Fmoc-Val-OH by using BTC.

Noting that the collidine hydrochloride salt precipitated during BTC activation, which could be a major hindrance to the application of this method to automated peptide synthesis, we then set out to deal with the precipitate and finally developed a novel procedure by using a special combination of bases for the activation of the amino acids. The key features of the procedure include: (1) performing the preactivation of the carboxylic acid with collidine in THF, racemization-free conditions validated by the research of Jung and Falb; (2) conversion of the resulting precipitate into the soluble *N*,*N*-diisopropylethylamine (DIEA) hydrochloride salt by the addition of a DIEA/DMF solution. For a typical coupling reaction on 150 mg resin (0.36 mmol g^{-1}), the Fmoc-protected amino acid (4 equiv.) and BTC (1.64 equiv.) were dissolved in dry THF (1 mL); then collidine (14 equiv.) was added slowly and a white solid precipitated immediately. This suspension was gently shaken for about 3 min before DIEA/DMF (5% v/v, 2 mL) was added to form a clear solution, which was transferred to the pretreated resin to generate the corresponding peptide.

Encouraged by these results, we investigated the efficiency of the optimized BTC protocol to form amide bonds in solid-phase peptide synthesis. For a model study, Fmoc-Ala-OH, Fmoc-Val-OH, and Fmoc-Ile-OH were chosen to prepare a short peptide on Wang resin preloaded with Fmoc-Gly. As reported in the total synthesis of cyclosporine O and omphalotin A, the previous BTC procedure was useless for the coupling of these amino acids. To our de-

light, the solid-phase synthesis of resin-bound Gly-Ala-Val-Ile-Fmoc proceeded smoothly by using the optimized BTC protocol. Cleavage of the tetrapeptide from the resin afforded the crude product in 86% purity; this is indicative of an average yield of 95% per coupling cycle (Figure 2).

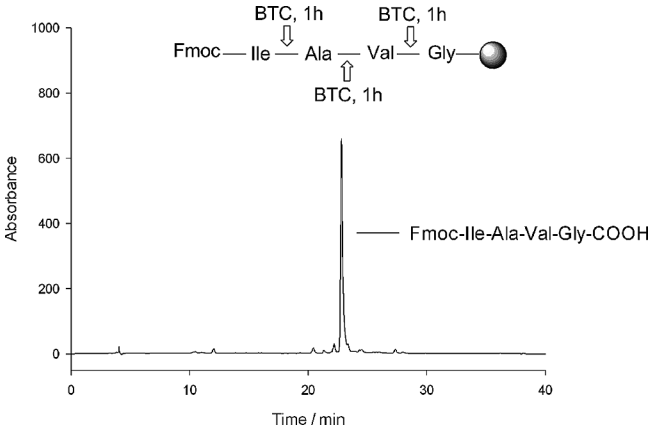


Figure 2. HPLC of crude linear tetrapeptide.

Another model reaction was used to check for racemization of the optimized BTC protocol. Coupling of racemic Fmoc-Ala-OH and Fmoc-L-Ala-OH or Fmoc-D-Ala-OH to resin-bound Gly-Val-NH_2 was performed, and the resulting tripeptide was subjected to HPLC (Figure 3). For the cases in which enantiomerically pure Fmoc-D-Ala-OH and Fmoc-L-Ala-OH were used, no racemization products could be detected.

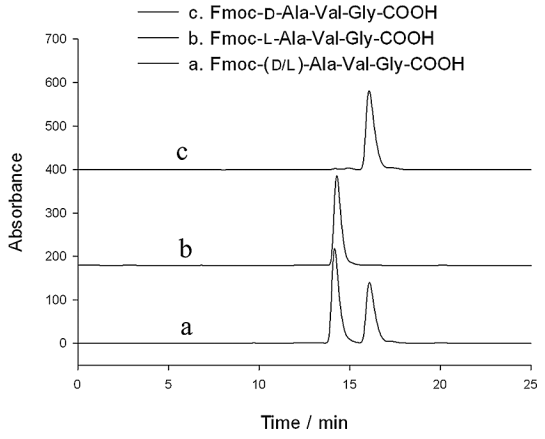


Figure 3. Comparison of the HPLC traces of tripetides resulting from BTC-mediated coupling of (a) racemic Fmoc-Ala-OH, (b) Fmoc-L-Ala-OH, and (c) Fmoc-D-Ala-OH (CHIRALPAK ID 0.46 cm × 25 cm × 5 μm, Shimadzu LC 20 with UV detector SPD-20A, 254 nm).

