

## Ligations of *O*-acyl threonine units to give native peptides *via* 5-, 8-, 9- and 10-membered cyclic transition states

Siva S. Panda,\*<sup>a</sup> Sumaira Liaqat,<sup>a,b</sup> Anand D. Tiwari,<sup>a</sup> Hadi M. Marwani,<sup>c</sup> Hassan M. Faidallah,<sup>c</sup> Abdul Rauf,<sup>b</sup> C. Dennis Hall,\*<sup>a</sup> and the late Alan R. Katritzky<sup>a,c,†</sup>

<sup>a</sup>*Center for Heterocyclic Compounds, Department of Chemistry,  
University of Florida, Gainesville, FL 32611-7200 (USA)*

<sup>b</sup>*Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan*

<sup>c</sup>*Department of Chemistry, King Abdulaziz University, Jeddah, 21589, Saudi Arabia*

*E-mail: [sspanda12@gmail.com](mailto:sspanda12@gmail.com), [charlesdennishall@gmail.com](mailto:charlesdennishall@gmail.com)*

<sup>†</sup>*Deceased on February 10, 2014*

**Dedicated to Prof. Manfred Schlosser in honor of his scientific achievements  
within his career**

DOI:<http://dx.doi.org/10.3998/ark.5550190.p008.796>

---

### Abstract

*N*-Acyl threonine isopeptides undergo acyl transfer in chemical ligations *via* 5-, 8-, 9- and 10-membered cyclic transition states to yield natural peptides, representing the first examples of successful isopeptide ligations from *N*-acyl threonine units.

**Keywords:** Chemical ligation, threonine, peptide, benzotriazole, acylation

---

### Introduction

Synthetic methods for peptides are of great interest: native chemical ligation (NCL), first reported by Wieland<sup>1</sup> and developed by Kent<sup>2,3</sup> is a chemo- and regio-selective reaction of a peptide-thioester with a *N*-terminal Cys-peptide that produces a long chain polypeptide with a native amide bond at the ligation site through rapid *S*- to *N*-acyl transfer within the initial thioester.

The chemical basis of such ligations is a regiospecific coupling of a C-terminal electrophile of one peptide with a *N*-terminal nucleophile of a second peptide, without any protection or activation step. The peptide segments can be synthetic<sup>4-7</sup> or biosynthetic in origin.<sup>8,9</sup> Many organic reactions have been enabled by ligations *via* a variety of chemical linkages, including amide,<sup>10</sup> thioester,<sup>11</sup> thiazolidine,<sup>12</sup> oxaproline,<sup>13</sup> oxime,<sup>14</sup> hydrazone,<sup>15</sup> and thioether moieties.<sup>16</sup>

NCL development as a synthetic tool for building peptides depends on additives to increase

both ligation rates (from hours to days) and yields. Ligations depend on factors such as steric demand, the exogenous thiol reactivity and the nature of the solvent.<sup>2,17-22</sup> In addition, the low abundance of cysteine (1.7% of the residues in protein sequences) is a major drawback of this methodology since NCL is restricted to Cysteine residues.

To address this limitation, efficient synthetic approaches have been developed<sup>20,23-31</sup> including our recent report on classic O- to N-acyl shifts *via* a 8 and 11-membered transition states in O-acyl serine<sup>32</sup> and 12- to 19-membered cyclic transition states in O-acyl tyrosine-peptides.<sup>33</sup> Thus, “traceless” chemical ligation involving O- to N-acyl shift (at Ser and Tyr site) involving neither Cys nor an auxiliary group at the ligation site is possible.<sup>34</sup>

Our focus has been on threonines, each possessing the 1,2-hydroxylamine bifunctionality<sup>34</sup> (corresponding to the SH/NH<sub>2</sub> in cysteine) and thus affording chemoselective ligation by O- to N-acyl transfer without the need of cysteine residues. We now report migrations of acyl groups from O-acylated threonine isopeptides *via* 5- 8-, 9- and 10-membered cyclic transition states to give natural peptides.

