Conformationally Restricted TRH Analogs: The Compatibility of a 6,5-Bicyclic Lactam-Based Mimetic with Binding to TRH-R

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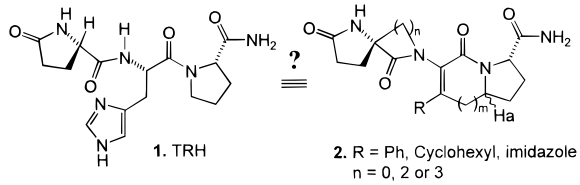
Abstract: A pair of conformationally restricted TRH analogs have been synthesized. Both analogs have the central histidine of TRH replaced by a cyclohexylalanine unit, and both analogs contain a 6,5-bicyclic lactam ring fusing the proline peptide bond into a trans conformation. The analogs differ at the bridgehead stereocenter. The synthesis of the analogs utilized an anodic amide oxidation reaction to selectively functionalize a proline derivative and a titanium tetrachloride-initiated cyclization−rearrangement sequence to assemble the desired bicyclic ring skeleton. The analogs were compared with the unrestricted cyclohexylalanine-containing TRH analog (cyclohexyl-Ala[2]-TRH) in order to determine how the added lactam ring affected the affinity and the potency of the analog for TRH-R. Both the affinity and potency of the restricted analogs were found to be critically dependent on the bridgehead stereochemistry of the bicyclic ring. The analog having *R* stereochemistry at the bridgehead was 478 times more potent than the *S* isomer. In addition, the *R* isomer was found to be approximately 4.7 times more potent than its unrestricted counterpart. Similarly, the *R* isomer was found to have an affinity for TRH-R that was approximately 3.4 times the affinity of the unrestricted cyclohexyl-Ala[2]-TRH.

Introduction

TRH is the hypothalamic tripeptide that controls the release of thyroid stimulating hormone from the anterior pituitary gland.[1] In addition to this endocrine activity, TRH displays effects in the brain, blood, spinal cord, and gut. The widespread biological activity of TRH, the existence of extensive reviews describing the structure−activity relationships of TRH analogs,[2] the close correlation between measured binding affinity and biological potency for TRH analogs (suggesting that binding and activity have similar structural requirements), and the fact that the conformation of TRH can be described to a first approximation with the use of only six torsional angles[3] combined to make TRH an ideal model system for developing chemical probes that are capable of elucidating the relationship between the predicted and actual biological activity of peptide conformations. This possibility was recognized by Garland Marshall and co-workers, and in the early 1980s they used the available analog data to predict several potential conformations for the binding of TRH to its endocrine receptor TRH-R. These predictions were then used to design a series of TRH analogs as conformational probes for examining the validity of the predictions and for refining the three-dimensional picture of the TRH receptor site.[4,5] One of the possible conformations predicted for the binding of TRH to TRH-R is illustrated in Scheme 1 (**1**), along with a family of proposed conformationally restricted analogs (**2**).[6]

Several items concerning the proposed restricted analogs deserve comment. First, the analogs were designed by examining conformation **1** and then replacing spacially close hydrogens with carbon bridges. The net result was to embed the peptide backbone into a polycyclic ring skeleton.[7] The advantages of such an approach included the ease with which new analogs could be designed, the preservation of both the peptide backbone and the amino acid side chains, and the increased proteolytic stability of the lactam rings. The principal disadvantage of this approach was the steric size of the bridges. If the bridges interfered with binding, then no new information about the conformational requirements of binding to TRH-R would be gained. Second, molecular modeling experiments were not able to differentiate the bridgehead isomers with respect to the potential of the analogs to bind TRH-R. Hence, both isomers at H_a were proposed for study. Finally, the initially designed analogs avoided the use of histidine as the central amino acid by replacing the histidine with either phenylalanine or cyclohexylalanine. Although analogs based on phenylalanine and cyclohexylalanine were not nearly as potent as TRH itself, they

Scheme 1



[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1996.

(1) For an outstanding series of reviews, see: *Thyrotropin-Releasing Hormone: Biomedical Significance*; Metcalf, G., Jackson, I. M. D., Eds.; *Ann. N. Y. Acad. Sci.* **1989**, *553.*

(2) For a summary, see: Marshall, G. R.; Gorin, F. A.; Moore, M. L. In *Annual Reports in Medicinal Chemistry*; Clarke, F. H., Ed.; Academic Press: New York, 1978; Vol. 13, p 227 and references therein.

(3) Reference 2, p 229.

(4) Font, J. Ph.D. Thesis, Washington University, St. Louis, MO, 1986, and unpublished results with Lawrence D. Rutledge.

(5) For alternative approaches to understanding the conformation of TRH binding, see: (a) Hinkle, P. M.; Woroch, E. L.; Tashjian, G. H. *J. Biol. Chem.* **1974**, *249*, 3085−3090. (b) Goren, H. J.; Bauce, L. G.; Vale, W. *Mol. Pharmacol.* **1977**, *13*, 606−614. (c) Kamiya, K.; Takamoto, M.; Wada, Y.; Fujino, M.; Nishikawa, M. *J. Chem. Soc., Chem. Commun.* **1980**, 438−439. (d) Sharif, N. A.; To, Z.; Whiting, R. L. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 1306−1311. (e) Vonhof, S.; Feuerstein, G. Z.; Cohen, L. A.; Labroo, V. M. *Eur. J. Pharmacol.* **1990**, *180*, 1−12.

(6) For an additional application of the models developed by Marshall and Font to the construction of TRH mimetics, see: Olson, G. L.; Cheung, H. C.; Chiang, E.; Madison, V. S.; Sepinwall, J.; Vincent, G. P.; Winokur, A.; Gary, K. A. *J. Med. Chem.* **1995**, *38*, 2866.

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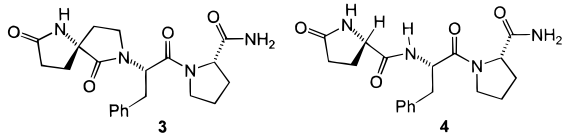
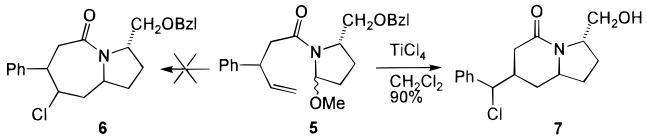
Chart 1



Scheme 2



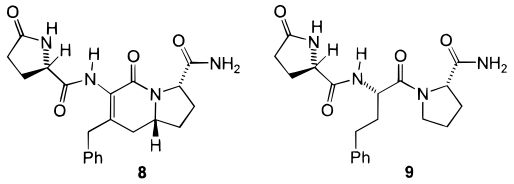
were both known to completely displace TRH from TRH-R and to lead to the same maximal extent of stimulation as TRH. By mimicking one or both of these TRH derivatives, it was hoped that the compatibility of the bridges used to restrict the analogs with binding and activation of the receptor could be determined before taking on the extra challenge of synthesizing restricted analogs having an imidazole substituent.

The bridge added to restrict the pyroglutamate region of **2** has been shown to be compatible with activation of TRH-R.[8] In this work, analog **3** (Chart 1) were synthesized and tested for its ability to bind and activate TRH-R. The resulting data were compared to similar data obtained for the unrestricted [Phe^2]TRH **4**. Analog **3** was found to have an approximately 3-fold lower affinity for TRH-R and a 3-fold lower potency for TRH-R than did the unrestricted analog **4**.

