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Ligations of *N*-acyl tryptophan units to give native peptides *via* 7-, 10-, 11- and 12-membered cyclic transition states†

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Vadim Popov,[a] Siva S. Panda[a] and Alan R. Katritzky*[a,b]

***N*-Acyl tryptophan isopeptides undergo acyl transfer in chemical ligations *via* 7-, 10-, 11- and 12-membered cyclic transition states to yield natural peptides, representing the first examples of successful isopeptide ligations from *N*-acyl tryptophan units.**

A recent important advance in peptide chemistry has been the development of orthogonal ligation methods which provide a powerful strategy to synthesize macromolecular peptides[1–6] difficult to obtain by conventional peptide synthesis. 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[1–4] or biosynthetic in origin.[5,6] Many organic reactions have been enabled by ligations *via* a variety of chemical linkages, including amide,[7] thioester,[8] thiazolidine,[9] oxaproline,[10] oxime,[11] hydrazone,[12] and thioether moieties.[13]

Native chemical ligation (NCL), while of great importance, is subject to limitations including (i) the requirement of a N-terminal cysteine residue at the ligation site to afford a peptide containing an internal cysteine, and (ii) the low abundance of cysteine in human proteins (1.7% of the residues).[14–17] In attempts to overcome the limitation of low abundance of cysteine, considerable effort has been devoted to developing auxiliary thiol groups, but their use in ligations was found: (i) difficult to complete due to steric hindrance[18–23] and (ii) problematic since extraneous groups present in the ligated product, can be difficult to remove.[18–23] Another strategy involves the conversion of the cysteine into a serine residue after NCL,[16,17] but this requires post-NCL modifications after NCL peptide synthesis.

In an alternative approach, our group developed[24–27] ligations of *S*-acylated cysteine peptides to form native peptides through a diversity of transition states with 8- to 19-membered rings. This methodology requires no auxiliary groups and enables the selective *S*-acylation of cysteine peptides by *N*-acylbenzotriazoles in good yields and under mild conditions followed by microwave-assisted chemical ligations of the *S*-acyl isopeptides. However, ligation through an 8-membered transition state can be a challenge and the low abundance of cysteine remains an obstacle. We recently reported the chemoselective *O*- to *N*-acyl migration of *O*-acyl serines *via* 5-, 8- and 11-membered transition states.[28]

We now demonstrate the first *N*- to *N*-acyl migration involving tryptophan isopeptides migrations *via* 7-, 10-, 11-, and 12-membered transition states. These chemical ligations have been achieved by migration of an *N*-peptidoyl unit of a tryptophan isopeptide unit to produce natural peptide and utilize neither cysteine residue nor an auxiliary group at the ligation site.

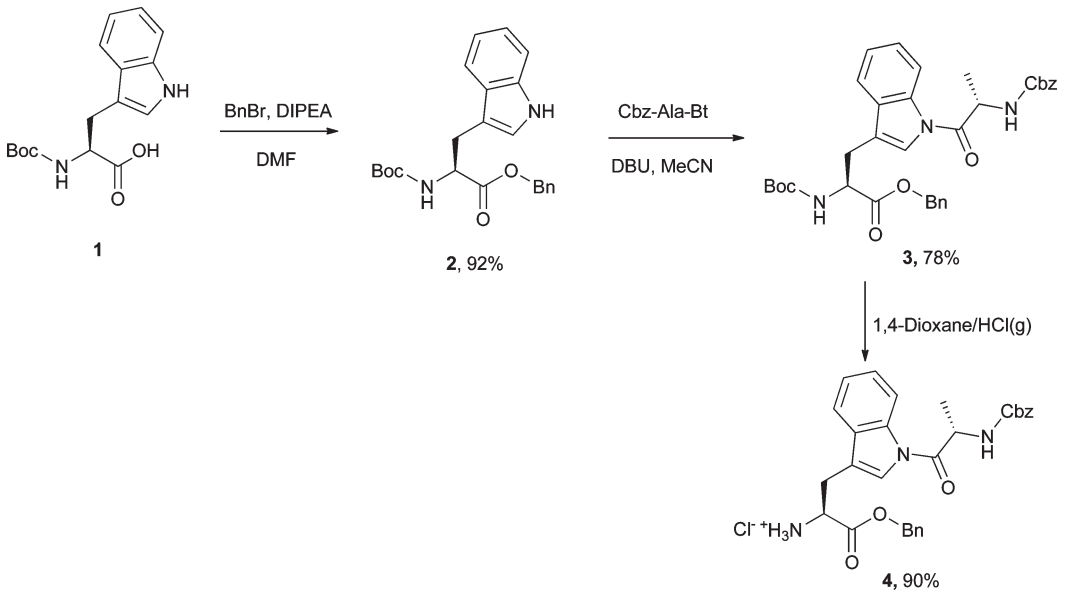
We synthesized the intermediate mono-isodipeptide **4** to study the *N*-acyl migration from the indole nitrogen to the N-terminal group of tryptophan amino acid sequence *via* a 7-membered transition state and also to serve as starting material to study the possibility of *N*- to *N*-acyl migration *via* 10-, 11- and 12-membered cyclic transition states. Compound **4** on coupling with α-, β- or γ-amino acids gave the starting mono-isotripeptides (**9a–c**) needed for the ligation studies involving these large transition states. To enhance migration rates, we used a glycine unit at the N-terminus of mono-isotripeptide **9a** and β- and γ-amino acid units in mono-isotripeptides **9b** and **9c** respectively.

