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Total synthesis of cyclic heptapeptide Rolloamide B†

Mirna El Khatib,[a] Mohamed Elagawany,[abc] Eray Çalışkan,[a] Emily Faith Davis,[a] Hassan M. Faidallah,[d] Said A. El-feky[b] and Alan R. Katritzky*[ad]

Cite this: *Chem. Commun.*, 2013, **49**, 2631

Received 30th December 2012,
Accepted 23rd January 2013

DOI: 10.1039/c3cc39291k

www.rsc.org/chemcomm

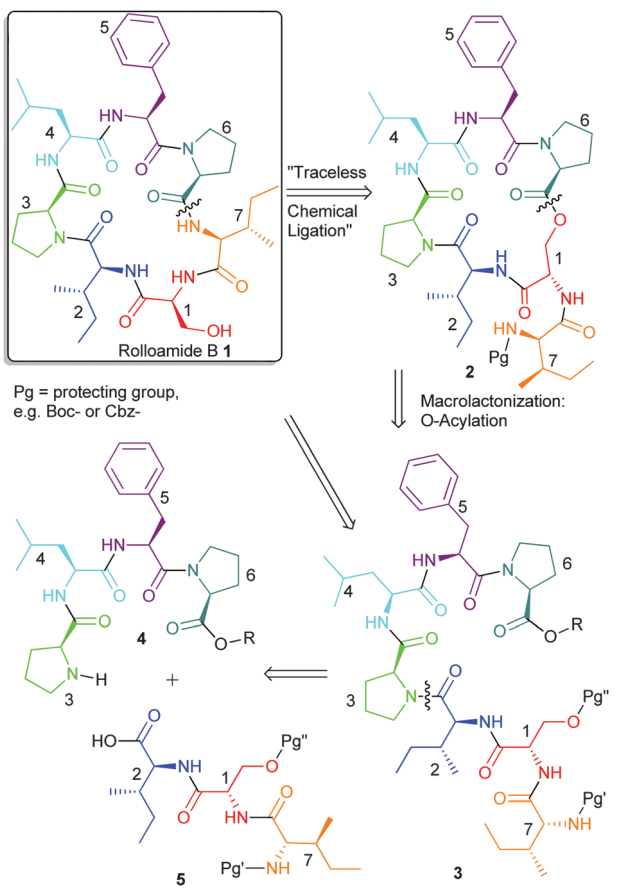
The first total synthesis of Rolloamide B, a cyclic proline-enriched heptapeptide, is reported. This work features solution phase benzotriazole-mediated peptide synthesis ligating native amino acids.

Since the first report of peptide composition by Emil Fischer,[1] solution-phase condensation strategies and orthogonal functional group protection[2–6] have become important synthetic tools for the preparation of native peptides and investigation of biological processes.[7–9] Protein synthesis gained further attention when natural protein modifications were identified as key elements for controlling complex cellular processes in living organisms.[3,10,11]

Peptides now draw considerable attention as potential therapeutics due to their roles as mediators of key biological functions together with low toxicity and high specificity. In 2010, four of the 60 peptide drugs on the market reached global sales in excess of US $ 1 billion, and more than 500 peptides are under clinical development.[12,13]

The topology of macrocyclic peptides reduces their susceptibility to attack by *exo*- and *endo*-peptidases;[14,15] thus, they play an important role in nature and are attractive targets for drug discovery and biomedical research.[16–20] Well-known examples of cyclic peptide drugs include the natural antibiotic vancomycin, hormone oxytocin, neuropeptide vasopressin and antibiotics cyclosporine and tyrocidine A.[21–27]

In 2009, Anderson and co-workers isolated 0.8 mg of Rolloamide B **1**, extracted from 2.6 kg of Dominican marine sponge *Eurypon laughlini*.[28] Chemical and spectroscopic analyses showed the structure of Rolloamide B **1** (Scheme 1) to be a cyclic heptapeptide[28] with attractive structural features, encouraging interest in its chemical synthesis. Marine sponges are a rich source of non-ribosomal biologically active cyclic peptides with seven to ten amino acid residues.[15,29–31] Their reduced zwitterionic character provides more lipophilicity and thus increases membrane permeability, by comparison with linear analogs.[30] The two prolines present in **1** render this natural product intriguing. Proline units reduce the backbone flexibility of cyclic peptides, thus enhancing the affinity and selectivity for protein binding.[30] Proline also facilitates the



Scheme 1 Retrosynthesis of **1**.