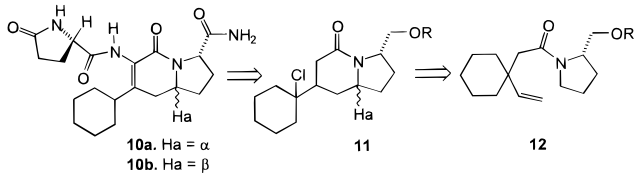
Restricting the X-Pro region of TRH proved to be more difficult. Our initial plan was to construct a seven-membered-ring lactam-based analog from compound **6** which would, in turn, be formed from the cyclization of **5**. However, the cyclization of **5** failed to form the desired seven-membered-ring analog and instead led to the six-membered-ring lactam compound **7** (Scheme 2).[9]

Bicyclic lactam **7** was converted into a TRH analog (**8**, Chart 2). Unfortunately, the extra benzylic methylene group interfered badly with binding to the TRH-R receptor. Even the unrestricted analog **9** with the extra methylene group failed to bind TRH-R. It was clear that compound **8** could not serve as a probe for determing the effectiveness of the added conformational constraint. A TRH analog that did not possess the extra methylene group was needed. We report herein the synthesis

Chart 2



Scheme 3



of a pair of such TRH analogs. These analogs were used to demonstrate that the six-membered-ring lactam constraint could serve to enhance the affinity and potency of a TRH analog for TRH-R and to identify the bridgehead stereochemistry required for binding and activity.

Synthesis of TRH Analogs 10

In principle, the rearrangement reaction outlined in Scheme 2 could be used to synthesize TRH analogs without the extra methylene group that interfered with the binding of both **8** and **9** to TRH-R. With this in mind, the construction of a pair of six-membered-ring lactam-based TRH analogs (**10a**,**b**) was designed as outlined in Scheme 3. As in earlier designs, these TRH analogs avoided the use of an imidazole group in order to circumvent potential synthetic problems (*vide infra*). In this case, a cyclohexyl group was chosen as the substituent in order to simplify the conversion of the initially formed six-membered-ring lactam derivative **11** into the eventual TRH analog. The plan for[10,11] synthesizing **11** called for the anodic methoxylation of amide **12**, followed by treatment of the resulting 5-methoxyproline derivative with $TiCl_4$ in order to initiate the desired cyclization−rearrangement sequence.

The forward syntheses of **10a**,**b** are outlined in Scheme 4. The syntheses started from compound **13**, which was utilized as a substrate for an ortho ester Claisen rearrangement. The product from the Claisen rearrangement was saponified to form an acid, the acid coupled to prolinol using EDCI coupling conditions, and the resulting amide alcohol benzylated to form the electrolysis substrate **12**. The anodic oxidation of **12** was accomplished at a carbon anode in methanol solvent using a constant current of 26.8 mA. A platinum cathode was used as the auxiliary electrode. After 2.1 F/mol had been passed, the reaction was stopped. A 74% isolated yield of the methoxylated amide **14** was obtained along with a 16% yield of the recovered starting material.

The cyclization−rearrangement sequence leading to the formation of the six-membered ring-lactam proceeded smoothly. In this experiment, the treatment of the methoxylated amide with titanium tetrachloride led to compound **11** in a 94% isolated yield. As illustrated in Scheme 5, the proposed mechanism for this reaction involves an initial cyclization to form a seven-membered ring, followed by migration of the C_3-C_4 bond to generate the tertiary carbocation and six-membered ring-lactam. The resulting cation was trapped by chloride to form **11**.

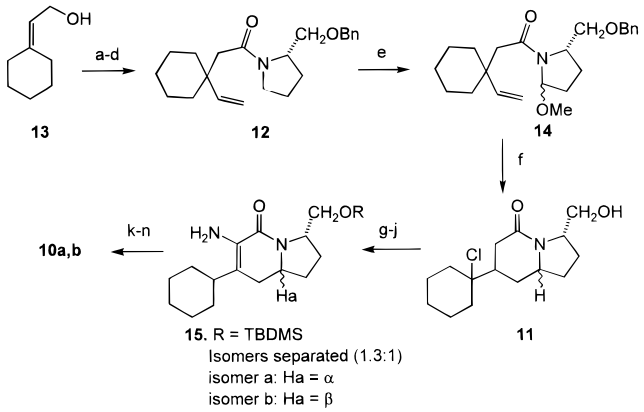
Intermediate **11** was converted into peptide building blocks **15a**,**b** as outlined in Scheme 4. In analogy to earlier syntheses involving the functionalization of six-membered-ring lactams, the oxidation of the carbon α to the amide with LDA and O_2

(7) For selected examples of lactam-based peptide mimetics, see: (a) Wolf, J. P.; Rapoport, H. *J. Org. Chem* **1989**, *54*, 3164−3173, as well as refs 1−8 therein. (b) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104−109. (c) Freidinger, R. M. *J. Org. Chem.* **1985**, *50*, 3631−3633. (d) Zydowsky, T. M.; Dellaria, J. F. Jr.; Nellans, H. N. *J. Org. Chem.* **1988**, *53*, 5607−5616. (e) Kempf, D. J.; Condon, S. L. *J. Org. Chem.* **1990**, *55*, 1390−1394. (f) Kemp, D. S.; McNamara, P. E. *J. Org. Chem.* **1985**, *50*, 5834−5838 and references therein. (g) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647−650. (h) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447−1449. (i) Paul, P. K. C.; Burney, P. A.; Campbell, M. M.; Osguthorpe, D. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 141−144. (j) Ward, P.; Ewan, G. B.; Jordon, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *33*, 1848−1851. (k) Deal, M. J.; Hagan, R. M.; Ireland, S. J.; Jordan, C. C.; McElroy, A. B.; Porter, B.; Ross, B. C.; Stephens-Smith, M.; Ward, P. *J. Med. Chem.* **1992**, *35*, 4195−4204. (l) Flynn, G. A.; Giroux, E. L.; Dage, R. C. *J. Am. Chem. Soc.* **1987**, *109*, 7914−7915. (m) Flynn, G. A.; Burkholder, T. P.; Huber, E. W.; Bey, P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 309−312. (n) Burkholder, T. P.; Huber, E. W.; Flynn, G. A. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 231−234. (o) Robl, J. A. *Tetrahedron Lett.* **1994**, *35*, 393−396, p. Lombart, H. G.; Lubell, W. D. *J. Org. Chem.* **1994**, *59*, 6147−6149. (q) Colombo, L.; Giacomo, M. D.; Papeo, G.; Carugo, O.; Scolastico, C.; Manzoni, L. *Tetrahedron Lett.* **1994**, *35*, 4031−4034.

(8) Rutledge, L. D.; Perlman, J. H.; Gershengorn, M. C.; Marshall, G. R.; Moeller, K. D. *J. Med. Chem.* **1996**, *39*, 1571.

(9) (a) Li, W.; Hanau, C. E.; d'Avignon, A.; Moeller, K. D. *J. Org. Chem.* **1995**, *60*, 8155. (b) Kao, J.; Li, W.; Moeller, K. D. Submitted for publication.

Scheme 4[a]



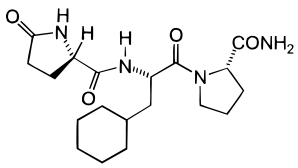
[a] Reagents: (a) $CH_3C(OEt)_3$, $CH_3CH_2CO_2H$, reflux; (b) KOH, MeOH, heat, 69% (over two steps); (c) L-prolinol, HOBt, EDCI, CH_2Cl_2, 86%; (d) (i) NaH, THF, 0 °C to RT, (ii) BnBr, THF, 0 °C to RT, 89%; (e) carbon rod anode, Pt cathode, 0.03 M Et_4NOTs, MeOH, 26.8 mA, 2.1 F/mole 74% (16% recovered starting material); (f) $TiCl_4$, CH_2Cl_2, 94%; (g) H_2, Pd on C, NaOMe, MeOH, 94%; (h) TBDMS, DBU, CH_2Cl_2, 95%; (i) (i) LDA, THF, −78 °C, (ii) O_2, 62%; (j) NH_3, MeOH, 91% (7% recovered starting material); (k) HOpGlu, HOBt, EDCI, CH_2Cl_2, isomer **a** 35%, isomer **b** 61%; (l) H_2SO_4, THF, H_2O, isomer **a** 96%, isomer **b** 78%; (m) Jones oxidation, isomer **a** 63%, isomer **b** 50%; (n) (i) *i*-BuOCOCl, CH_2Cl_2, *N*-methylmorpholine, (ii) NH_3, isomer **a** 90%, isomer **b** 84%.

