Pergamon

Tetrahedron 56 (2000) 9791–9800

TETRAHEDRON

Constrained Peptidomimetics for TRH: *cis*-Peptide Bond Analogs

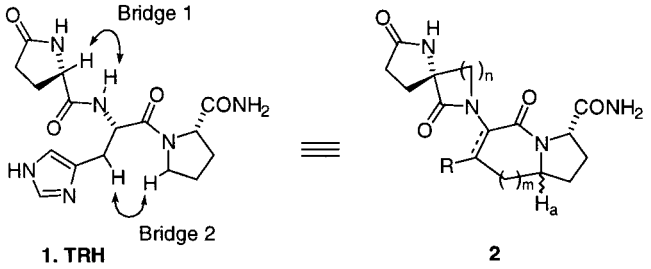
Yunsong Tong,[a] Jacek Olczak,[b,d] Janusz Zabrocki,[b,d] Marvin C. Gershengorn,[c] Garland R. Marshall[b] and Kevin D. Moeller[a,*]

[a]*Department of Chemistry, Washington University, St. Louis, MO 63130, USA*
[b]*Department of Molecular Biology and Pharmacology, and the Center for Molecular Design, Washington University, St. Louis, MO 63130, USA*
[c]*Division of Molecular Medicine, Department of Medicine, Cornell University Medical College and The New York Hospital, New York, NY 10021, USA*
[d]*Institute of Organic Chemistry, Technical University, Zeromiskiego, 116, 90-924 Lodz, Poland*

Received 28 April 2000; accepted 14 August 2000

Abstract—Two constrained analogs of the tripeptide hormone thyroliberin (TRH) have been synthesized. In both, the HisPro peptide bond of the hormone has been 'locked' into a *cis*-conformation. In the first analog, the constraint used was identical to the constraint used in an earlier *trans*-peptide bond analog that was a potent agonist for the TRH endocrine receptor TRH-R_1. The second analog was built in order to take advantage of a tetrazole ring as the *cis*-peptide bond constraint. Neither analog showed agonist or antagonist behavior toward TRH-R_1 suggesting that the *cis*-peptide bond conformation may not play a role in the affinity of TRH for TRH-R_1.

Thyroliberin (TRH) is the hypothalamic tripeptide (*p*-GluHisPro-NH_2) that controls the release of thyroid stimulating hormone from the pituitary gland.[1] In addition, it is active in the central nervous system.[2] Because of its biological relevance, TRH has been extensively studied, and a number of proposals have been made concerning the conformation responsible for the binding of TRH to its endocrine receptor (TRH-R_1).[3] Marshall and coworkers used the active analog approach to computer assisted drug design to predict an active conformation for the binding of TRH to TRH-R_1.[4,5] In this work, two main conformational possibilities were proposed. Of these two possibilities, the more frequently populated conformation is illustrated in Scheme 1 (**1**). In order to probe whether this proposed conformation represented an 'active' conformation of the hormone, a series of analogs having the overall structure of **2** were examined. Analogs having only the pyroglutamate region constrained (m=0),[6] analogs having only the HisPro region of the molecule constrained (n=0),[7] and fully constrained analogs[8] have all been studied. The studies have resulted in several constrained analogs that bind the TRH-R_1 receptor more tightly than their unconstrained counterparts[7,8] and at least one analog that also shows improved potency relative to its unconstrained counterpart.[7]

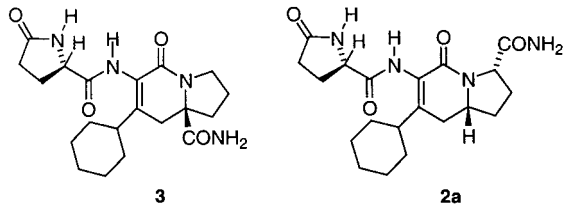


Scheme 1.

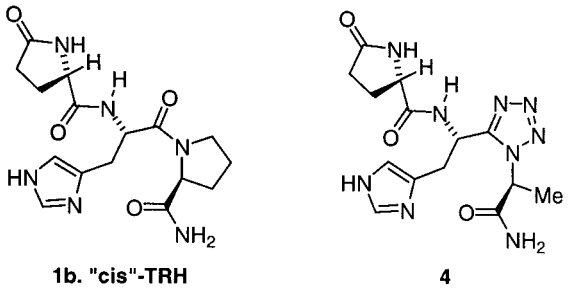
The conformation of TRH illustrated by **1** was determined by assuming that all of the analogs used in the original basis set for the active analog approach had a *trans*-peptide bond connecting the central amino acid to the proline moiety. This assumption represented a very reasonable starting point. However, because proline residues involve a tertiary amide, the presence of a proline in a peptide is known to destabilize *trans*-peptide bonds relative to their *cis*-peptide bond counterparts. A significant percentage (ca. 10%)[9] of the proline residues found in natural proteins have a *cis*-conformation for the N-terminal peptide bond. In a number of cases, the *cis*-conformation for this peptide bond has been implicated as the conformation responsible for receptor binding and the observed biological activity.[10] Could a *cis*-peptide bond conformation involving the proline amide bond also play a role in the binding of TRH to TRH-R_1? While the observation that analogs constraining the HisPro region of TRH into a *trans* conformation were potent agonists for TRH-R_1 certainly implicated that the *trans*-conformation was important for potency, one could

Keywords: tripeptide hormone thyroliberin; tetrazole ring; TRH endocrine receptor.
* Corresponding author. Tel.: +314-935-4270; fax: +314-935-4481; e-mail: moeller@wuchem.wustl.edu

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00886-3



Scheme 2.



