

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

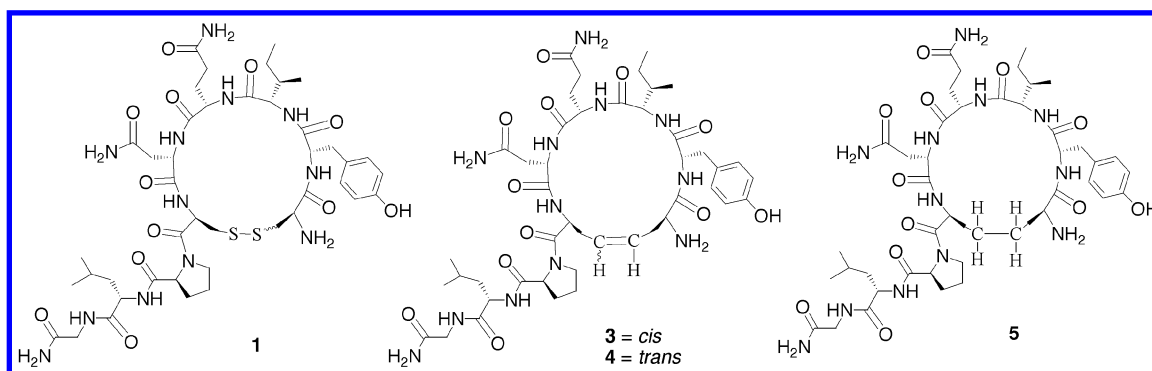
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Received October 23, 2002

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).

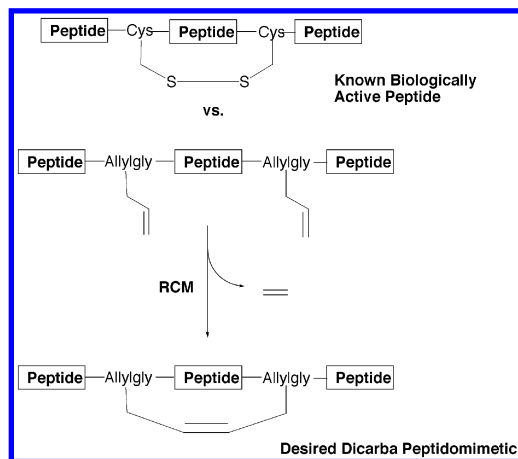


Figure 1. RCM Strategy for Peptidic Dicarba Analogues

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 neurophysseal carrier protein.¹⁰

The synthesis is initiated by first building the linear peptide backbone **2** using allylglycine in place of cysteine residues

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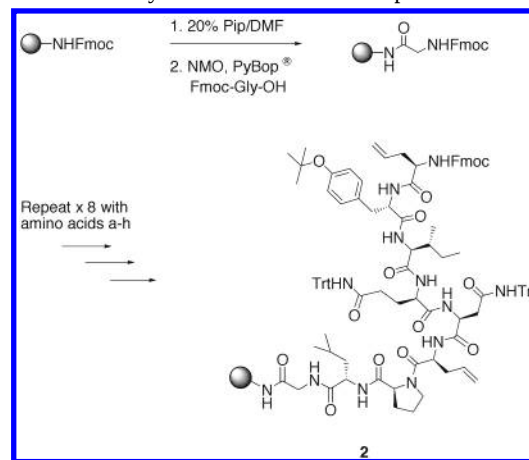
(8) Examples of RCM on resin-bound peptides. (a) Schmiedeborg, N.; Kessler, H. *Org. Lett.* **2002**, 4 (1), 59–62. (b) Jarvo, E. R.; Copeland, G. T.; Papaioannou, N.; Bonitatebus, P. J., Jr.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, 121, 11638–11643. (c) Schafmeister, C. E.; Po, J.; Verdine, G. L. *J. Am. Chem. Soc.* **2000**, 122, 5891–5892.

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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

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.

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.

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 procedure¹⁴ 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).

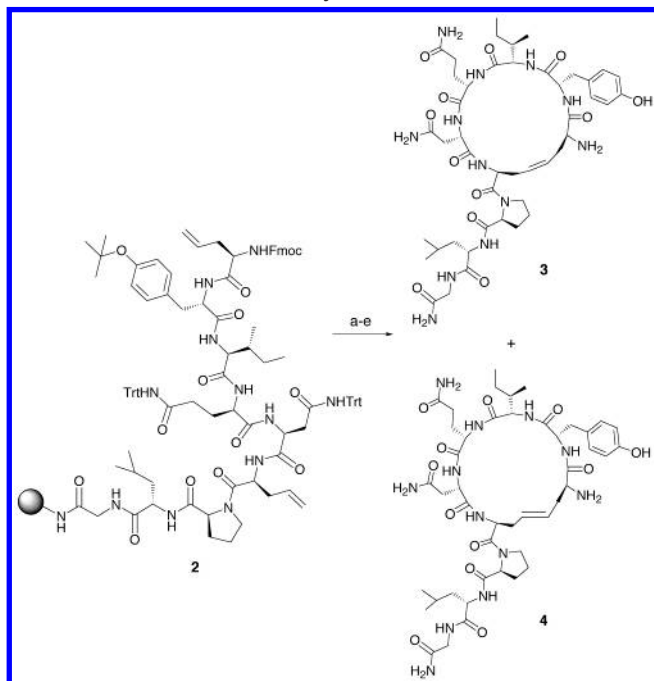
(11) (a) Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamadaka, V.; Lyttle, M. H.; Songster, M. F. *J. Org. Chem.* **1998**, 63, 3706–3716. (b) Sieber, P.; Rinker, B. *Tetrahedron Lett.* **1991**, 32, 739–742.

(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 % (PCy₃)₂Cl₂Ru(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**.

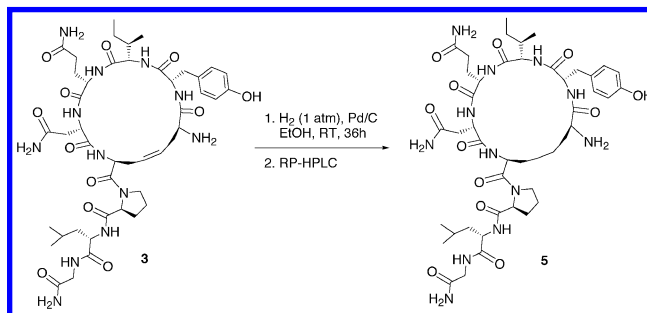
The geometry of the double bond could be determined by NMR spectrometry using multiple decoupling experiments in which all methylene protons adjacent to the olefin protons in **3** or **4** (assigned by gCOSY) are simultaneously irradiated using two frequencies. This allows the coupling constants of 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 H₂ 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).

(16) Cusack, N. J. *Tetrahedron* **1976**, 32, 2157–2162.

(17) No "Fmoc" group was detectable by ¹H NMR, and only the olefin protons of trans olefinic isomer **6** were detected.

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.

Acknowledgment. We thank Dr. T. Nakashima for assistance with simultaneous double-decoupling experiments. These investigations were supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), Boehringer Ingelheim Canada, Ltd., Bio ChemPharma Shire, Ltd., and the Canada Research Chair in Bioorganic and Medicinal Chemistry (J.C.V.).

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>.

OL027160V

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