Boc-protected tryptophan was treated with benzyl bromide in presence of Hunig's base in DMF to obtain (*S*)-benzyl 2-((*tert*-butoxycarbonyl)amino)-3-(1*H*-indol-3-yl)propanoate (**2**) in 92% yield. The *N*-acylation of the indole of compound **2** was carried out by react with Cbz-Ala-Bt in presence of DBU in acetonitrile to afford Boc-protected mono-isodipeptide **3** (78%), which on deprotection with dioxane–HCl solution afforded unprotected mono-isodipeptide **4** (90%) (Scheme 1).

[a]*Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA. E-mail: katritzky@chem.ufl.edu; Fax: +1 352-392-9199; Tel: +1 352-392-0554*

[b]*Department of Chemistry, King Abdulaziz University, Jeddah, 21589 Saudi Arabia*

†Electronic supplementary information (ESI) available: ^{1}H, ^{13}C NMR, CHN/HRMS for all compounds and HPLC chromatograms and ESI-MS spectra of ligation studies. See DOI: 10.1039/c3ob27421g



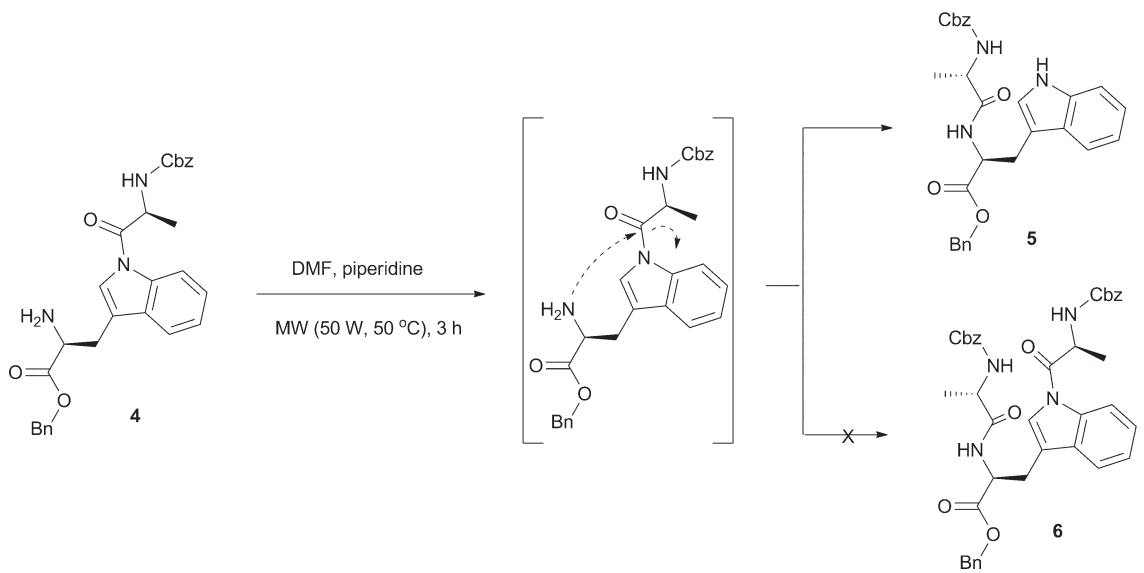
Scheme 1 Synthesis of *N*-acyl tryptophan isodipeptide **4**.