## Results and Discussion

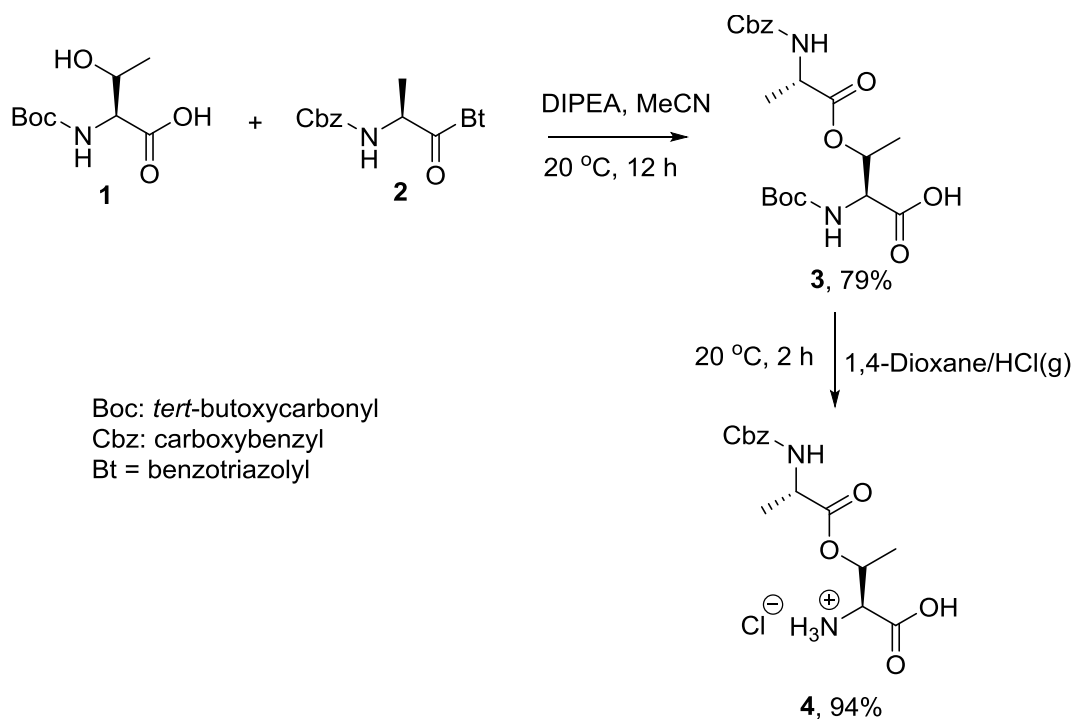
We synthesized the intermediate mono-isodipeptide **4** to study the O-acyl migration from the oxygen to the N-terminal group of threonine amino acid sequence *via* a 5-membered transition state and also to serve as starting material to study the possibility of O- to N-acyl migration *via* 8-, 9- and 10-membered cyclic transition states. Compound **4** on coupling with  $\alpha$ -,  $\beta$ - or  $\gamma$ -amino acids gave the starting mono-isotripeptides (**8a-c**) needed for the ligation studies. To avoid steric problems and enhance migration rates, we used a glycine unit at the N-terminus of mono-isotripeptide **8a** and  $\beta$ - and  $\gamma$ -amino acid units in mono-isotripeptides **8b** and **8c** respectively.

### Preparation of mono-isodipeptide **3**

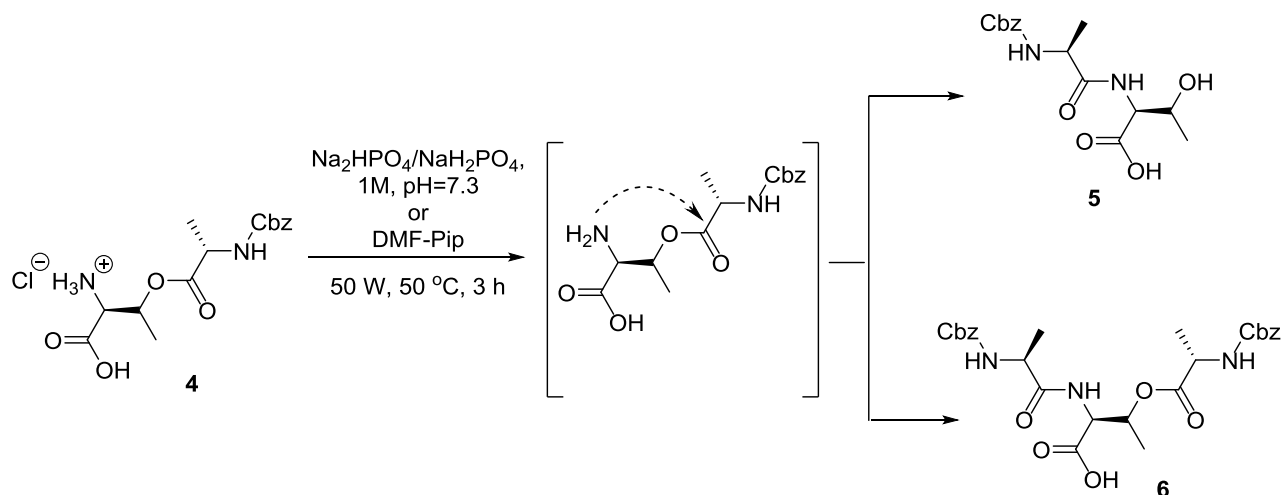
The O-acylation of Boc-protected threonine **1** was carried out by reaction with Cbz-L-Ala-Bt **2** in the presence of DIPEA in acetonitrile to afford Boc-protected mono-isodipeptide **3** (79%), which on deprotection with dioxane-HCl(g) solution afforded unprotected mono-isodipeptide **4** (94%) (see Scheme 1).

### Study of O→N acyl migration *via* a 5-membered cyclic transition state

Chemical ligation *via* a 5-membered cyclic transition state was investigated by subjecting mono-isodipeptide **4** to microwave irradiation at 50 °C, 50 W for 3 h using aqueous conditions (pH 7.3, 1 M buffer strength) as well as basic condition (DMF-piperidine). (Scheme 2) The reaction was allowed to cool to room temperature and acidified with 2 N HCl to pH = 1. The mixture was extracted with ethyl acetate (3 x 20 mL), the combined organic extracts were dried over MgSO<sub>4</sub> and solvent was removed under reduced pressure. The ligation mixture was weighed and then a solution in methanol (1 mg mL<sup>-1</sup>) was analyzed by HPLC-MS.