We then investigated whether the BTC-based coupling methodology could be applied to the preparation of biologically active cyclopeptides. Brachystemin A (**1**) is an octacyclopeptide that was isolated from *Brachystemma calycinum* D. Don (Caryophyllaceae), a Chinese folk medicine that has been used to treat rheumatoid arthritis, limb numbness, impotence, and gonorrhea.[7] In 2011, the structure of brachystemin A, originally proposed as cyclo-(Pro^1-Phe^2-Leu^3-Ala^4-Thr^5-Pro^6-Ala^7-Gly^8), was revised to cyclo-(Pro^1-Pro^2-Ala^3-Gly^4-Leu^5-Ala^6-Thr^7-Phe^8) as a result of QTOF/MS and X-ray diffraction analysis (Figure 4).[8] It was shown that bachystemin A, at a concentration of 10 μM, could significantly inhibit the secretion of IL-6, CCL-2, and collagen IV. In addition, its effects on collagen I, reactive oxygen species (ROS), production of the superoxide anion ($O_2^{\cdot-}$), cell viability in mesangial cells, and nitric oxide production in macrophage cells were evaluated, and it was revealed that brachystemin A was a promising agent for the treatment of diabetic nephropathy (DN).[9] Further exploration of this octacyclopeptide in drug discovery was, however, prevented by the lack of its availability from natural sources. Therefore, we initiated the total synthesis of bachystemin A by head-to-tail cyclization of a linear peptide. Furthermore, this octacyclopeptide not only contains the glycine and alanine residues used in our model study, but it also contains special residues such as Phe, Thr, and Pro; it therefore appeared as an ideal target on which to test the efficiency of our newly developed BTC protocol.

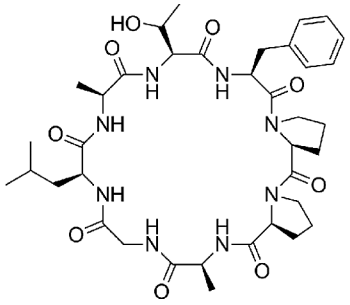
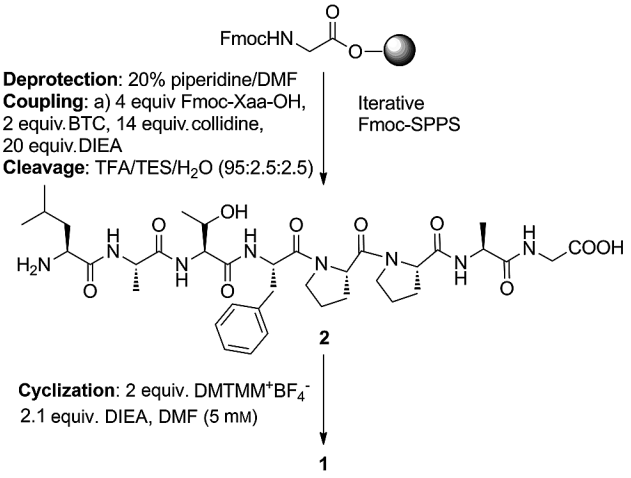


Figure 4. Brachystemin A (**1**).

It was envisioned that the existence of two proline residues, which can induce conformational turns, should encourage head-to-tail peptide cyclization. With circumspection, a cyclization point at the Gly^4-Leu^5 junction was chosen to give the linear precursor NH_2-Leu-Ala-Thr-Phe-Pro-Pro-Ala-Gly-OH (**2**). This precursor was expected to cyclize readily while failing to support any C-terminal epimerization during the cyclization.

Linear octapeptide **2** was prepared by using Fmoc-based SPPS techniques with Fmoc-Gly-Wang resin as the solid support and BTC as the coupling reagent (Scheme 2). As described in the model study, the activation of Fmoc-Xaa-OH was completed with BTC (0.41 equiv.) in 5 min; coupling times of 30 min to 1 h were typically required for quantitative conversion to the coupled products according to the ninhydrin test (for the free primary amine) and the chloranil test (for the free secondary amine of Pro). Deprotection of the Fmoc group was performed with 20% piperidine in DMF. Cleavage of the precursor from the resin was achieved with trifluoroacetic acid (TFA)/triethylsilane (TES)/H_2O (95:2.5:2.5).[10] Owing to the high purity, the linear peptide could be cyclized after precipitation from cold diethyl ether without further purification.[11]



Scheme 2. Total synthesis of brachystemin A.