[a] *Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA. E-mail: katritzky@chem.ufl.edu*
[b] *Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig-44519, Egypt*
[c] *Department of Organic Chemistry, College of Pharmacy, Misr University for Science and Technology, Al-Motamayez District, 6th of October, P.O. Box: 77, Egypt*
[d] *Department of Chemistry, King Abdulaziz University, Jeddah, 21589, Saudi Arabia*
† Electronic supplementary information (ESI) available: Experimental procedures and ^{1}H and ^{13}C NMR for all compounds. See DOI: 10.1039/c3cc39291k

formation of β-turns and increases the structural diversity as a result of *cis–trans* isomerism in cyclic peptides.[32–34] Rolloamide B **1** also possesses a β-hydroxy-α-amino acid (L-Ser) with a solubilizing effect. β-Hydroxy-α-amino acid units, Ser/Thr, are present in numerous natural products possessing a wide range of biological activities such as antibiotics and immunosuppressants (for example, vancomycin and echinocandin D);[35,36] phosphorylation of proteins on Ser and Thr residues is fundamental to signal transduction processes in cells.[37]

Herein, we report the first total synthesis of Rolloamide B *via* a solution-phase benzotriazole-mediated synthesis demonstrating a novel route of peptide building of peptide-based macrocycles complementing the route of isolating such materials from natural sources.

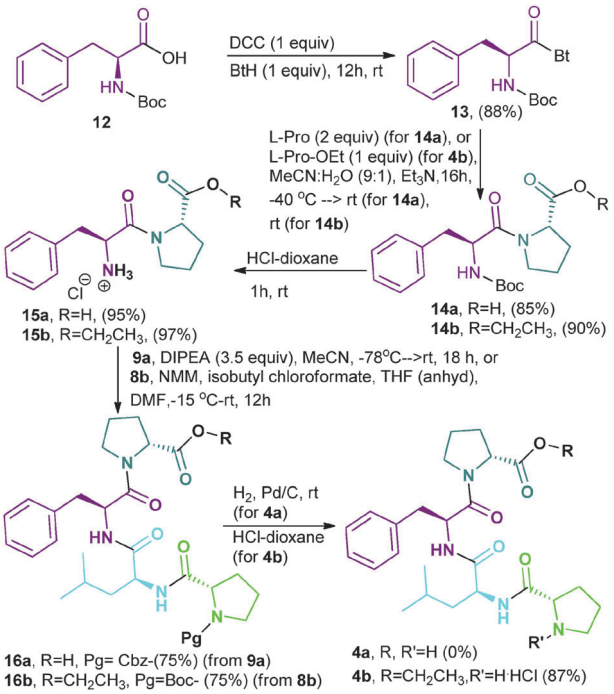
We envisaged the synthesis of Rolloamide B **1** (Scheme 1) from two possible routes, both involving cyclization as the final stages of the synthesis: (i) macrocyclization of a linear heptapeptide chain followed by functional group deprotection and (ii) functional group deprotection of the hydroxyl group in the Ser-unit, assisting the cyclization step by 'traceless' chemical ligation. The latter step involves an initial macrolactonization followed by O- to N- acyl transfer, a route previously developed by our group for linear peptides *via* cyclic membered transition states.[38,39] The initial generation of the lactone has been reported to yield a more conformationally flexible peptide bringing the N-terminus in close proximity to the C-terminus.[15,40,41]

We planned to assemble linear heptapeptide **3** in solution phase from tetrapeptide **4** and tripeptide **5** each prepared by benzotriazole activation of naturally occurring amino acids utilizing orthogonal protection and deprotection strategies (Scheme 1).

Starting from Pg-L-Pro-OH **6a** (Pg=Cbz-)coupling of amino acid residues resulted in linear tripeptide **10a** (Scheme 2) but preparation of tri-peptide-Bt **11a** failed.

Our alternative route to key intermediate **4** was coupling dipeptide fragment **15** (Scheme 3) with already prepared dipeptide **9** or **8** (Scheme 2). Compound **12** was converted into its benzotriazolide **13** (88%) using DCC in methylene chloride. Coupling **13** with L-Pro-OH and L-Pro-OEt followed by Boc-deprotection using commercial HCl–dioxane solution gave compounds **15a** and **15b** (Scheme 3).

Coupling of **9a** with **15a** in MeCN and DIPEA at −78 °C gave **16a** (75%) (Scheme 3). Although direct esterification of tetrapeptide

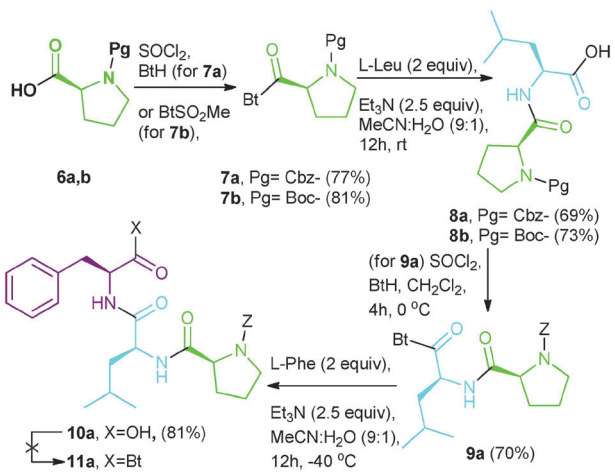


Scheme 3 Synthetic scheme for preparation of intermediate **4**.