**Scheme 1.** Synthesis of *N*-acyl threonine isodipeptide **4**.



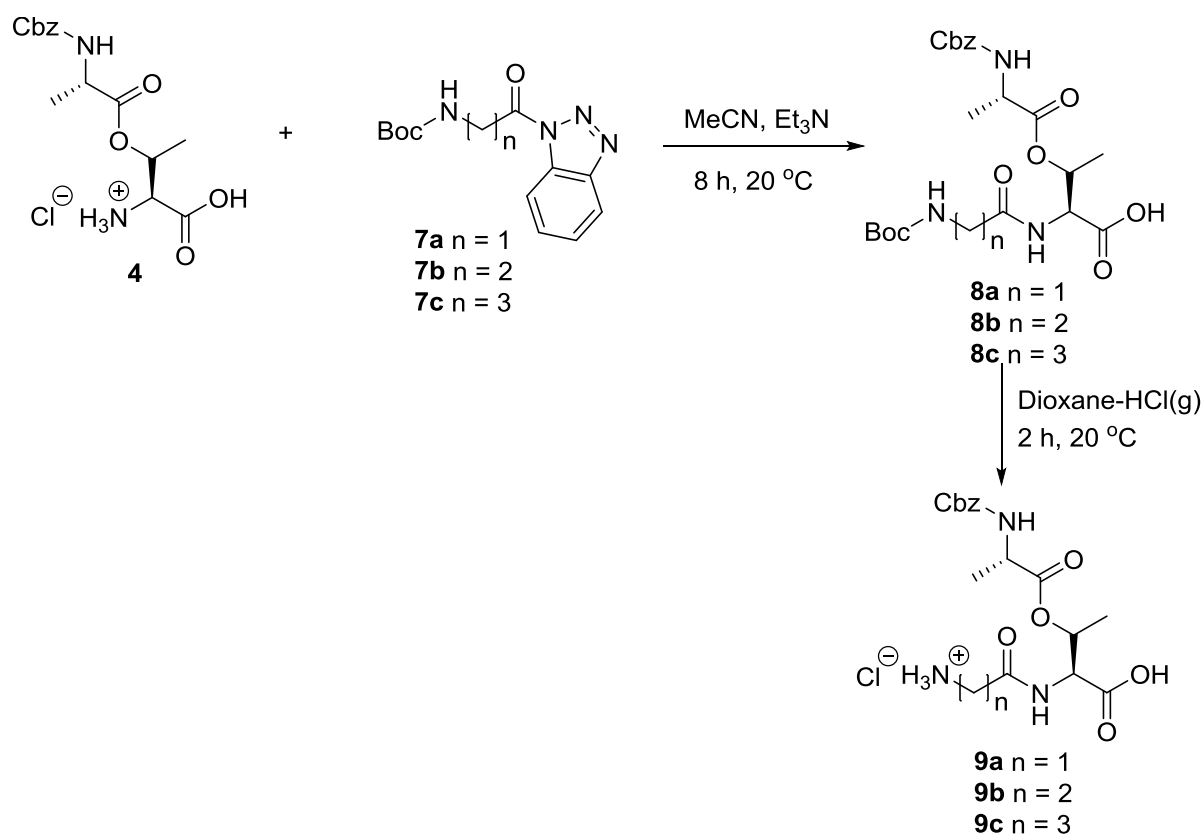
**Scheme 2.** Study of the O→N acyl migration *via* a 5-membered cyclic transition state.

HPLC-MS (ESI) analysis of the ligated mixtures showed both in aqueous buffer as well as DMF-piperidine the expected migration product **5** (rt 38.08,  $m/z$  325.0) together with intermolecular bis-acylation product **6** (rt 60.58,  $m/z$  530.1) in. HPLC-HRMS, *via* (+) ESI-MS, confirmed that the ligated product **5** (rt 38.08,  $m/z$  325.0) and starting mono-isohexapeptide **4** (rt 34.61,  $m/z$  325.0) produced different MS patterns. The result indicates the formation of ligated product **5** with 89% and 86% relative abundance in aqueous buffer and DMF-piperidine

respectively, which infers that O- to N-acyl group migration *via* a 5-membered transition state is preferred over intermolecular acylation. Thus, acyl migration *via* a 5-membered cyclic transition state is feasible and may afford a promising approach for the synthesis of native peptide analog containing threonine.

### Preparation of mono-isotripeptide **9a–c**

Unprotected mono-isodipeptide **4**, on coupling with benzotriazolide of Boc protected  $\alpha$ -,  $\beta$ - or  $\gamma$ -amino acids **7a–c** at room temperature in acetonitrile in the presence of 1.5 equiv. of triethylamine gave mono-isotripeptides **8a–c** in good yields. Compounds **8a–c** on deprotection with dioxane-HCl solution afforded unprotected mono-isotripeptides **9a–c** in good yields, which were fully characterized by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and HRMS analysis (Scheme 3).