(step i in Scheme 4) led directly to the formation of a 1,2-dicarbonyl compound.[9a] The dicarbonyl was converted into the desired enamine building blocks with the use of ammonia in methanol. The bridgehead stereoisomers were then separated at this point of the synthesis by gravity flow chromatography through silica gel. Each isomer was then carried on independently. The stereochemistry of isomers **15a**,**b** was assigned by analogy to previously synthesized building blocks having the same ring system.[9] Specifically, enamine-based 6,5-bicyclic lactam building blocks like **15a**,**b** fall into two "stereochemical families" that can be readily distinguished by proton NMR. Initially the stereochemistry of the two families was identified by the use of 2D-NOE data at the building block stage[9a] and by a combination of 2D-NOE data and coupling constant information once the building block was incorporated into a peptide.[9b] Once the stereochemical families were characterized, three proton NMR signals could be used to identify which stereochemical family a new analog belonged to. An isomer having *S* stereochemistry at the bridgehead could be characterized by a methine signal at 4.2 ppm, an AM of an AMX pattern where A was centered around 4.0 ppm, M was centered around 3.8 ppm, and J_{AX} was larger than J_{MX}, and a methine signal at 1.6−1.5 ppm. An isomer having *R* stereochemistry at the bridgehead could be characterized by a methine signal at 4.1 ppm (the appearance of which was significantly different than that for the *S* isomer), an AM of an AMX pattern where A was centered around 3.8 ppm, M was centered around 3.6 ppm, and J_{MX} was larger that J_{AX}, and a methine buried under other signals at approximately 1.8 ppm. For the cyclohexyl case examined here, the methine signal around 1.6−1.5 ppm in **16a** was partially obscured by the cyclohexyl protons. However, the other two characteristic signals could be easily seen for both **15a** and **15b**.

Once obtained, **15a**,**b** were converted into TRH analogs using standared peptide coupling techniques. The yield for the coupling of isomer **15a** with pyroglutamic acid was never optimized due to the inability of **10a** to serve as a potent ligand for TRH-R (*vide infra*). The ease with which these syntheses were completed served to illustrate the generality of the overall synthetic approach.

Biological Data

Once synthesized, the analogs were tested for their ability to activate TRH-R and compared with the unrestricted cyclohexyl-Ala^2-TRH.[12] The EC_{50}s for second messenger inositol phosphate (IP) formation, that is, potencies, and the K_is of binding, that is, affinities, were obtained according to previously published procedures.[13] The maximal extents of stimulation of IP formation were similar for TRH, cyclohexylAla^2-TRH, **10a**, and **10b**, and at high concentrations all three cyclohexyl-based analogs displaced 100% of TRH-R-bound [^{3}H]-[N$^{\tau}$*Me*-His]TRH. Comparison of the restricted analogs with the unrestricted cyclohexyl-Ala^2-TRH indicated that the added bridge in **10a** badly interfered with both the binding and the potency of the analog for TRH-R. However, analog **10b** proved to be approximately 4.7 times *more potent* than its unrestricted counterpart. Similarly, analog **10b** had an affinity for TRH-R that was approximately 3.4 times the affinity of the unrestricted cyclohexyl-Ala^2-TRH. The results of these studies are summarized in Table 1.[14]



[CyclohexylAla^2]TRH

Clearly, the added lactam ring in analog **10b** did not interfere with binding but rather served to enhance both the potency and the affinity of the analog for TRH-R. It would appear that the

(10) For pioneering work with anodic amide oxidations, see: (a) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Am. Chem. Soc.* **1964**, *86*, 4139. (b) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Org. Chem.* **1966**, *31*, 128. (c) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Am. Chem. Soc.* **1966**, *88*, 4657. For reviews, see: (d) Shono, T. *Tetrahedron* **1984**, *40*, 811. (e) Shono, T.; Matsumura, Y.; Tsubata, K. In *Organic Synthesis*; Saucy, G., Ed.; Organic Synthesis Inc., 1984; Vol. 63, p 206 and references therein. (f) Shono, T. In *Topics in Current Chemistry*; Steckhan, E. Ed.; Springer-Verlag: Berlin-Heidelberg-New York, 1988; Vol. 148, p 131.

(11) For examples of amide oxidations being used to functionalize peptides, see refs 8 and 10 as well as the following: (a) Shono, T.; Matsumura, Y.; Inoue, K. *J. Org. Chem.* **1983**, *48*, 1388. (b) Thaning, M.; Wistrand, L.-G. *Helv. Chim. Acta* **1986**, *69*, 1711. (c) Renaud, P.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 843. (d) Renaud, P.; Seebach, D. *Helv. Chim. Acta* **1986**, *69*, 1704. (e) Seebach, D.; Charczuk, R.; Gerber, C.; Renaud, P.; Berner, H.; Schneider, H. *Helv. Chim. Acta* **1989**, *72*, 401. (f) Papadopoulos, A.; Heyer, J.; Ginzel K.-D.; Steckhan, E. *Chem. Ber.* **1989**, *122*, 2159. (g) Ginzel, K.-D.; Brungs, P.; Steckhan, E. *Tetrahedron* **1989**, *45*, 1691. (h) Papadopoulos, A.; Lewall, B.; Steckhan, E.; Ginzel, K.-D.; Knoch, F.; Nieger, M. *Tetrahedron* **1991**, *47*, 563. (i) Barrett, A. G. M.; Pilipauskas, D. *J. Org. Chem.* **1991**, *56*, 2787. (j) Moeller, K. D.; Rutledge, L. D. *J. Org. Chem.* **1992**, *57*, 6360. (k) Cornille, F.; Fobian, Y. M.; Slomczynska, U.; Beusen D. D.; Marshall, G. R.; Moeller, K. D. *Tetrahedron Lett.* **1994**, *35*, 6989−6992. (l) Cornille, F.; Slomczynska, U.; Smythe, M. L.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. *J. Am. Chem. Soc.* **1995**, *117*, 909. (m) Slomczynska, U.; Chalmers, D. K.; Cornille, F.; Smythe, M. L.; Beusen, D. D.; Moeller, K. D.; Marshall, G. R. *J. Org. Chem.* **1996**, *61*, 1198−1204. (n) Fobian, Y. M.; d'Avignon, D. A.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 315−318.

(12) The unrestricted analog was synthesized from proline amide using standard peptide coupling conditions.

(13) Perlman, J. H.; Thaw, C. N.; Laakkonen, L.; Bowers, C. Y.; Osman, R.; Gershengorn, M. C. *J. Biol. Chem.* **1994**, *269*, 1610. Straub, R. E.; Frech, G. C.; Joho, R. H.; Gershengorn, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9514.

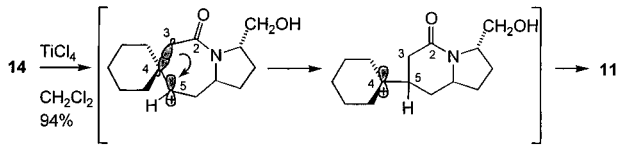
(14) For a detailed experimental discussion concerning biological data and an interpetation of the data with respect to a second model for the binding of TRH to TRH-R based on receptor mutants, see: Laakkonen, L.; Li, W.; Perlman, J. H.; Guarnieri, F.; Osman, R.; Moeller, K. D. and Gershengorn, M. C. *Mol. Pharmacol.* **1996**, *49*, 1092.