In the following study, the coupling reagents that could affect the head-to-tail cyclization of linear precursor **2** were screened. Ring closure was effected by EDC·HCl, HATU, HBTU, or 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate ($DMTMM^+BF_4^-$) at high dilution (5 mM) in the various yields presented in Table 1. As expected, octacyclopeptide **1** was isolated in every case in moderate to good yield. Recently, $DMTMM^+BF_4^-$ was found to be effective in fragment coupling and cyclization to provide high yields and a low degree of epimerization for a wide range of amino acid and peptide substrates.[12] Cyclization of **2** by using this coupling reagent gave the highest yield of product (72%). Use of the much cheaper chloride salt, $DMTMM^+Cl^-$, provided **1** in 59% yield, which was lower than that obtained with HATU/DIEA as the coupling reagents but higher than that obtained with EDCI/HOBt or HBTU/DIEA as the coupling reagents. Purification of the crude product by preparative reverse-phase HPLC followed by lyophilization afforded **1** as a white powder. The ^1H NMR and ^{13}C NMR (500 and 75 MHz) spectra of **1** and the mass of the HRMS parent ion were identical to those of natural brachystemin A (see the Supporting Information).

Table 1. Yields of isolated **1** formed by cyclization of **2** by using different coupling reagents.

Coupling reagents	Yield [%][a]
EDC·HCl/HOBt	53
HBTU/DIEA	53
HATU/DIEA	68
$DMTMM^+BF_4^-$/DIEA	72
$DMTMM^+Cl^-$/DIEA	59

[a] The cyclizations were performed in DMF at high dilution (5 mM) of linear peptide **2**.

Conclusions

We demonstrated that increasing the molar ratio of triphosgene (BTC) to the amino acids significantly improved the activation efficiency of unmethylated proteinogenic amino acids, which resulted in the development of a reproducible, low-costing, and very efficient protocol for BTC-mediated solid-phase peptide synthesis. The optimized BTC method was successfully applied to the first total synthesis of brachystemin A, a naturally occurring, bioactive cyclic octapeptide. This precedent will broaden the application of BTC in the synthesis of a wide range of peptides including aromatic amino acids, *N*-alkylated amino acid, as well as proteinogenic amino acids. It also shows potential for automation in multiple, parallel peptide synthesis, which we are currently investigating in our laboratory. We anticipate that the automated synthesis of brachystemin A will provide sufficient material for further pharmacokinetics and pharmacodynamics studies in animals.

Experimental Section

General: All reagents were HPLC or peptide-synthesis grade. Dry DMF and dry THF were obtained from Sigma–Aldrich. Collidine (2,4,6-collidine) was purchased from J&K Scientific. BTC, DIEA, and TFA were purchased from Aladdin. Fmoc-Gly-Wang resin and the amino acids were purchased from GL Biochem. All commercial reagents were used as received. Analytical reverse-phase HPLC was performed at room temperature with a Shimadzu LC 20 with a UV detector SPD-20A by using an Inertsil ODS-SP column (4.6 × 250 mm, 5 μm, 100 Å). The reverse-phase HPLC gradient was started at 10% of B (MeCN), then increased to 100% of B over 30 min (A: 0.1% TFA in water). ^1H NMR (^{13}C NMR) spectra were recorded with a Bruker Avance AV500, Bruker Avance AV400, or DPX-300 at 500 (125), 400 (100), or 300 MHz (75 MHz), respectively. Chemical shifts are referenced to either tetramethylsilane as an internal standard or the signals resulting from the residual solvent. Mass spectra were measured with an ABI Q-star Elite.

BTC Activation of Fmoc-Val-OH: Fmoc-Val-OH (1.0 equiv.) and BTC (0.41 equiv. or test equiv.) were dissolved in dry THF (1 mL). Collidine (14 equiv.) was added slowly to the resulting solution, and the suspension was gently shaken for about 2 min before an excess amount of dry MeOH (1.0 mL) was added. The mixture was stirred at room temperature for 2 h before a small portion was taken out for HPLC analysis. ***CAUTION: Phosgene and CO_2 gas were evolved; this operation must be performed in a fume hood!***