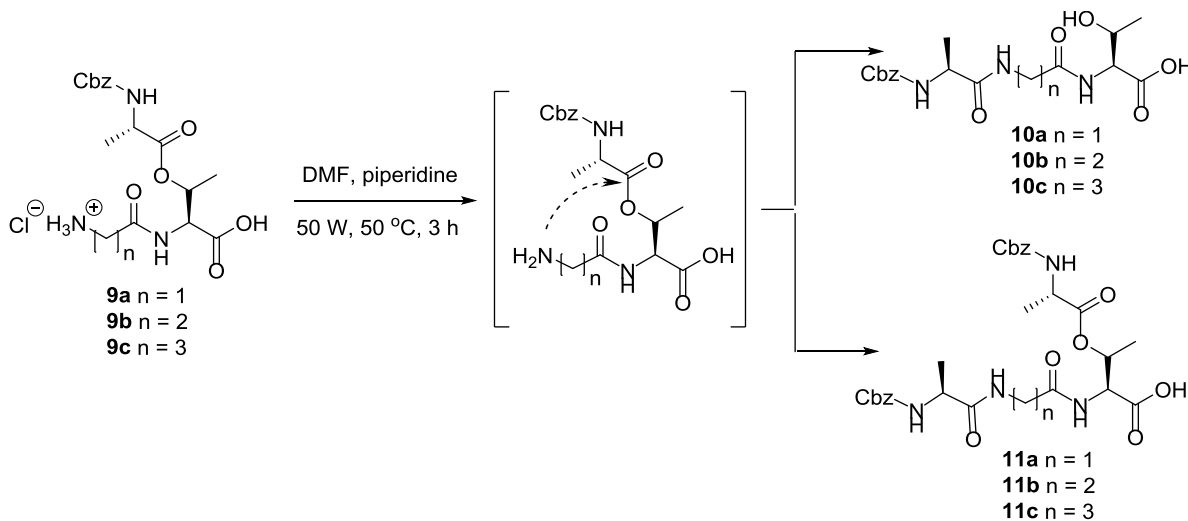


**Scheme 3.** Synthesis of mono-isotripeptides **9a–c**.

### Study of O→N acyl migration *via* a 8-, 9- and 10-membered cyclic transition state

When treated under *aqueous conditions*, (pH 7.3, 1 M buffer strength, MW 50 °C, 50 W, 3 h), **9a–c** did not form the desired ligated products **10a–c** or bis-acylated products **11a–c** (Scheme 4, Table 1). Microwave irradiation of **9b** in *piperidine–DMF* at 50 °C, 50 W for 3 h gave **10b** (57%) and **11b** (36%) as observed by HPLC-MS. We also observed bis-acylated product **11c** in case of **9c**. The retention times and fragmentation patterns of **9b** and **10b** were and HPLC-MS,

via (-)ESI-MS/MS, confirmed that **9b** and **10b**, had different fragmentation patterns, thus proving the formation of intramolecular ligated product **10b** via a 9-membered TS (see Supplementary Material).



**Scheme 4.** Study of O→N acyl migrations via a 8-, 9- and 10-membered cyclic transition states.

**Table 1.** Chemical ligation of N-acyl isotriptides **9a–c** in DMF-piperidine

React	Cyclic TS size	Total crude yield (%) of products isolated	Relative area (%) <sup>a,b</sup>			Product characterization by HPLC-MS			
			React	LP (RT)	BA (RT)	Ligated peptide (LP)		Bis-acylated product (BA)	
						LP [M+H] <sup>+</sup> found	TA	[M+H] <sup>+</sup> found	
<b>9a</b>	8	85	99.0 (34.63)	-	-	<b>10a</b>	-	<b>11a</b>	-
<b>9b</b>	9	90	28.6 (42.37)	57.0 (59.43)	35.8 (59.43)	<b>10b</b>	571.1	<b>11b</b>	601.0
<b>9c</b>	10	89	13.9 (30.61)	-	86.1 (53.22)	<b>10c</b>	-	<b>11c</b>	615.0

<sup>a</sup>Determined by HPLC-MS semiquantitative. The area of ion-peak resulting from the sum of the intensities of the [M+H]<sup>+</sup> and [M+Na]<sup>+</sup> ions of each compound was integrated (corrected for starting material); <sup>b</sup> LP=ligated peptide, BA= Bis-acylated product

## Conclusions

Threonine isopeptides with  $\alpha$ -,  $\beta$ -, or  $\gamma$ -amino acid units were synthesized and acyl migration from threonine oxygen to terminal  $\text{NH}_2$  was studied under microwave irradiation. From the experiments two conclusions can be drawn: (i) intramolecular acyl transfer through 5- and 9-membered transition states was favoured over 8- and 11-membered transition state and (ii) intramolecular acyl migration occurs more readily in basic non-aqueous media relative to aqueous buffered conditions.