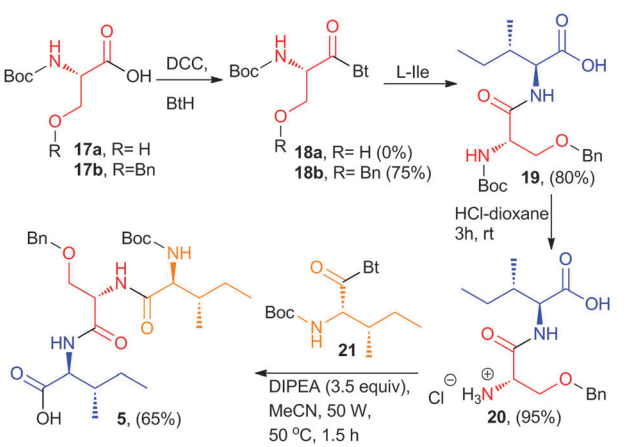
16a failed, direct coupling of **15b** with **8b** (both prepared using benzotriazole activation)[42,43] gave the desired **16b**, reducing the number of steps to key intermediate **4** (Scheme 3). Although attempted Cbz deprotection of **16a** with hydrogen and catalytic Pd/C consistently failed to give **4a** (Scheme 3), removal of the Boc-protecting group in **16b** provided **4b** (87%).

For the synthesis of intermediate **5** (Scheme 4), activation of the carboxyl of Boc-L-Ser-OH **17a** to give **18a** failed, but the corresponding benzyl ether **17b** gave benzotriazolide **18b** (75%). Compound **18b** was coupled with L-Ile to give **19** followed by deprotection of the Boc group affording **20**. Coupling **20** with **21** (prepared by activating L-Ile with benzotriazole) was completed under microwave irradiation to provide key intermediate **5**.

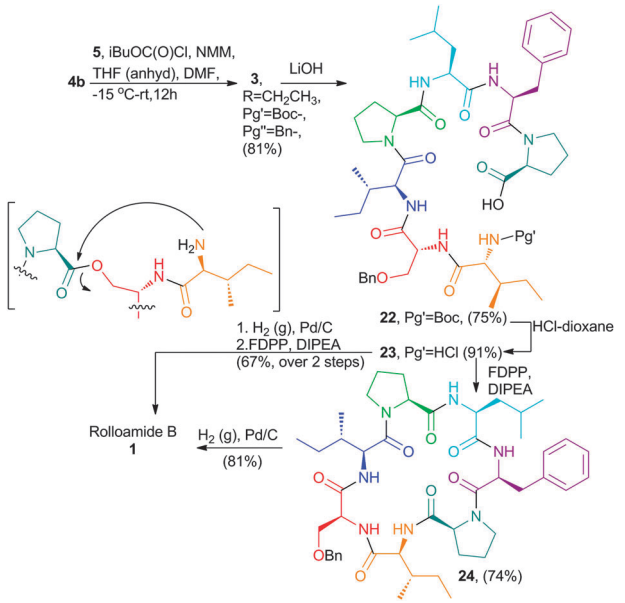
With both the tetrapeptide **4** and tripeptide **5** in hand, the next goal was fragment ligation of **4** with **5** to prepare the requisite linear



Scheme 2 Attempted preparation of intermediate **4** by linear peptide coupling.



Scheme 4 Preparation of intermediate **5**.



Scheme 5 Macrocyclization to **1**.

heptapeptide **3** (Scheme 5). Indeed, fragment **4** was readily coupled with fragment **5** to give linear heptapeptide **3** (81%). Stepwise and selective removal of the ethyl ester in **3** with LiOH gave **22**, and subsequent Boc-group removal with HCl–dioxane afforded **23** (Scheme 5). Key intermediate **23** was cyclized by the FDPP peptide coupling reagent in the presence of DIPEA to give macrocycle **24** in 74% yield. Removal of the benzyl protecting group gave the desired macrocycle peptide natural product, Rolloamide B (**1**) (Scheme 5), on a 130 mg scale ($[\alpha]_D^{20}$ −112.8 (*c* 1.0, CH_3OH)).

We also examined initial deprotection of the benzyl group, followed by an attempt to cyclize affording **1** (Scheme 5). Initial benzyl deprotection provides a free hydroxyl group which is known to aid in the cyclization through 'traceless' chemical ligation using serine sites, a methodology developed earlier by our group.[38,39] The generation of the lactone, by the reaction of the free OH group in Ser- with the carboxylic group, gives a more conformationally flexible peptide bringing the N-terminus in close proximity to the C-terminus and thus facilitating an O- to N- acyl transfer to afford the cyclic native peptide.[15,40,44]

In conclusion, we have achieved the first total synthesis of cyclic heptapeptide Rolloamide B **1** on a scale of 130 mg in a total of 9 steps (shortest route, 16 steps for the longest route) and an overall yield of 12.8% (3.6% for the longest route) and an average yield of 79.8% (82.0% for longest route).

The authors thank the University of Florida, the Kenan foundation, and also acknowledge King Abdulaziz University for technical and financial support of this work. We thank Dr C. D. Hall for helpful discussions.

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