Synthesis of Linear Octapeptide 2: Polyamide synthesis was performed manually in a 10 mL peptide synthesis vessel by solid-phase Fmoc chemistry. Fmoc-Gly-Wang resin was used as a solid support at a substitution level of 0.36 mmol g^{-1} (150 mg, 0.054 mmol). The Fmoc group was deprotected by using 20% piperidine/DMF. After washing with DMF (4 × 2 mL), the resin was treated with dry DMF (2 mL) for 2 min. Meanwhile, the following Fmoc-protected amino acid (4.0 equiv.) and BTC (1.64 equiv.) were dissolved in dry THF (1 mL), and the resulting solution was cooled to 0 °C in an ice/water bath. Collidine (14 equiv.) was added slowly to the THF solution, and a white solid precipitated immediately. After activating for 3 min, DIEA/DMF (5% v/v, 2 mL) was added to form a clear solution, which was transferred to the deprotected Wang resin. The mixture was shaken for 0.5 to 1 h (until the Kaiser test was negative for the free primary amine or the chloranil test was negative for the free secondary amine of proline), and it was then drained from the resin and rinsed with DMF (4 × 2 mL). This procedure was repeated until a peptide sequence (NH_2-Leu-Ala-Thr-Phe-Pro-Pro-Ala-Gly) bound to Wang resin was obtained. After washing with DMF (4 × 2 mL) and MeOH (4 × 2 mL), the resin

was then dried under N_2. Peptide **2** was cleaved off the Wang resin by using TFA/TES/H_2O (95:2.5:2.5, 1.0 mL) at room temperature for 4 h. The TFA/Wang resin/peptide mixture was then filtered to remove the resin. The crude peptide was precipitated by adding eight volumes of diethyl ether and cooling to 0 °C. The crude product was collected by centrifugation and dried under vacuum to produce a white powdery solid, which was used for cyclization without further purification (39 mg). HRMS (ESI): calcd. for $C_{37}H_{57}N_8O_{10}$ $[M + H]^+$ 773.4198; found 773.4196.

Typical Procedure for the Synthesis of Cyclopeptide 1: DIEA (19 μL, 0.11 mmol) and $DMTMM^+BF_4^-$ (33 mg, 0.1 mmol) were added sequentially to a solution of octapeptide **2** (39 mg, 0.05 mmol) in DMF (10 mL). The resulting solution was stirred at room temperature for 72 h, and then the solvent was removed under vacuum, and the residue was precipitated with water. The crude product was collected by centrifugation and purified by semipreparative reverse-phase HPLC followed by lyophilization to afford **1** as a white powder (27 mg, 72%). Data for synthetic **1**: ^{1}H NMR (500 MHz, C_5D_5N): δ = 10.56 (s, 1 H), 8.94 (t, J = 6.0 Hz, 1 H), 7.94 (d, J = 9.5 Hz, 1 H), 7.70 (d, J = 5.5 Hz, 1 H), 7.50 (d, J = 7.5 Hz, 2 H), 7.32–7.28 (m, 2 H), 7.28–7.14 (m, 3 H), 5.38 (t, J = 4.5 Hz, 1 H), 5.22–5.15 (m, 1 H), 5.05–4.95 (m, 3 H), 4.79 (d, J = 8.5 Hz, 1 H), 4.62–4.54 (m, 2 H), 4.31–4.29 (m, 1 H), 3.98 (t, J = 9.0 Hz, 1 H), 3.83 (dd, J = 17.0, 6.0 Hz, 1 H), 3.45–3.38 (m, 3 H), 3.30 (t, J = 12.0 Hz, 1 H), 3.13 (t, J = 8.0 Hz, 1 H), 2.31–2.27 (m, 2 H), 2.18–2.10 (m, 1 H), 2.08–1.96 (m, 3 H), 1.93–1.84 (m, 1 H), 1.84 (d, J = 7.5 Hz, 3 H), 1.80–1.75 (m, 1 H), 1.56 (d, J = 7.0 Hz, 3 H), 1.62–1.45 (m, 2 H), 1.39 (d, J = 6.0 Hz, 3 H), 1.33–1.23 (m, 1 H), 0.96 (d, J = 6.5 Hz, 3 H), 0.74 (d, J = 6.0 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, C_5D_5N): δ = 177.2, 173.9, 173.7, 172.3, 172.1, 171.9, 169.7, 169.5, 138.8, 130.0, 128.9, 127.2, 66.5, 64.6, 64.0, 59.7, 55.7, 53.5, 50.1, 48.7, 47.5, 47.2, 44.2, 44.0, 36.5, 29.1, 28.2, 26.4, 25.1, 24.7, 23.8, 21.9, 21.1, 18.8, 16.8 ppm. HRMS (ESI): calcd. for $C_{37}H_{54}N_8O_9Na$ $[M + Na]^+$ 777.3912; found 777.3906.

Supporting Information (see footnote on the first page of this article): Copies of the ^{1}H NMR and HR mass spectra of all key intermediates and final products.