## Experimental Section

**General.** All commercial materials (Aldrich, Fluka) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher Solvents were dried using standard protocols kept under a dry atmosphere of nitrogen. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$ ,  $\text{DMSO}-d_6$  or  $\text{CD}_3\text{OD}$  using a 300 MHz spectrometer (with TMS as an internal standard) at ambient temperature unless otherwise stated. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (2.1×150 mm; 5  $\mu\text{m}$ ) + guard column (2×4 mm) or Thermoscientific Hypurity C8 (5 $\mu\text{m}$ ; 2.1×100 mm + guard column) using 0.2% acetic acid in  $\text{H}_2\text{O}$ /methanol as mobile phases; wavelength = 254 nm; and mass spectrometry was done with electro spray ionization (ESI).

***O*-(((Benzyloxy)carbonyl)-L-alanyl)-*N*-(*tert*-butoxycarbonyl)-L-threonine (3).** *N,N*-Diisopropylethylamine (DIPEA) (0.18 mL, 0.15 mmol) was added to a solution of Boc-L-Thr-OH (0.22 g, 1 mmol) in MeCN (15 mL). Cbz-L-Ala-OH (0.32 g, 1 mmol) dissolved in MeCN (5 mL) was added and the mixture was stirred for 8 h at room temperature. Completion of the reaction was judged by the disappearance of starting material in TLC. The solution was acidified with 2 N HCl, evaporated, the residue diluted with ethyl acetate and washed with 2 N HCl. The organic portion was dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and dried to give *O*-(((benzyloxy)carbonyl)-L-alanyl)-*N*-(*tert*-butoxycarbonyl)-L-threonine as oil (0.33 g, 0.78 mmol). Yield, 79%;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 300 MHz)  $\delta_{\text{H}}$ : 7.71 (d, *J* 8.1 Hz, 1H), 7.35 (s, 5H), 7.01 (d, *J* 9.4 Hz, 1H), 5.23 (s, 1H), 5.04 (s, 2H), 4.34–3.84 (m, 2H), 1.41 (s, 9H), 1.25 (d, *J* 7.2 Hz, 3H), 1.14 (d, *J* 6.4 Hz, 3H).  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 75 MHz)  $\delta_{\text{C}}$ : 171.4, 171.2, 156.0, 155.7, 137.0, 128.4, 127.9, 127.8, 78.6, 70.3, 65.5, 56.9, 49.7, 28.2, 17.7, 16.5.

**O-(((Benzyloxy)carbonyl)-L-alanyl)-L-threonine hydrochloride (4).** Boc-L-Thr(Cbz-Ala)-OH **3** (0.42g, 1.0 mmol) was dissolved in dioxane-HCl (g) (6 ml) at 25 °C and stirred for 2 hours. The reaction mixture was evaporated and the residue was recrystallized from diethyl ether to give the hydrochloride salt of O-(((benzyloxy)carbonyl)-L-alanyl)-L-threonine as sticky white solid (0.33 g, 0.91 mmol). Yield 94%; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δ<sub>H</sub>: 7.74 (d, *J* 8.1 Hz, 1H), 7.47–7.16 (m, 5H), 5.38–5.16 (m, 1H), 5.02 (s, 2H), 4.30–3.99 (m, 2H), 1.53–1.11 (m, 6H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δ<sub>C</sub>: 171.3, 168.3, 155.8, 137.0, 128.4, 127.9, 127.8, 68.7, 65.6, 55.5, 49.5, 17.5, 16.6. HRMS (ESI) *m/z* [M-H]<sup>-</sup> Calcd for C<sub>15</sub>H<sub>21</sub>ClN<sub>2</sub>O<sub>6</sub> 323.1249, found 323.1261.

**General procedure for synthesis of compounds (8a–c).** L-Thr(Cbz-L-Ala)-OH hydrogen chloride **4** (0.36 g, 1.0 mmol) and the benzotriazolide of Boc-protected α-, β- or γ-amino acid **7a–c** (1.0 mmol) were dissolved in MeCN (15 mL) and triethylamine (0.15 mL, 1.5 mmol) was added to the mixture. The reaction was stirred for 8 h at room temperature. After completion of the reaction, solvent was removed under reduced pressure and diluted with 2 N hydrochloric acid. The residue was dissolved in 50 mL of ethyl acetate and washed with 2 N hydrochloric acid (3\*20 mL). The organic layer was dried over magnesium sulfate and evaporated to obtain the corresponding isotriptides **8a–c**.

**O-(((Benzyloxy)carbonyl)-L-alanyl)-N-((tert-butoxycarbonyl)glycyl)-L-threonine (8a).** Oil; Yield: 77%; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz) δ<sub>H</sub>: 12.92 (br s, 1H), 7.96 (s, 1H), 7.67 (s, 1H), 7.36 (s, 5H), 7.01 (s, 1H), 5.26 (s, 1H), 5.03 (s, 2H), 4.58 (s, 1H), 4.28–3.96 (m, 1H), 3.67 (s, 2H), 1.49–1.19 (m, 12H), 1.12 (s, 3H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 75 MHz) δ<sub>C</sub>: 171.7, 170.7, 170.0, 155.9, 155.7, 136.9, 128.4, 127.9, 127.8, 78.1, 70.6, 65.5, 54.8, 49.4, 43.1, 28.2, 17.3, 16.4. HRMS (ESI) *m/z* [M+H]<sup>+</sup> Calcd for C<sub>22</sub>H<sub>31</sub>N<sub>3</sub>O<sub>9</sub> 482.2133, found 482.2114.