Chemical ligation *via* a 7-membered cyclic transition state was investigated by subjecting mono-isodipeptide **4** to microwave irradiation at 50 °C, 50 W irradiation power for 3 h using 1 M NaH_2PO_4/Na_2HPO_4 phosphate buffer to maintain pH 7.4 (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 × 20 mL), the combined organic extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The ligation mixture was weighed and then a solution in methanol (1 mg mL^{-1}) was analyzed by HPLC-MS.

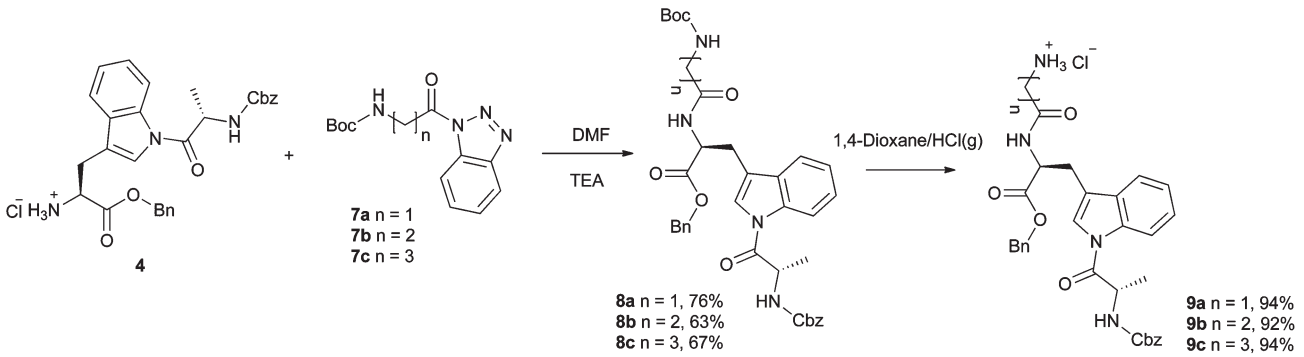
HPLC-MS analysis indicates the ligation did not take place in aqueous conditions (pH 7.3, 1 M buffer strength, MW 50 °C, 50 W, 3 h). We also examined the HPLC-MS of the ligation mixture under basic condition (DMF, piperidine, MW 50 °C, 50 W, 3 h) which disclosed a small amount (2%) the ligated product **5**, together with a major peak corresponding to **4**.

Unprotected mono-isodipeptide **4**, on coupling with benzotriazolide of Boc protected α-, β- or γ-amino acids **7a–c** at room temperature in DMF in the presence of 2 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 ^1H, ^{13}C NMR and CHN/HRMS analysis (Scheme 3).

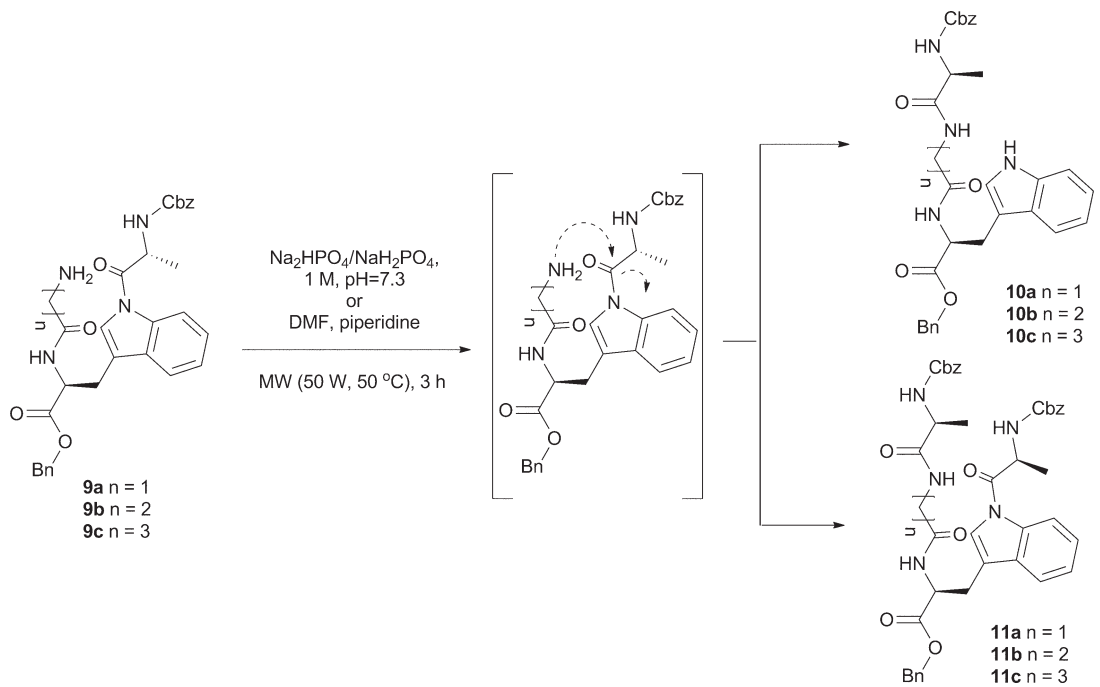
When compounds **9b–c** were subjected to ligation (Scheme 4) under *aqueous conditions*, (pH 7.3, 1 M buffer



