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Synthesis of Biologically Active Dicarba Analogues of the Peptide Hormone Oxytocin Using Ring-Closing Metathesis

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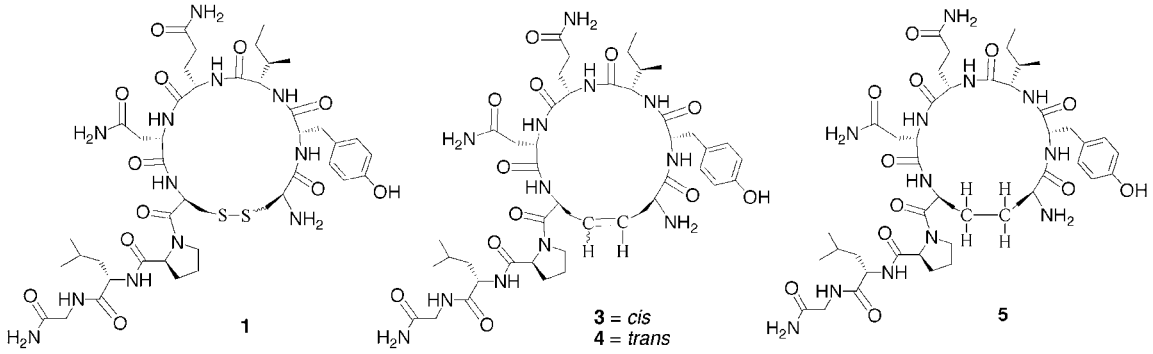
ABSTRACT



Facile synthesis of cis and trans olefinic analogues of oxytocin 1 that have carbon in place of sulfur is achieved via ring-closing metathesis (RCM) on a resin-bound linear precursor peptide. Hydrogenation of the cis olefin, 3, proceeds selectively to generate the previously reported saturated derivative 5. Biological testing on rat uterus strips shows that cis compound 3 has an EC50 value of 38 ng/mL (EC50 for oxytocin is 2.7 ng/mL) whereas 5 and trans olefin 4 are less active.

The synthesis of diamino acids and cyclic peptide derivatives using ring-closing metathesis (RCM) affords the opportunity to substitute a metabolically less stable disulfide bridge with two methylene groups, a structural change that results in replacement of LL-cystine with LL-diaminosuberic acid.[1] Although such replacement should in principle impose negligible change on the overall peptide structure, the polarity of the sulfurs and their preference for a close to 90° dihedral angle is clearly different from the two methylenes in the "dicarba" analogue. Oxytocin **1** is a mammalian nonapeptide hormone that controls mammary and uterine smooth muscle contraction,[2] has neurotransmitting properties in the central nervous system, and displays autocrine and/or paracrine functions in the ovaries and testes.[2,3] The synthesis of a fully saturated dicarba analogue of oxytocin has been described,[4] but it involves a cumbersome multistep procedure to incorporate a selectively protected diaminosuberic acid moiety. The compound was reported as being "less active"

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(1) For some early reports, see: (a) Miller, S. J.; Blackwell, H. E.; Grubbs, R. H. *J. Am. Chem. Soc.* **1996**, *118*, 9606−9614. (b) Williams, R. M.; Liu, J. *J. Org. Chem.* **1998**, *63*, 2130−2132. (c) Gao, Y.; Lane-Bell, P.; Vederas, J. C. *J. Org. Chem.* **1998**, *63*, 2133−2143.

(2) For a review, see: (a) Lehninger, A. L.; Nelson, D. L.; Cox, M. *Principles of Biochemistry*, 2nd ed.; Worth Publishers, Inc.: New York, 1993; pp 750−752. For examples of oxytocin analogues see: (b) Bélec, L.; Slaninova, J.; Lubell, W. D. *J. Med. Chem.* **2000**, *43*, 1448−1445. (c) ZhongQing, Y.; Blomberg, D.; Sethson, I.; Brickman, K.; Ekholm, K.; Johansson, B.; Nilsson, A.; Kihlberg, J. *J. Med. Chem.* **2002**, *45*, 2512−2519.

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than the parent hormone **1**, which may be due to greater conformational flexibility at the bis-methylene unit. In the present study, we show that substitution of the cysteines with L-allylglycine residues allows RCM for facile generation of more rigid olefinic analogues of **1** and provides access to the saturated dicarba derivative (Figure 1).