**O-(((Benzyloxy)carbonyl)-L-alanyl)-N-(3-((tert-butoxycarbonyl)amino)propanoyl)-L-threonine (8b).** Oil; Yield: 79%; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ<sub>H</sub>: 7.35 (br s, 5H), 5.42 (br s, 1H), 5.10 (s, 2H), 4.55–4.35 (m, 1H), 4.31–4.19 (m, 1H), 3.32 (br s, 2H), 2.48 (br s, 2H), 1.48–1.34 (m, 12H), 1.23 (s, 3H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz) δ<sub>C</sub>: 173.7, 172.8, 158.2, 138.1, 129.4, 129.0, 128.8, 80.0, 73.7, 67.6, 59.3, 51.2, 37.9, 37.1, 28.8, 18.0, 17.5. HRMS (ESI) *m/z* [M+Na]<sup>+</sup> Calcd for C<sub>23</sub>H<sub>33</sub>N<sub>3</sub>O<sub>9</sub> 518.2109, found 518.2124.

**O-(((Benzyloxy)carbonyl)-L-alanyl)-N-(4-((tert-butoxycarbonyl)amino)butanoyl)-L-threonine (8c).** Oil; Yield: 78%; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ<sub>H</sub>: 7.35 (br s, 5H), 5.42 (br s, 1H), 5.10 (s, 2H), 4.55–4.35 (m, 1H), 4.31–4.19 (m, 1H), 3.32 (br s, 2H), 2.48 (br s, 2H), 1.48–1.34 (m, 12H), 1.23 (s, 3H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz) δ<sub>C</sub>: 175.3, 173.7, 158.3, 138.0, 129.4, 128.9, 128.7, 79.8, 73.7, 67.5, 59.2, 51.2, 40.7, 34.2, 28.8, 27.1, 17.9, 17.6. HRMS (ESI) *m/z* [M+Na]<sup>+</sup> Calcd for C<sub>24</sub>H<sub>35</sub>N<sub>3</sub>O<sub>9</sub> 532.2266, found 532.2291.

**General procedure for synthesis of compounds (9a–c).** Boc-protected isotriptides **8a–c** (1.00 mmol) were dissolved in dioxane-HCl (g) (6 ml) at 25 °C and stirred for 4 h. The reaction mixtures were evaporated and the residues were recrystallized from diethyl ether to give corresponding hydrogen chloride salts of unprotected isodipeptides **9a–c**.

***O*-(((Benzyloxy)carbonyl)-L-alanyl)-*N*-glycyl-L-threonine hydrochloride (9a).** Off white sticky solid; Yield: 97%; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ<sub>H</sub>: 7.31 (s, 5H), 5.52–5.29 (m, 1H), 5.05 (s, 2H), 4.74–4.65 (m, 1H), 4.16 (d, *J* 7.3 Hz, 1H), 3.79 (s, 2H), 1.33 (d, *J* 7.7 Hz, 3H), 1.25 (d, *J* 6.3 Hz, 3H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz) δ<sub>C</sub>: 173.5, 167.9, 163.1, 158.3, 138.1, 129.5, 129.0, 128.8, 72.0, 67.6, 57.3, 51.0, 41.6, 17.6, 17.2. HRMS (ESI) *m/z* [M+Na]<sup>+</sup> Calcd for C<sub>17</sub>H<sub>24</sub>ClN<sub>3</sub>O<sub>7</sub> 382.1609, found 382.1622.

***N*-(3-Aminopropanoyl)-*O*-(((benzyloxy)carbonyl)-L-alanyl)-L-threonine hydrochloride (9b).** Off white sticky solid; Yield: 97%; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ<sub>H</sub>: 7.31 (br s, 5H), 5.41 (br s, 1H), 5.06 (br s, 2H), 4.69 (br s, 1H), 4.19 (br s, 1H), 3.19 (br s, 2H), 2.77 (br s, 2H), 1.39–1.19 (m, 6H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz) δ<sub>C</sub>: 173.4, 172.8, 171.1, 158.2, 138.1, 129.4, 129.0, 128.7, 72.0, 67.6, 56.9, 51.1, 37.1, 32.6, 17.7, 17.2. HRMS (ESI) *m/z* [M+H]<sup>+</sup> Calcd for C<sub>18</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>7</sub> 396.1765, found 396.1784.