Table 1. Binding and Activation of TRH-R Receptors by TRH Analogs **10a** and **10b**

analog	K_i (nM)[b]	EC_{50} (nM)[c]
[N$^{\tau}$*Me*-His]TRH	1.2 (0.92−1.5)[d]	nd
TRH	nd	1.1 (0.95−1.3)[d]
cyclohexyl-Ala^2-TRH	6500 (5400−7900)[d]	430 (380−480)[d]
analog **10a**	290 000 (220 000−370 000)[d]	44 000 (37 000−52 000)[d]
analog **10b**	1900 (1600−2200)[d]	91 (76−110)[d]

[a] Experiments were performed with intact AtT-20 mouse pituitary tumor cells stably expressing TRH receptors. [b] For binding, cells were incubated with 1 nM [^{3}H]-[N$^{\tau}$*Me*-His]TRH in the absence or presence of various doses of unlabelled TRH analogs for 1 h at 37 °C. The data are means of duplicate determinations in two or three experiments. [c] For activation, cells prelabeled with [^{3}H]myoinositol were incubated with various doses of TRH analogs for 1 h at 37 °C, and inositol phosphate formation was measured. Maximal extents of stimulation were similar for all analogs. The data are means of duplicate determinations in two or three experiments. [d] 95% confidence intervals. [e] nd, not determined.

Scheme 5



lactam ring used to restrict **10b** is an excellent constraint for beginning to experimentally "map" the TRH-R receptor. In addition, the data indicated that the *R* configuration at the bridgehead stereocenter was essential for both high potency and high affinity for the receptor.

Conclusions

In summary, we have found that using electrochemistry to systematically embed the X-Pro region of a TRH analog into a 1-aza-2-oxobicyclo[4.3.0]nonane ring skeleton can lead to a new, conformationally restricted analog with enhanced potency and affinity for TRH-R. The potency and affinity of the analog for TRH-R were critically dependent on the bridgehead stereochemistry of the bicyclic ring system. The *R* isomer at the bridgehead was 478 times more potent than the *S* isomer. The synthesis of future analogs will have to focus on controlling the stereochemistry at this position. At this point, both bridges proposed by Marshall and Font in the design of analog **2** appear to be compatible with the receptor site. Efforts aimed at synthesizing and testing the fully restricted analogs and at synthesizing and testing TRH analogs having the imidazole ring present are underway.

Experimental Section[15]

(1′-Vinylcyclohexyl)acetic Acid. A solution of triethyl phosphonoacetate (22.419 g, 100 mmol) in 120 mL of DME was added dropwise to a suspension of NaH (4.00 g of 60% mixture with mineral oil, 100 mmol) in 80 mL of DME at 0 °C. The mixture was allowed to warm to room temperature, stirred for 1 h, cooled to 0 °C, and treated with cyclohexanone (9.815 g, 100 mmol). The resulting mixture was stirred at room temperature for an additional 30 min. The reaction mixture was then poured into a separatory funnel and washed with water. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo* to afford 16.011 g (95%) of the crude product. A solution of this crude product (12.979 g, 77.3 mmol) in 100 mL of dry ether was added dropwise to a suspension of $LiAlH_4$ (4.398 g, 115.9 mmol) in 50 mL of dry ether at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched by adding small chips of ice. The aqueous layer was extracted with ether, and then the combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to afford 8.710 g (89%) of the crude alcohol **13**. The crude alcohol **13** (6.512 g, 51.7 mmol) was placed in a flame-dried 250 mL round-bottom flask along with triethyl orthoacetate (41.949 g, 258.4 mmol) and propionic acid (0.229 g, 3.1 mmol). To the flask was attached a Dean−Stark trap and a condenser. The reaction was then raised to reflux and heated until about 42 mL of ethanol was removed from the reaction. At this point, the reaction was cooled, MeOH (100 mL) and KOH (5.8 g, 103.4 mmol) were added, and the mixture was again raised to reflux for an additional 5 h. The MeOH was then removed under reduced pressure, and the crude oil was partitioned between a saturated $NaHCO_3$ solution and Et_2O. The aqueous layers were combined, acidified to pH = 1, and extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude oil was distilled to afford 6.000 g (69%) of the desired acid product. The spectral data for the acid were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 10.50 (br s, 1H), 5.80 (dd, 1H, J = 17.7, 10.5 Hz), 5.15−5.01 (m, 2H), 2.35 (s, 2H), 1.71−1.64 (m, 2H), 1.55−1.45 (m, 8H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 177.9, 144.5, 113.6, 45.7, 39.1, 35.5, 26.1, 22.0; IR (neat/NaCl) 3083, 2928, 2853, 1706, 1455, 1448, 1411, 1302, 1278, 1260, 914, 676 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 170 (M + 2, 5), 169 (M + 1, 22), 167 (10), 152 (12), 151 (56), 133 (17), 123 (17), 110 (19), 109 (100), 108 (10), 107 (16); HRMS (EI) *m*/*e* calcd for $C_{10}H_{16}O_2$ 168.1150, found 168.1148.

(2*S*)-2-(Hydroxymethyl)-1-(1′-vinylcyclohexylacetoyl)pyrrolidine. To a mixture of the acid generated above (6.000 g, 35.7 mmol) in 70 mL of CH_2Cl_2 were added HOBt (6.755 g, 50.0 mmol) and L-prolinol (4.335 g, 42.9 mmol). The mixture was stirred at 0 °C for 5 min, and then 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) (8.876 g, 46.4 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred at room temperature overnight. The reaction was then poured into a separatory funnel and washed with saturated $NaHCO_3$, 10% citric acid, and an aqueous saturated NaCl solution. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was chromatographed through ca. 300 g of silica gel using a 5% MeOH in Et_2O solution as eluant to afford 7.703 g (86%) of the amide as a colorless oil. The spectral data for the amide were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 5.80 (dd, 1H, J = 17.4, 10.8 Hz), 5.24 (br s, 1H), 5.15−5.02 (m, 2H), 4.26−4.18 (m, 1H), 3.65−3.50 (m, 3H), 3.47−3.39 (m, 1H), 2.32 and 2.31 (two s, 2H), 2.07−1.95 (m, 1H), 1.93−1.73 (m, 5H), 1.60−1.43 (m, 8H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 172.6, 144.5, 113.5, 67.3, 60.7, 49.0, 46.0, 40.0, 36.1, 35.7, 28.2, 26.1, 24.3, 22.0; IR (neat/NaCl) 3394 br, 2926, 2855, 1701, 1662, 1617, 1586, 1447, 1429, 1401, 1052, 914, 786, 657 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 280 (M + 29, 12), 253 (M + 2, 27), 252 (M + 1, 100), 251 (M, 9), 250 (17), 102 (27); HRMS (EI) *m*/*e* calcd for $C_{15}H_{25}O_2N$ 251.1885, found 251.1874.