Scheme 2 Study of the feasibility of *N*→*N* acyl migration *via* a 7-membered cyclic transition state.



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



Scheme 4 Study of the feasibility of *N*→*N* acyl migrations *via* a 10-, 11- and 12-membered cyclic transition states.

Table 1 Chemical ligations of *N*-acyl isotripeptides **9a–c** in buffer

			Relative area[a,b] (%)			Product characterization by HPLC-MS			
						Ligated peptide (LP)		Transacylation product (TA)	
React	Cyclic TS size	Total crude yield (%) of products isolated	React	LP (RT)	TA (RT)	LP	$[M + H]^+$ found	TA	$[M + H]^+$ found
9a	10	nd		nd	nd	**10a**	nd	**11a**	nd
9b	11	87	36.9 (49.9)	29.1 (59.2)	33.9 (65.52)	**10b**	571.2	**11b**	776.1
9c	12	92	86.4 (48.8)	6.3 (59.5)	7.3 (65.4)	**10c**	585.2	**11c**	790.1

[a] Determined by HPLC-MS semiquantitative. The area of ion-peak resulting from the sum of the intensities of the $[M + H]^+$ and $[M + Na]^+$ ions of each compound was integrated (corrected for starting material). [b] LP = ligated peptide, TA = transacylation product.

strength, MW 50 °C, 50 W, 3 h), we obtained the desired ligated products **10b** (29%) and **10c** (6%) *via* 11- and 12-membered transition states (Scheme 4, Table 1). However **10a** (44%), **10b** (71%) and **10c** (99%) were obtained *via* 10-, 11- and 12-membered cyclic transition state observed when **9a–c** were subjected to ligation reaction under microwave irradiation in

Table 2 Chemical ligation of *N*-acyl isotripeptides **9a–c** in DMF–piperidine

			Relative area[a,b] (%)			Product characterization by HPLC-MS			
						Ligated peptide (LP)		Transacylation product (TA)	
React	Cyclic TS size	Total crude yield (%) of products isolated	React	LP (RT)	TA (RT)	LP	$[M + H]^+$ found	TA	$[M + H]^+$ found
9a	10	92	36.3 (48.2)	44.4 (57.3)	19.2 (63.2)	**10a**	557.1	**11a**	762.1
9b	11	91	28.6 (48.2)	71.4 (57.1)	0.00	**10b**	571.1	**11b**	—
9c	12	90	0.9 (45.2)	99.1 (54.2)	0.00	**10c**	585.1	**11c**	—

[a] Determined by HPLC-MS semiquantitative. The area of ion-peak resulting from the sum of the intensities of the $[M + H]^+$ and $[M + Na]^+$ ions of each compound was integrated (corrected for starting material). [b] LP = ligated peptide, TA = transacylation product.

piperidine–DMF at 50 °C, 50 W for 3 h (Scheme 4, Table 2). HPLC-MS indicated the formation of desired intramolecular ligated products **10a–c**, transacylated products **11a–c** and unreacted starting material **9a–c**. The retention times and fragmentation patterns of **9a–c** and **10a–c** were also studied with control experiments (HPLC-MS of pure **9a–c**). Thus HPLC-MS, *via* (−)ESI-MS/MS, confirmed that compounds **9a–c** and **10a–c**, have very different fragmentation patterns, proving the formation of intramolecular ligated product **10a–c** (see ESI†). The identity of **10c** was further confirmed by HRMS.

Conclusion

Tryptophan isopeptides with α-, β-, or γ-amino acid units were synthesized and acyl migration form indole nitrogen to terminal NH_2 was studied under microwave irradiation. Intramolecular acyl transfer through 10-, 11- and 12-membered transition states was favoured over 7-membered transition state and acyl migration occur more readily in basic non-aqueous media relative to aqueous buffered conditions. This observations form a starting point for development of a ligation method.

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