***N*-(4-Aminobutanoyl)-*O*-(((benzyloxy)carbonyl)-L-alanyl)-L-threonine hydrochloride (9c).** Off white sticky solid; Yield: 96%; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 300 MHz) δ<sub>H</sub>: 7.37–7.07 (m, 5H), 5.49–5.23 (m, 1H), 5.00 (s, 2H), 4.60 (s, 1H), 4.27–4.00 (m, 1H), 3.00–2.85 (m, 2H), 2.55–2.29 (m, 2H), 2.01–1.79 (m, 2H), 1.38–1.18 (m, 6H); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 75 MHz) δ<sub>C</sub>: 175.1, 173.4, 173.1, 158.3, 138.1, 129.4, 129.1, 128.7, 72.1, 67.6, 56.97, 51.1, 40.3, 33.3, 24.4, 17.8, 17.2. HRMS (ESI) *m/z* [M+H]<sup>+</sup> Calcd for C<sub>18</sub>H<sub>26</sub>ClN<sub>3</sub>O<sub>7</sub> 410.1922, found 410.1941.

**General procedure for the synthesis of ligated products 5 and 6 in buffer.** Isotetrapeptide (**4**) (0.20 mmol) were each suspended in deoxygenated phosphate buffer (NaH<sub>2</sub>PO<sub>4</sub>/Na<sub>2</sub>HPO<sub>4</sub>) (1 M, pH = 7.4, 8 mL) and irradiated with microwave (50 °C, 50 W, 3 h). Each reaction mixture was allowed to cool to room temperature, acidified with 2 N HCl to pH = 1, and extracted with ethyl acetate (3×10 mL). The combined organic extracts were dried over Na<sub>2</sub>SO<sub>4</sub> and the solvent was removed under reduced pressure. Each ligation mixture was weighed and a solution in methanol (1 mg mL<sup>-1</sup>) was analysed by HPLC-MS.

**General procedure for synthesis of ligated products 5, 6, 10b and 11b in DMF-piperidine.** Isotriptides (**4** & **9**) (0.20 mmol) were each dissolved in a mixture of DMF-piperidine (5mL/1.5mL) and the mixture was irradiated with microwave (50 °C, 50 W, 3 h) in a microwave tube. After cooling to room temperature the reaction mixtures were acidified with 2 N HCl to pH = 1. Each mixture was extracted with ethyl acetate (3×10 mL), the combined organic extracts were dried over sodium sulfate and the solvent removed under reduced pressure. Each ligation mixture was weighed and then a solution in methanol (1 mg mL<sup>-1</sup>) was analysed by HPLC-MS.

## Acknowledgments

We thank the University of Florida and the Kenan Foundation for financial support. This paper was also funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under Grant no. (24-3-1432/HiCi). The authors, therefore, acknowledge the technical and financial support of KAU.



## References

1. Wieland, T.; Bokelmann, E.; Bauer, L.; Lang, H. U.; Lau, H.; Schafer, W. *Liebigs Ann.* **1953**, 583, 129–149.  
<http://dx.doi.org/10.1002/jlac.19535830110>
2. Kent, S. B. H. *Chem. Soc. Rev.* **2009**, 38, 338–351.  
<http://dx.doi.org/10.1039/b700141j>
3. Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. *Science* **1994**, 266, 776–779.  
<http://dx.doi.org/10.1126/science.7973629>
4. Zhang, L.; Torgerson, T. R.; Liu, X.-Y.; Timmons, S.; Colosia, A. D.; Hawiger, J.; Tam, J. P. *Proc. Natl. Acad. Sci. USA* **1998**, 95, 9184–9189.  
<http://dx.doi.org/10.1073/pnas.95.16.9184>
5. Rao, C.; Tam, J. P. *J. Am. Chem. Soc.* **1994**, 116, 6975–6976.  
<http://dx.doi.org/10.1021/ja00094a078>
6. Spetzler, J. C.; Tam, J. P. *Int. J. Pept. Protein Res.* **1995**, 45, 78–85.  
<http://dx.doi.org/10.1111/j.1399-3011.1995.tb01570.x>
7. Kochendoerfer, G. G.; Salom, D.; Lear, J. D.; Wilk-Orescan, R.; Kent, S. B. H.; DeGrado, W. F. *Biochemistry* **1999**, 38, 11905–11913.  
<http://dx.doi.org/10.1021/bi990720m>
8. Muir, T. W.; Sondhi, D.; Cole, P. A. *Proc. Natl. Acad. Sci. USA* **1998**, 95, 6705–6710.  
<http://dx.doi.org/10.1073/pnas.95.12.6705>
9. Evans, T. C. Jr.; Benner, J.; Xu, M. Q. *J. Biol. Chem.* **1999**, 274, 3923–3926.  
<http://dx.doi.org/10.1074/jbc.274.7.3923>
10. Tam, J. P.; Lu, Y. A.; Liu, C. F.; Shao, J. *Proc. Natl. Acad. Sci. USA* **1995**, 92, 12485–12489.  
<http://dx.doi.org/10.1073/pnas.92.26.12485>
11. Schnolzer, M.; Kent, S. B. H. *Science* **1992**, 256, 221–225.  
<http://dx.doi.org/10.1126/science.1566069>
12. Liu, C.-F.; Tam, J. P. *Proc. Natl. Acad. Sci. USA* **1994**, 91, 6584–6588.  
<http://dx.doi.org/10.1073/pnas.91.14.6584>
13. Tam, J. P.; Miao, Z. *J. Am. Chem. Soc.* **1999**, 121, 9013–9022.  
<http://dx.doi.org/10.1021/ja991153t>
14. Fruchart, J.-S.; Gras-Masse, H.; Melnyk, O. *Tetrahedron Lett.* **1999**, 40, 6225–6228.  
[http://dx.doi.org/10.1016/S0040-4039\(99\)01161-2](http://dx.doi.org/10.1016/S0040-4039(99)01161-2)
15. Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, 117, 3893–3899.  
<http://dx.doi.org/10.1021/ja00119a001>
16. Englebretsen, D. R.; Garnham, B. G.; Bergman, D. A.; Alewood, P. F. *Tetrahedron Lett.* **1995**, 36, 8871–8874.  
[http://dx.doi.org/10.1016/0040-4039\(95\)01843-7](http://dx.doi.org/10.1016/0040-4039(95)01843-7)
17. Payne, R. J.; Wong, C.-H. *Chem. Commun.* **2010**, 46, 21–43.  
[http://dx.doi.org/10.1016/0040-4039\(95\)01843-7](http://dx.doi.org/10.1016/0040-4039(95)01843-7)