(2*S*)-2-(Benzyloxymethyl)-1-(1′-vinylcyclohexylacetonyl)pyrrolidine (12). A solution of the above amide (7.228 g, 28.8 mmol) in 40 mL of THF was added dropwise to a suspension of NaH (1.728 g of a 60% mixture with mineral oil, 43.2 mmol) in 20 mL of THF at 0 °C. The mixture was allowed to warm to room temperature, stirred for 30 min, cooled to 0 °C, and treated with benzyl bromide (5.418 g, 31.7 mmol). The reaction was allowed to warm to room temperature, stirred overnight, and then quenched by adding an aqueous saturated NaCl solution. The emulsion was transferred to a separatory funnel, diluted with Et_2O, and washed with water. The aqueous layers were combined and extracted with Et_2O. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was chromatographed through ca. 250 g of silica gel using a 30% hexane in Et_2O solution as eluant to afford 8.723 g (89%) of the protected amide as a colorless oil. The spectral data for the protected amide were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 7.36−7.26 (m, 5H),

(15) For a general experimental section, see: Wong, P. L.; Moeller, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 11434.

5.86−5.73 (m, 1H), 5.08−4.94 (m, 2H), 4.56−4.45 (m, 2H), 4.34−4.27 (m, 0.7H), 4.08−4.01 (m, 0.3H), 3.65 (dd, 1H, J = 9.3, 3.0 Hz), 3.52−3.35 (m, 3H), 2.28 and 2.27 (two s, 2H), 2.04−1.71 (m, 6H), 1.60−1.41 (m, 8H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 170.2, 170.0, 145.1, 138.5, 128.4, 128.2, 127.7, 127.5, 113.0, 73.2, 73.0, 71.0, 70.1, 57.2, 56.1, 48.2, 45.9, 45.2, 39.8, 36.8, 35.9, 35.7, 35.2, 28.6, 27.5, 26.2, 24.2, 22.2, 22.1, 22.0, 21.7; IR (neat/NaCl) 2928, 2856, 1636, 1452, 1412, 1363, 1197, 1102, 911, 736, 698 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 370 (M + 29, 13), 343 (M + 2, 55), 342 (M + 2, 100), 341 (M, 12), 340 (18); HRMS (EI) *m*/*e* calcd for $C_{22}H_{31}O_2N$ 341.2355, found 341.2368.

(2*S*)-2-(Benzyloxymethyl)-5-methoxy-1-(1′-vinylcyclohexylacetoyl)-pyrrolidine (14). An oven-dried three-neck flask was charged with the protected amide (13.321 g, 39.1 mmol), MeOH (78 mL), and tetraethylammonium tosylate (0.705 g, 2.34 mmol). The flask was equipped with a carbon anode, a platinum wire cathode, and a nitrogen inlet. The reaction mixture was degassed by sonication while a slow stream of nitrogen was passed through the solution for 5 min. The mixture was then electrolyzed at a constant current of 26.8 mA until 2.1 F/mol had been passed. When complete, the MeOH was removed under reduced pressure and the crude oil chromatographed through ca. 600 g of silica gel using a gradient elution from 60% ether/hexane to 80% ether/hexane. The column afforded 10.748 g (74%) of the desired methoxylated amide as a mixture of diastereomers, along with 2.179 g (16%) of the recovered starting material. The spectral data for the mixture of diastereoisomers were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 7.36−7.25 (m, 5H), 5.87−5.73 (m, 1H), 5.59 (d, 0.3H, J = 5.1 Hz), 5.11−4.93 (m, 2H), 4.90 (d, 0.7H, J = 3.3 Hz), 4.58−4.33 (m, 2H), 4.30−4.09 (m, 0.7H), 3.91 (dd, 0.3H, J = 9.0, 3.6 Hz), 3.59 (dd, 0.5H, J = 9.6, 3.3 Hz), 3.54−3.32 (m, 1.5H), 3.28 and 3.23 (two s, 3H), 2.41 and 2.35 (two s, 2H), 2.10−1.61 (m, 6H), 1.54−1.26 (m, 8H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 171.6, 145.4, 145.2, 138.5, 128.4, 128.3, 127.7, 127.6, 127.5, 127.4, 113.3, 113.0, 112.9, 89.8, 87.4, 74.7, 73.3, 73.1, 72.1, 69.9, 57.1, 56.7, 56.3, 55.7, 53.9, 45.5, 45.3, 39.8, 37.0, 36.8, 36.6, 35.4, 35.2, 31.0, 30.5, 29.0, 27.0, 26.3, 26.2, 26.1, 24.7, 22.2, 22.1, 22.0; IR (neat/NaCl) 2929, 2855, 1701, 1689, 1647, 1622, 1559, 1539, 1528, 1457, 1438, 1415, 1399, 1085, 757, 698 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 372 (M + 1, 6), 341 (10), 340 (25), 218 (8), 191 (14), 190 (100), 91 (7); HRMS (EI) *m*/*e* calcd for $C_{23}H_{33}O_3N$ 371.2460, found 371.2463.

(9*S*)-1-Aza-4-(1′-chlorocyclohexyl)-9-(hydroxymethyl)-2-oxobicyclo-[4.3.0]nonane (11). To a −78 °C solution of the methoxylated amide (10.745 g, 29.0 mmol) in 150 mL of CH_2Cl_2 was added 72.4 mL of a 1 M $TiCl_4$ in CH_2Cl_2 solution. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was then quenched by the addition of a 30% solution of Rochelle's salt in water. The reaction mixture was transferred to a separatory funnel, the layers were separated, and the aqueous layer was extracted 10 times with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was chromatographed through 600 g of silica gel using a 10% solution of MeOH in ether as the eluant to afford 7.770 g (94%) of the cyclized compound as a yellow oil. The spectral data for the mixture of diastereoisomers obtained were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 4.29−4.15 (m, 1H), 3.76−3.42 (m, 3H), 2.69−2.42 (m, 2H), 2.30 (t, 1H, J = 12.3 Hz), 2.18−1.90 (m, 5H), 1.82−1.14 (m, 12H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 171.1, 76.6, 68.2, 67.6, 61.4, 61.3, 60.5, 59.0, 45.1, 44.5, 37.7, 37.0, 36.9, 33.0, 32.7, 32.5, 31.4, 30.2, 30.1, 27.4, 26.3, 25.2, 22.0, 21.8; IR (neat/NaCl) 3391 br, 2934, 2863, 1618, 1459, 1448, 1413, 1328, 1058, 1048, 873, 686 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 286 (M + 1, 100), 250 (37), 154 (36), 136 (42), 117 (78), 115 (42), 91 (48), 89 (34); HRMS (FAB) *m*/*e* calcd for $C_{15}H_{25}O_2ClN$ 286.1573 (M + 1), found 286.1584.

(9*S*)-1-Aza-4-cyclohexyl-9-(hydroxymethyl)-2-oxobicyclo[4.3.0]-nonane. To a solution of the cyclized compound (7.720 g, 27.0 mmol) in 54 mL of MeOH were added 1.93 g of 5% Pd on activated carbon and 2.921 g (54.1 mmol) of sodium methoxide. The mixture was stirred under a hydrogen balloon overnight. The catalyst was then removed with the use of a fritted glass funnel and the filtrate concentrated in vacuo. The crude product was chromatographed through 500 g of silica gel using 10% MeOH in ether as the eluant to afford 6.381 g (94%) of the hydrogenated product. The spectral data for the mixture of diastereomers obtained were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 4.27−4.12 (m, 1H), 3.73−3.38 (m, 3H), 2.52 (td, 1H, J = 17.4, 6.0 Hz), 2.19−1.93 (m, 4H), 1.77−1.62 (m, 6H), 1.57−1.32 (m, 2H), 1.20−0.91 (m, 8H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 172.0, 68.1, 67.6, 61.3, 61.2, 61.0, 59.6, 58.2, 42.2, 41.8, 38.8, 38.2, 35.7, 35.4, 32.7, 32.5, 31.3, 29.8, 29.7, 27.3, 26.4, 26.2, 18.3; IR (neat/NaCl) 3276 br, 2931, 2854, 1694, 1546, 1455, 1419, 1321, 1265, 730 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 292 (M + 41, 10), 280 (M + 29, 24), 254 (M + 3, 2), 253 (M + 2, 44), 252 (M + 1, 100), 234 (3), 220 (3); HRMS (EI) *m*/*e* calcd for $C_{15}H_{25}O_2N$ 251.1885, found 251.1887.