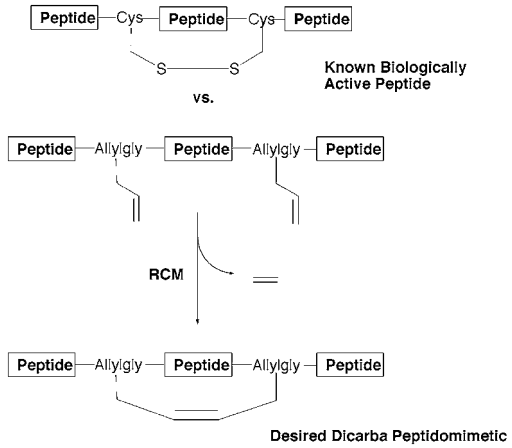
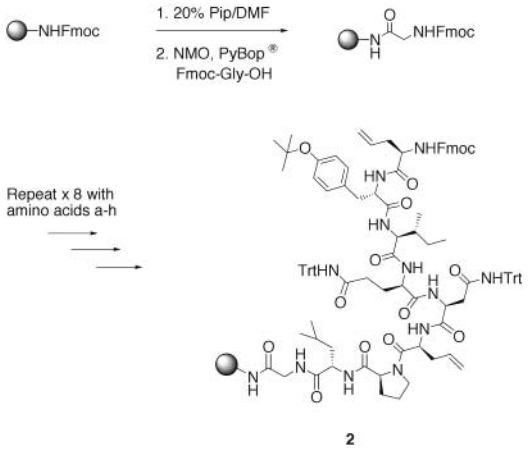


Figure 1. RCM Strategy for Peptidic Dicarba Analogues

Ruthenium-catalyzed RCM has been previously achieved[5,6] on an assortment of peptidic diene systems[1,7,8] with varying yields. To date, the cyclization of bis-allylglycine-containing peptides to make hormone analogues has not been reported, nor have such olefinic analogues been investigated for biological activity. It is interesting that the X-ray crystal structure of free oxytocin **1** does not allow exact determination of the conformation at the disulfide,[9] although this is clearly fixed in a single orientation upon binding to a neurophyseal carrier protein.[10]

The synthesis is initiated by first building the linear peptide backbone **2** using allylglycine in place of cysteine residues of oxytocin on Rink amide NovaGel resin in conjunction with standard Fmoc chemistry for amino acid coupling (Scheme 1).[11] This resin conveniently provides the C-terminal amide functionality upon cleavage. Protection of the side chains of tyrosine (O-tBu), asparagine (N-Trt), and glutamine (N-Trt) with acid-labile groups is essential. The trityl (Trt) groups ensure optimal coupling by preventing tandem cyclization and dehydration of the primary amide side chains,[12,13] whereas the *tert*-butyl group is necessary to avoid interference of the phenol in the RCM reaction.

Scheme 1. Synthesis of the Linear Peptide Backbone[a]



[a] Conditions: (a) Fmoc-Leu-OH, (b) Fmoc-Pro-OH, (c) Fmoc-allylglycine-OH, (d) Fmoc-Asn(N-Trt)-OH, (e) Fmoc-Gln(N-Trt)-OH, (f) Fmoc-Ile-OH, (g) Fmoc-Tyr(O-tBu)-OH, (h) Fmoc-allylglycine-OH.

Resin-bound linear peptide **2** could then be cyclized using 10 mol % Grubbs (benzylidene-bis(tricyclohexyl-phosphine)-dichlororuthenium) catalyst[6,7] to give a mixture of olefinic products. Upon completion of this reaction, it is essential to add DMSO (50 equiv relative to the catalyst loading) to the resin-bound peptide. Failure to do so results in the production of a mixture of products and ruthenium-containing contaminants that is exceedingly difficult to separate, even by reverse-phase HPLC. This technique is an adaptation of a literature procedure[14] wherein DMSO was added to a solution-phase RCM reaction unrelated to peptide synthesis.[15] Removal of the remaining Fmoc group followed by acidic cleavage from the resin with concomitant side chain deprotection affords a 4:1 mixture of cis and trans isomers **3** and **4**, respectively (Scheme 2).

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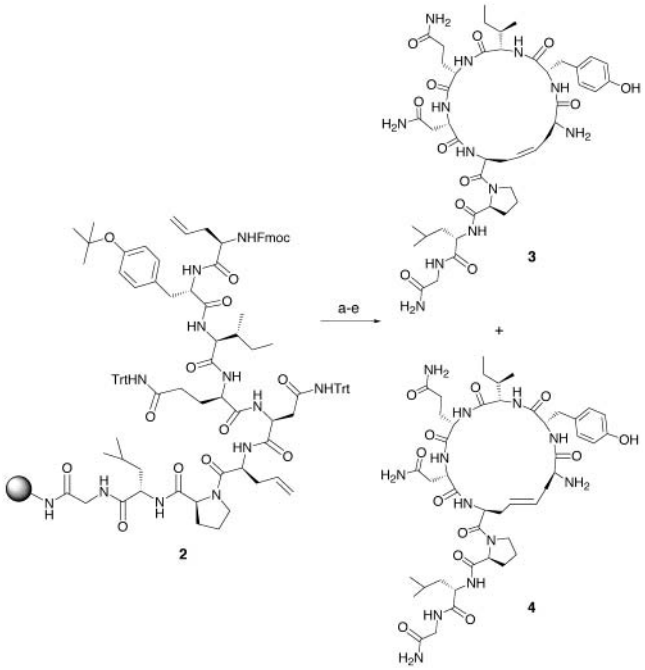
(12) Novabiochem Catalog 2002−2003; Calbiochem-Novabiochem Inc., San Diego, CA.