18. Coltart, D. M. *Tetrahedron* **2000**, *56*, 3449–3491.  
[http://dx.doi.org/10.1016/S0040-4020\(00\)00147-2](http://dx.doi.org/10.1016/S0040-4020(00)00147-2)
19. Coin, I. J. *J. Pept. Sci.* **2010**, *16*, 223–230.  
<http://dx.doi.org/10.1002/psc.1224>
20. Hackenberger, C. P. R.; Schwarzer, D. *Angew. Chem. Int. Ed.* **2008**, *47*, 10030–10074.  
<http://dx.doi.org/10.1002/anie.200801313>
21. Dirksen, A.; Dawson, P. E. *Curr. Opin. Chem. Biol.* **2008**, *12*, 760–766.  
<http://dx.doi.org/10.1016/j.cbpa.2008.10.009>
22. Johnson, E. C. B.; Kent, S. B. H. *J. Am. Chem. Soc.* **2006**, *128*, 6640–6646.  
<http://dx.doi.org/10.1021/ja058344i>
23. Restituyo, J. A.; Comstock, L. R.; Petersen, S. G.; Stringfellow, T.; Rajski, S. R. *Org. Lett.* **2003**, *5*, 4357–4360.  
<http://dx.doi.org/10.1021/ol035635s>
24. Hojo, H.; Ozawa, C.; Katayama, H.; Ueki, A.; Nakahara, Y.; Nakahara, Y. *Angew. Chem. Int. Ed.* **2010**, *49*, 5318–5321.  
<http://dx.doi.org/10.1002/anie.201000384>
25. Macmillan, D.; Anderson, D. W. *Org. Lett.* **2004**, *6*, 4659–4662.  
<http://dx.doi.org/10.1021/ol048145o>
26. Kawakami, T.; Aimoto, S. *Tetrahedron Lett.* **2003**, *44*, 6059–6061.  
[http://dx.doi.org/10.1016/S0040-4039\(03\)01463-1](http://dx.doi.org/10.1016/S0040-4039(03)01463-1)
27. Offer, J.; Boddy, C. N. C.; Dawson, P. E. *J. Am. Chem. Soc.* **2002**, *124*, 4642–4646.  
<http://dx.doi.org/10.1021/ja016731w>
28. Botti, P.; Carrasco, M. R.; Kent, S. B. H. *Tetrahedron Lett.* **2001**, *42*, 1831–1833.  
[http://dx.doi.org/10.1016/S0040-4039\(01\)00036-3](http://dx.doi.org/10.1016/S0040-4039(01)00036-3)
29. Wu, B.; Chen, J.; Warren, J. D.; Chen, G.; Hua, Z.; Danishefsky, S. J. *Angew. Chem. Int. Ed.* **2006**, *45*, 4116–4125.  
<http://dx.doi.org/10.1002/anie.200600538>
30. Okamoto, R.; Kajihara, Y. *Angew. Chem. Int. Ed.* **2008**, *47*, 5402–5406.  
<http://dx.doi.org/10.1002/anie.200801097>
31. Zheng, W.; Zhang, Z.; Ganguly, S.; Weller, J. L.; Klein, D. C.; Cole, P. A. *Nat. Struct. Biol.* **2003**, *10*, 1054–1057.  
<http://dx.doi.org/10.1038/nsb1005>
32. El Khatib, M.; Elagawany, M.; Jabeen, F.; Todadze, E.; Bol'shakov, O.; Oliferenko, A.; Khelashvili, L.; El-Feky, S. A.; Asiri, A.; Katritzky, A. R. *Org. Biomol. Chem.* **2012**, *10*, 4836–4838.  
<http://dx.doi.org/10.1039/c2ob07050b>
33. Popov, V.; Panda, S. S.; Katritzky, A. R. *J. Org. Chem.* **2013**, *78*, 7455–7461.  
<http://dx.doi.org/10.1021/jo4009468>
34. Li, X.; Lam, H. Y.; Zhang, Y.; Chan, C. K. *Org. Lett.* **2010**, *12*, 1724–1727.  
<http://dx.doi.org/10.1021/ol1003109>