(9*S*)-1-Aza-9-[(*tert*-butyldimethylsiloxy)methyl]-4-cyclohexyl-2-oxobicyclo[4.3.0]nonane. To a solution of the hydrogenated compound (3.341 g, 13.3 mmol) in 30 mL of CH_2Cl_2 were added TBDMSCl (4.028 g, 26.6 mmol) and DBU (4.197 g, 28.0 mmol). The mixture was stirred at room temperature for 1 h and then transferred to a separatory funnel. The organic layer was separated and then washed with a 10% citric acid solution followed by a saturated aqueous $NaHCO_3$ solution. The CH_2Cl_2 was removed under reduced pressure and the crude product chromatographed through ca. 250 g of silica gel using 80% ether/hexane as eluant to afford 4.251 g of the protected amide (95%). The spectral data for the mixture of diastereoisomers were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 4.18−4.11 (m, 0.5H), 4.04−3.98 (m, 0.5H), 3.95 (dd, 0.5H, J = 10.2, 4.5 Hz), 3.86 (dd, 0.5H, J = 9.6, 5.1 Hz), 3.73 (dd, 0.5H, J = 9.9, 2.7 Hz), 3.67 (dd, 0.5H, J = 10.2, 2.4 Hz), 3.48−3.32 (m, 1H), 2.42 (td, 1H, J = 18.6, 6.6 Hz), 2.10−1.80 (m, 5H), 1.78−1.55 (m, 6H), 1.39−0.90 (m, 8H), 0.88 and 0.87 (two s, 9H), 0.03, 0.02, 0.01 and 0 (four s, 6H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 169.6, 169.1, 63.0, 62.1, 60.1, 59.9, 58.1, 57.8, 42.5, 42.0, 39.3, 38.7, 35.9, 33.1, 33.0, 32.4, 31.2, 29.9, 29.8, 29.7, 26.6, 26.5, 26.3, 25.9, 25.8, 25.7, 24.8, 18.1, −3.6, −5.4, −5.5; IR (neat/NaCl) 2927, 2854, 1620, 1463, 1413, 1361, 1327, 1253, 1088, 1049, 889, 834, 773, 665 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 395 (M + 30, 6), 394 (M + 29, 18), 368 (M + 3, 8), 367 (M + 2, 29), 366 (M + 1, 100), 365 (M, 9), 364 (26), 351 (13), 350 (49), 309 (8), 308 (40); HRMS (EI) *m*/*e* calcd for $C_{21}H_{39}O_2NSi$ 365.2750, found 365.2775.

(9*S*)-1-Aza-9-[(*tert*-butyldimethylsiloxy)methyl]-4-cyclohexyl-3-hydroxy-2-oxobicyclo[4.3.0]non-3-ene. An oven-dried two-neck flask was charged with 15 mL of THF and 4.071 g (40.2 mmol) of diisopropylamine. To this mixture was added 15.7 mL (39.3 mmol) of a 2.5 M *n*-butyllithium in hexanes solution at −78 °C. The mixture was stirred at −78 °C for 0.5 h, and then a solution of the protected amide from above (6.991 g, 19.2 mmol) in 20 mL of THF was cannulated slowly into the flask. The flask originally containing the protected amide was washed with an additional 5 mL of THF, and these washings were added to the reaction. The resulting mixture was stirred at −78 °C for another 45 min. Following this period, oxygen was bubbled through the reaction for 30 min at −78 °C. The reaction was quenched by the addition of a saturated aqueous $NaHSO_3$ solution to the flask. The reaction was then transferred to a separatory funnel, the layers were separated, and the aqueous layer was extracted with ether. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was chromatographed through 400 g of silica gel using a gradient elution from 30% ether/hexane to 70% ether/hexane as eluant to afford 4.524 g (62%) of the product as a white solid. The spectral data for the mixture of diastereoisomers were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 6.29 and 6.28 (two s, 1H), 4.19−4.06 (m, 2H), 3.85−3.56 (m, 3H), 2.80−2.68 (m, 1H), 2.39 (dd, 1H, J = 16.5, 6.3 Hz), 2.27−1.99 (m, 3H), 1.82−1.53 (m, 6H), 1.46-1.11 (m, 5H), 0.93 and 0.91 (two s, 9H), 0.09, 0.07, 0.06 and 0.05 (four s, 6H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 162.5, 161.6, 137.1, 136.7, 123.2, 121.2, 62.9, 62.3, 58.5, 58.1, 57.7, 57.5, 37.4, 37.3, 32.2, 30.9, 30.8, 29.1, 28.9, 28.6, 26.9, 26.2, 26.1, 25.9, 25.8, 18.2, 18.1, −5.5; IR (neat/NaCl) 3393 br, 2928, 2854, 1637, 1455, 1450, 1330, 1257, 1111, 1056, 845, 837, 777, 667 cm^{-1}; LRMS (PCI) *m*/*e* (relative intensity) 408 (M + 29, 13), 382 (M + 3, 11), 381 (M + 2, 26), 380 (M + 1, 100), 379 (M, 7), 378 (20), 364 (29), 322 (28); HRMS (EI) *m*/*e* calcd for $C_{21}H_{37}O_3NSi$ 379.2543, found 379.2527.

(6*S* and 6*R*,9*S*)-1-Aza-3-amino-9-[(*tert*-butyldimethylsiloxy)-methyl]-4-cyclohexyl-2-oxobicyclo[4.3.0]non-3-ene (15a,b). Ammonia gas was passed through a solution of the above 1,2-dicarbonyl compound (4.497 g, 11.9 mmol) in 500 mL of MeOH at 0 °C until the solution was saturated. The reaction mixture was stirred at room

temperature under a rubber septum for 3 days. The MeOH was removed under reduced pressure, and the crude product was chromatographed through 400 g of silica gel using 50% ether/hexane as the eluant in order to afford 2.324 g (52%) of isomer **a**, 1.728 g (39%) of isomer **b**, and 0.333 g (7%) of recovered starting material. The spectral data for isomer **a** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 4.21−4.14 (m, 1H), 3.98 (A of AMX, 1H, J_{AM} = 9.6 Hz, J_{AX} = 4.8 Hz), 3.78 (M of AMX, 1H, J_{MA} = 9.6 Hz, J_{MX} = 2.7 Hz), 2.35−2.28 (m, 1H), 2.31 (dd, 1H, J = 15.9, 5.1 Hz), 2.23−2.13 (m, 1H), 2.10−1.94 (m, 3H), 1.87−1.66 (m, 4H), 1.61−1.48 (m, 2H), 1.40−1.09 (m, 6H), 0.88 (s, 9H), 0.06 and 0.03 (two s, 6H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 162.0, 129.3, 120.8, 62.6, 58.5, 56.8, 39.1, 32.1, 30.5, 29.4, 29.0, 26.4, 26.3, 26.1, 26.0, 25.8, −5.5; IR (neat/NaCl) 3450, 3354, 2928, 2856, 1663, 1631, 1583, 1450, 1361, 1323, 1255, 1189, 1155, 1116, 1035, 1007, 939, 837, 776, 736, 666 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 380 (M + 2, 57), 379 (M + 1, 100), 378 (M, 43), 322 (41), 321 (78), 233 (38), 154 (31); HRMS (FAB) *m*/*e* calcd for $C_{21}H_{39}O_2N_2Si$ 379.2780 (M + 1), found 379.2788.