(13) Several peptide syntheses were done using asparagine and glutamine subunits without side chain protection in the presence of an extra 1 equiv of HOBt.[10] In all attempts, a substantial amount of dehydrated product was obtained, as shown by ES/MS as a (M-18) peak.

(14) Ahn, Y. M.; Yang, K.; Georg, G. I. *Org. Lett.* **2001**, *3*, 9, 1411−1413.

(15) To our knowledge, this is the first time ruthenium byproducts have been removed using simple DMSO injection and filtration when working with resin-bound peptides.

Scheme 2 Cyclization of **2**[a]



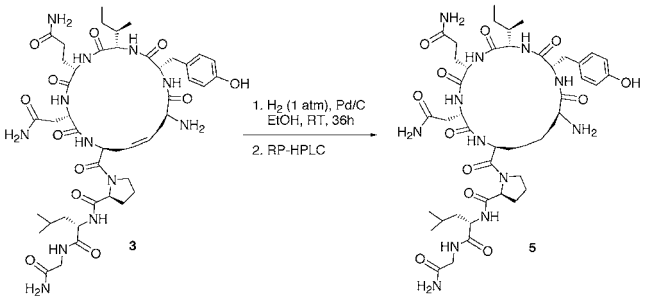
[a] Conditions: (a) 10 mol % $(PCy_3)_2Cl_2Ru(CHPh)$ (Grubbs catalyst), DCM, reflux, 24h. (b) 50 equiv of DMSO, rt, 12 h. (c) 20% Pip/DMF, rt, 5 min. (d) 90% TFA/5% DCM/5% anisole, 1 h. (e) RP-HPLC.

Separation of **3** and **4** is facile using reverse-phase HPLC. The combined yield of these pure olefinic products is ca. 45% from resin-bound material **2**.

The geometry of the double bond could be determined by NMR spectrometry using multiple decoupling experiments in which all methylene protons adjacent to the olefin protons in **3** or **4** (assigned by gCOSY) are simultaneously irradiated using two frequencies. This allows the coupling constants of the AB quartet formed by the two olefin protons in **3** and **4** to be determined. The J_{AB} for **3** (cis isomer) is 10.9 Hz, whereas the corresponding J_{AB} value for **4** (trans isomer) is 15.9 Hz.

Hydrogenation of a mixture of **3** and **4** using a variety of conditions selectively reduces **3** to bis-methylene analogue **5** without altering **4**. Initial attempts at reducing the cis/trans mixture of free cyclic peptides after acidic cleavage but without Fmoc removal using *N*,*N*-dimethylacetamide (DMAc) with H_2 at 1 atm gave only starting material. Attempted reduction using in situ diimide production (triisopropyl benzenesulfonyl hydrazide (TPSH) and base)[7,16] yielded fully deprotected peptides with selective hydrogenation of **3** to **5**.[17] Purified cis isomer **3** is soluble in ethanol and easily hydrogenated in quantitative yield (Scheme 3).

Scheme 3. Reduction of **3**



Testing for biological activity of **3**−**5** utilized freshly excised rat uterus strips according to established literature procedures.[18] Preliminary results show that cis isomer **3** is the most active dicarba analogue with an EC50 value of 38 ng/mL, which is approximately 14-fold less than that of oxytocin **1**, whose EC50 is 2.7 ng/mL. Trans isomer **4** and saturated dicarba analogue **5** are about an order of magnitude less potent than **3** with EC50 values of 242 and 338 ng/mL, respectively.

In summary, RCM on resin-bound allylglycine-containing peptides provides an effective and rapid method for the synthesis of cyclic dicarba analogues of biologically active peptide hormones such as oxytocin **1**. The ability of RCM to introduce more rigid ethylene bridges in place of metabolically less stable disulfide moieties may prove to be useful for generation of analogues of a host of biologically active peptides and proteins. The yields of resin-bound olefin metathesis are acceptable when compared with other peptide RCM cyclizations[7,19] and may potentially be improved by use of second generation catalysts.[6] The synthesis of other analogues of oxytocin using carbon bridges of varying lengths is currently under investigation, as is application of the methodology to other biologically active peptides.

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Supporting Information Available: Characterization and biological data for compounds **3**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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