Acknowledgments

This work was supported by the "Hundred Talents Program" of the Chinese Academy of Sciences, the Shenzhen Sciences & Technology Innovation Council (CXZZ20120831175831692, KQCX20130628112914285) and the National Natural Science Foundation of China (Grant No. 21402232). The authors are grateful for the assistance of the NMR and mass facility from Peking University, Shenzhen Graduate School. We are grateful to Dr. G. W. A. Milne for manuscript revision.

[1] a) A. El-Faham, F. Albericio, *Chem. Rev.* **2011**, *111*, 6557–602; b) E. Valeur, M. Bradley, *Chem. Soc. Rev.* **2009**, *38*, 606–31; c) C. A. G. N. Montalbetti, V. Falque, *Tetrahedron* **2005**, *61*, 10827–52; d) S. Y. Han, Y. Kim, *Tetrahedron* **2004**, *60*, 2447–67; e) B. L. Bray, *Nat. Rev. Drug Discovery* **2003**, *2*, 587–93; f) S. Kent, *J. Pept. Sci.* **2003**, *9*, 574–93; g) W. Zhang, *Tetrahedron* **2003**, *59*, 4475–89; h) C. Najera, *Synlett* **2002**, *9*, 1388–403.

[2] a) H. Eckert, B. Forster, *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 894–895; *Angew. Chem.* **1987**, *99*, 922–923; b) E. Falb, T. Yechezkel, Y. Salitra, C. Gilon, *J. Pept. Res.* **1999**, *53*, 507–17.

[3] a) B. Thern, J. Rudolph, G. Jung, *Tetrahedron Lett.* **2002**, *43*, 5013–5016; b) B. Thern, J. Rudolph, G. Jung, *Angew. Chem. Int. Ed.* **2002**, *41*, 2307–2309; *Angew. Chem.* **2002**, *114*, 2401–2403.

[4] W. Su, S. J. Gray, R. Dondi, G. A. Burley, *Org. Lett.* **2009**, *11*, 3910–3913.

[5] H. M. Konig, T. Gorelik, U. Kolb, A. F. M. Kilbringer, *J. Am. Chem. Soc.* **2007**, *129*, 704–708.

[6] a) S. Fuse, N. Tanabe, T. Takahashi, *Chem. Commun.* **2011**, *47*, 12661–12663; b) S. Fuse, Y. Mifune, T. Takahashi, *Angew. Chem. Int. Ed.* **2014**, *53*, 851–855; *Angew. Chem.* **2014**, *126*, 870–874.

[7] a) Y. X. Cheng, J. Zhou, N. H. Tan, Z. T. Ding, *Chin. Chem. Lett.* **2000**, *11*, 595–596; b) Y. X. Cheng, J. Zhou, N. H. Tan, *Acta Bot. Sin.* **2001**, *43*, 760–765.

[8] J. Zhao, L. L. Zhou, X. Li, H. B. Xiao, F. F. Hou, Y. X. Cheng, *J. Nat. Prod.* **2011**, *74*, 1392–400.

[9] a) J. F. Navarro, C. J. Mora, *J. Am. Soc. Nephrol.* **2008**, *19*, 433–42; b) J. F. Navarro, C. Mora, *Nephrol. Dial. Transplant.* **2005**, *20*, 2601–2604; c) C. Mora, J. F. Navarro, *Curr. Diab. Rep.* **2006**, *6*, 463–468; d) K. R. Tuttle, *J. Am. Soc. Nephrol.* **2005**, *16*, 1537–1538; e) L. J. He, M. Liang, F. F. Hou, Z. J. Guo, D. Xie, X. J. Zhang, *Endocrine* **2009**, *200*, 347–55.

[10] Notably, the hydroxy group of threonine was protected as a *tert*-butyl ether during the linear peptide Synthesis Deprotection took place simultaneous to cleavage of octapeptide **2** from the resin by using a mixture of TFA/TES/H_2O.

[11] A major and minor set of resonances in a 2:1 ratio is clearly observed in the ^{1}H NMR spectrum (D_2O), which indicates that linear peptide **2** exists as rotational conformers.

[12] a) Z. J. Kaminski, B. Kolesinska, J. Kolesinska, G. Sabatino, M. Chelli, P. Rovero, M. Blaszczyk, M. L. Glowka, A. M. Papini, *J. Am. Chem. Soc.* **2005**, *127*, 16912–20; b) R. E. Thompson, R. J. Payne, K. A. Jolliffe, *Org. Lett.* **2012**, *14*, 5110–5113.

Received: August 29, 2014
Published Online: October 21, 2014