The spectral data for isomer **b** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 4.12−4.06 (m, 1H), 3.79 (A of AMX, 1H, J_{AM} = 10.2 Hz, J_{AX} = 3.0 Hz), 3.64 (M of AMX, 1H, J_{MA} = 10.2 Hz, J_{MX} = 6.0 Hz), 3.59−3.53 (m, 2H), 2.37−2.30 (m, 1H), 2.33 (dd, 1H, J = 16.8, 4.5 Hz), 2.11−1.92 (m, 3H), 1.89−1.66 (m, 6H), 1.57−1.53 (m, 1H), 1.41−1.11 (m, 6H), 0.89 (s, 9H), 0.06 and 0.04 (two s, 6H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 163.0, 129.4, 123.0, 62.8, 57.8, 57.7, 39.3, 31.2, 30.7, 29.2, 29.0, 26.5, 26.4, 26.3, 26.1, 25.9, 18.2, −5.4, −5.5; IR (neat/NaCl) 33445, 3349, 2925, 2846, 1659, 1625, 1575, 1442, 1358, 1337, 1320, 1255, 1190, 1101, 1063, 1005, 936, 835, 775, 734, 663 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 379 (M + 1, 12), 172 (14), 150 (100), 148 (61), 119 (64); HRMS (FAB) *m*/*e* calcd for $C_{21}H_{39}O_2N_2Si$ 379.2780 (M + 1), found 379.2775.

(6*S* and 6*R*,9*S*,12*S*)-1-Aza-9-[(*tert*-butyldimethylsiloxy)methyl]-4-cyclohexyl-2-oxo-3-(pyroglutamylamino)bicyclo[4.3.0]non-3-ene. To a solution of **15a** (2.304 g, 6.09 mmol) in 30 mL of CH_2Cl_2 were added HOBt (1.070 g, 8.53 mmol) and L-pyroglutamic acid (0.944 g, 7.31 mmol). The mixture was stirred at 0 °C for 5 min, and then EDCI (1.398 g, 7.92 mmol) was added. The resulting mixture was allowed to warm to room temperature and stirred overnight. When complete, the solution was transferred to a separatory funnel, the layers were separated, and the organic phase was washed with saturated $NaHCO_3$, 10% citric acid in water, and a saturated solution of aqueous NaCl. The CH_2Cl_2 was removed under reduced pressure, and the crude product was chromatographed through 150 g of silica gel using 7% MeOH in ether to afford 1.253 g (35%) of the tripeptide (isomer **a**) as a white solid along with 0.413 g (14%) of the silyl-deprotected amide. The spectral data for the product from **15a** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 7.97 (s, 1H), 7.02 (s, 1H), 4.30 (dd, 1H, J = 8.7, 5.1 Hz), 4.15−4.08 (m, 1H), 3.90−3.72 (m, 1H), 3.87 (A of ABX, 1H, J_{AB} = 9.9 Hz, J_{AX} = 4.8 Hz), 3.74 (B of ABX, 1H, J_{BX} = 9.9 Hz, J_{BX} = 3.0 Hz), 2.61−2.47 (m, 4H), 2.42−2.21 (m, 4H), 2.16−1.95 (m., 3H), 1.91−1.70 (m, 4H), 1.64−1.49 (m, 2H), 1.32−1.13 (m, 5H), 0.87 (s, 9H), 0.04 and 0.01 (two s, 6H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 178.7, 171.2, 161.3, 148.3, 121.2, 62.6, 58.8, 57.3, 56.1, 41.3, 32.0, 30.6, 30.2, 29.4, 28.9, 26.3, 26.2, 26.1, 26.0, 25.8, 18.1, −5.4, −5.5; IR (neat/NaCl) 32.56 br, 2929, 2855, 1700, 1653, 1617, 1522, 1448, 1254, 1102, 837, 778, 735 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 492 (M + 3, 10), 491 (M + 2, 35), 490 (M + 1, 100), 488 (11), 432 (14), 386 (8); HRMS (FAB) *m*/*e* calcd for $C_{26}H_{44}O_4N_3Si$ (M + 1) 490.3101, found 490.3094.

15b was carried on in an identical fashion with a yield of 61%. The spectral data for the product from **15b** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.33 (s, 1H), 7.54 (s, 1H), 4.25 (dd, 1H, J = 9.3, 5.1 Hz), 4.08−4.01 (m, 1H), 3.78−3.67 (m, 1H), 3.76 (A of ABX, 1H, J_{AB} = 10.2 Hz, J_{AX} = 5.4 Hz), 3.70 (B of ABX, 1H, J_{BA} = 10.2 Hz, J_{BX} = 2.4 Hz), 2.64−2.46 (m, 3H), 2.43−2.22 (m, 3H), 2.15−1.99 (m, 3H), 1.94−1.68 (m, 6H), 1.50−1.47 (m, 1H), 1.34−1.13 (m, 5H), 0.89 (s, 9H), 0.03 and 0.01 (two s, 6H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.2, 172.1, 162.3, 151.3, 121.8, 62.7, 58.0, 57.8, 56.7, 41.3, 31.3, 30.4, 30.2, 29.4, 29.2, 27.0, 26.3, 26.1, 25.9, 18.2, −5.5; IR (neat/NaCl) 3263 br, 2930, 2855, 1700, 1653, 1618, 1522, 1447, 1362, 1321, 1258, 1096, 1055, 838, 778, 735 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 491 (M + 2, 33), 490 (M + 1, 100), 432 (15), 307 (16); HRMS (FAB) *m*/*e* calcd for $C_{26}H_{44}O_4N_3Si$ (M + 1) 490.3101, found 490.3100.

(6*S* and 6*R*,9*S*,12*S*)-1-Aza-4-cyclohexyl-9-(hydroxymethyl)-2-oxo-3-(pyroglutamylamino)bicyclo[4.3.0]non-3-ene. The tripeptide (isomer **a**, 6*S*) (0.110 g, 0.23 mmol) was dissolved in a solution comprised of 1 mL of 2 N H_2SO_4 and 3 mL of THF. The mixture was stirred at room temperature overnight. The solution was transferred into a separatory funnel, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was then chromatographed through silica gel with the use of 40% MeOH in ether as eluant to afford 81.5 mg (96%) of the deprotected product (isomer **a**) as a white solid. The spectral data for isomer **a** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.20 (s, 1H), 7.33 (s, 1H), 4.31 (dd, 1H, J = 8.4, 4.8 Hz), 4.10−4.08 (m, 1H), 3.88−3.71 (m, 2H), 3.56−3.50 (m, 1H), 2.59−2.45 (m, 2H), 2.42−2.08 (m, 8H), 1.80-1.67 (m, 4H), 1.61−1.49 (m, 3H), 1.30−1.10 (m, 5H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.2, 172.2, 162.9, 150.7, 121.6, 65.5, 62.0, 57.2, 56.3, 41.0, 32.0, 30.5, 30.2, 29.5, 28.6, 27.0, 26.2, 25.9; IR (neat/NaCl) 3387 br, 3267 br, 2931, 2854, 1685, 1645, 1616, 1522, 1449, 1319, 1265, 1098, 735 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 386 (M + 11, 19), 376 (M + 1, 44), 308 (26), 307 (100), 289 (46), 235 (15), 219 (15); HRMS (FAB) *m*/*e* calcd for $C_{29}H_{30}O_4N_3$ (M + 1) 376.2236, found 376.2235.

Isomer **b** (6*R*) was carried on in an identical fashion using pyridine and HCl instead of H_2SO_4 with a yield of 78%. The spectral data for isomer **b** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.26 (s, 1H), 7.89 (s, 1H), 4.29 (dd, 1H, J = 7.8, 3.0 Hz), 4.18−4.11 (m, 1H), 3.81−3.69 (m, 2H), 3.52−3.44 (m, 1H), 2.59 (dd, 1H, J = 16.8, 3.6 Hz), 2.52−2.28 (m, 5H), 2.18−2.07 (m, 3H), 2.01−1.93 (m, 2H), 1.83−1.67 (m, 5H), 1.49−1.45 (m, 1H), 1.32−1.11 (m, 5H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.1, 172.1, 163.7, 152.7, 122.1, 65.0, 59.5, 57.5, 57.3, 41.2, 31.0, 30.6, 30.2, 29.5, 29.0, 27.0, 26.2, 26.0, 25.9, 25.5; IR (neat/NaCl) 3386 br, 3256, 2930, 2853, 1685, 1647, 1617, 1522, 1447, 1262, 1046, 736 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 386 (M + 11, 29), 377 (M + 2, 24), 376 (M + 1, 100), 374 (14), 371 (15), 307 (10); HRMS (FAB) *m*/*e* calcd for $C_{29}H_{30}O_4N_3$ (M + 1) 376.2236, found 376.2232.

(6*S* and 6*R*,9*S*,12*S*)-1-Aza-9-carboxy-4-cyclohexyl-2-oxo-3-(pyroglutamylamino)bicyclo[4.3.0]non-3-ene. To a solution of the deprotected alcohol (isomer **a**, 6*S*, 0.413 g, 1.10 mmol) in 3 mL of acetone was added Jones reagent (about 1.1 mL, 1.43 mmol) dropwise at 0 °C. The mixture was stirred at 0 °C for 0.5 h and then at room temperature for 1.5 h. When complete, the reaction was diluted with MeOH, and then the salt in the solution was removed by filtration through a plug of glass wool. The filtrate was concentrated *in vacuo* to afford a crude oil, which was dissolved in water. Ammonium chloride was added in order to saturate the solution, and then the mixture was extracted with ethyl acetate. The organic layers were combined, dried over $MgSO_4$, filtered through a glass fritted funnel, and concentrated *in vacuo*. The crude acid was further purified by HPLC using 35% MeOH/H_2O as eluant to afford the acid (isomer **a**) as a white solid (0.263 g, 63%). The spectral data for isomer **a** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.64 (s, 1H), 7.84 (s, 1H), 4.61−4.52 (m, 1H), 4.47−4.40 (m, 1H), 4.02−3.91 (m, 1H), 2.62−2.38 (m, 5H), 2.32−2.09 (m, 4H), 2.02−1.85 (m, 2H), 1.80−1.59 (m, 5H), 1.53−1.45 (m, 1H), 1.24−1.05 (m, 5H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 180.6, 174.0, 172.3, 162.1, 150.5, 121.6, 58.5, 57.5, 56.1, 41.2, 32.6, 30.3, 29.9, 29.7, 29.0, 28.5, 26.4, 26.3, 26.2, 26.0; IR (neat/NaCl) 3268 br, 2926, 2847, 1716, 1685, 1653, 1616, 1521, 1448, 1320, 1262, 728, 626 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 391 (M + 2, 7), 390 (M + 1, 27), 246 (8), 219 (7), 186 (9), 185 (100); HRMS (FAB) *m*/*e* calcd for $C_{20}H_{28}O_5N_3$ (M + 1) 390.2029, found 390.2042.

Isomer **b** (6*R*) was carried on in an identical fashion with a yield of 50%. The spectral data for isomer **b** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.42 (s, 1H), 8.17 (s, 1H), 4.51−4.45 (m, 1H), 4.38−4.32 (m, 1H), 3.90−3.78 (m, 1H), 2.58−2.38 (m, 6H), 2.25−2.11 (m, 5H), 1.80−1.61 (m, 5H), 1.58−1.51 (m, 1H), 1.32−1.07 (m, 5H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.9, 173.6, 172.9, 161.6, 152.5, 122.7, 57.9, 57.6, 56.1, 40.5, 31.6, 30.5, 29.9, 29.7, 28.7, 28.3, 26.1, 25.8, 25.7, 25.6; IR (neat/NaCl) 3260 br, 2927, 2854, 1680, 1653, 1517, 1449, 1265, 1215, 733 cm^{-1}; LRMS (FAB) *m*/*e* (relative intensity) 390 (M

+ 1, 21), 384 (19), 307 (100), 289 (52), 280 (15); HRMS (FAB) *m*/*e* calcd for $C_{20}H_{28}O_5N_3$ (M + 1) 390.2029, found 390.2033.

(6*S* and 6*R*,9*S*,12*S*)-1-Aza-9-carboxamoyl-4-cyclohexyl-2-oxo-3-(pyroglutamylamino)bicyclo[4.3.0]non-3-ene (10a,b). Isobutyl chloroformate (79 mg, 0.58 mmol) was added to a solution of the acid (isomer **a**) (224 mg, 0.58 mmol) in 6 mL of CH_2Cl_2, followed by *N*-methylmorpholine (64 mg, 0.64 mmol) at 0 °C. The mixture was stirred at 0 °C for 30 min, and then ammonia gas was passed through it for 1 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated *in vacuo*. The crude product was further purified by HPLC using 50% MeOH/H_2O as eluant to afford 200 mg (90%) of **10a**. The spectral data for **10a** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.69 (s, 1H), 7.50 (s, 1H), 7.37 (s, 1H), 6.64 (s, 1H), 4.38 (dd, 1H, J = 8.1, 5.1 Hz), 4.29 (t, 1H, J = 7.8 Hz), 2.68−2.10 (m, 10H), 2.04−1.91 (m, 1H), 1.78−1.57 (m, 5H), 1.32−1.12 (m, 5H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.2, 175.1, 173.3, 162.0, 152.7, 121.9, 59.9, 56.9, 56.2, 40.9, 32.8, 30.5, 30.1, 29.8, 28.9, 28.5, 26.2, 26.0, 25.8; IR (neat/NaCl) 3457 br, 2925, 2850, 1701, 1670, 1636, 1617, 1457, 1449, 1419, 730, 657; LRMS (FAB) *m*/*e* (relative intensity) 389 (M + 1, 64), 306 (100), 288 (50), 233 (20); HRMS (FAB) *m*/*e* calcd for $C_{20}H_{29}N_4O_4$ (M + 1) 389.2189, found 389.2207.

Analog **10b** was made in an identical fashion with a yield of 84%. The spectral data for **10b** were as follows: ^{1}H NMR (300 MHz/$CDCl_3$) δ 8.69 (s, 1H), 7.80 (s, 1H), 7.39 (s, 1H), 6.41 (s, 1H), 4.35 (d, 1H, J = 8.4 Hz), 4.29 (dd, 1H, J = 8.4, 4.8 Hz), 3.81−3.70 (m, 1H), 2.59−2.29 (m, 6H), 2.26−1.97 (m, 4H), 1.94−1.54 (m, 6H), 1.30−1.10 (m, 5H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 179.3, 174.4, 173.1, 162.5, 153.8, 122.3, 58.7, 57.1, 56.7, 40.9, 31.7, 30.7, 30.0, 29.7, 28.8, 28.2, 26.1, 26.0, 25.9, 25.8; IR (neat/NaCl) 3388 br, 2930, 2851, 1674, 1636, 1617, 1539, 1521, 1506, 1457, 1448, 1436, 1420, 1294, 1262, 667; LRMS (FAB) *m*/*e* (relative intensity) 389 (M + 1, 100), 306 (59), 288 (33); HRMS (FAB) *m*/*e* calcd for $C_{20}H_{29}N_4O_4$ (M + 1) 389.2189, found 389.2201.

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Supporting Information Available: Proton and carbon NMR data for all new compounds (36 pages). See any current masthead page for ordering and Internet access instructions.

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