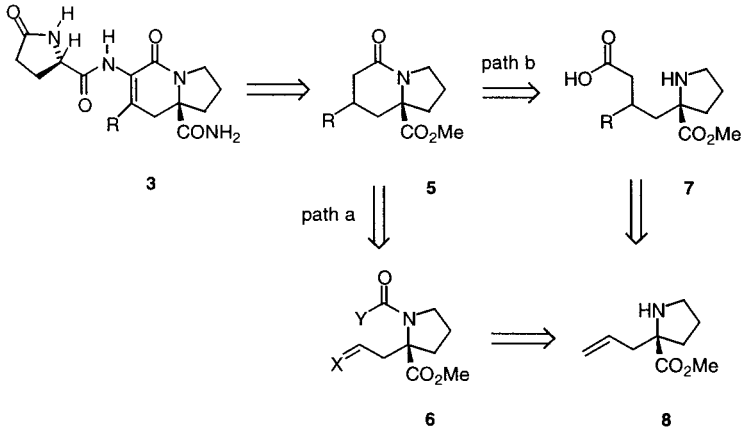
Scheme 3.

imagine a scenario where TRH initially bound TRH-R_1 with a *cis*-peptide bond conformation in the HisPro region and then equilibrated to a *trans*-peptide bond conformation during the reorganization of the ligand/receptor complex that leads to signaling. In this case, the potency of the constrained *trans*-peptide mimetics might have resulted from the analogs either inducing a fit with the receptor or 'draining off' an equilibrium between various receptor conformations. If this were the case, then a constrained analog fixed into a *cis*-conformation might prove to be an antagonist for the receptor. Such an analog would bind TRH-R_1, but then be unable to undergo the conformational change needed for potency.

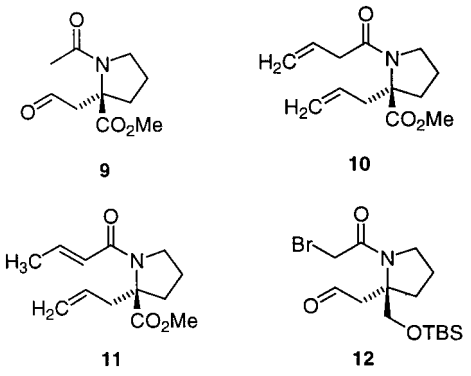
Initially, two approaches were taken in order to investigate this possibility. In one, a TRH analog having a *cis*-peptide bond constraint (**3**) identical to the one used successfully for the *trans*-peptide bond analog **2a** was designed (Scheme 2). The only difference between the two analogs was the position of the primary amide group on the fused bicyclic ring system used to lock the C-terminal region of the molecule in place. In the second approach, a tetrazole ring was used as the *cis*-peptide bond mimetic (**4**/Scheme 3).[11] In this case, the constraint incorporated the nitrogen of the peptide bond; an alteration that forced a replacement of the proline amino acid at the C-terminal end of the hormone. For TRH, replacements of this type can be made without losing activity. TRH analogs having a methylalanine (which has the same steric parameters as the proline) group in the C-terminal position retain both their affinity and potency for TRH-R_1.[12]

Two approaches to the synthesis of **3** seemed reasonable (Scheme 4). Both plans called for taking advantage of what had been learned during the synthesis of **2a** in order to convert the a bicyclic lactam **5** into the desired TRH analog. In turn, the bicyclic building block would be constructed from the known allylated proline **8**.[13] The two routes differed in how the lactam ring constraint was to be built. For path a, the amine in **8** would be converted into an amide and then the two sidechains coupled to form the lactam ring. For path b, the sidechain in **8** would be developed into a longer chain carboxylic acid derivative and then the lactam ring synthesized by an intramolecular amide formation. On paper both routes had their advantages. Path a appeared more divergent and compatible with the rapid incorporation of substituents at the carbon beta to the amide carbonyl in **5**, while path b was directly analogous to the route used by others to make unsubstituted (R=H) building blocks like **5**.[14,15]

In practice, efforts along path a were not successful. Three different approaches were attempted including an intramolecular aldol reaction (substrate **9**), a ring closing olefin metathesis reaction (substrates **10** and **11**),[16] and an intramolecular Barbier reduction (substrate **12**).[17] (Scheme 5) Neither the intramolecular aldol nor the ring closing metathesis reaction led to the formation of any cyclized product. A variety of conditions were tried for each, but in each case only starting material was recovered. The lack of reactivity for these substrates was rationalized by suggesting that the steric size of the quaternary center forced the amide bond to adopt a conformation that did not allow for the two ends of the proposed cyclizations to approach each other. The Barbier route to effecting the cyclization (SmI_2) did lead to low yields of the bicyclic product (23–34%), but the reaction could not be optimized further.



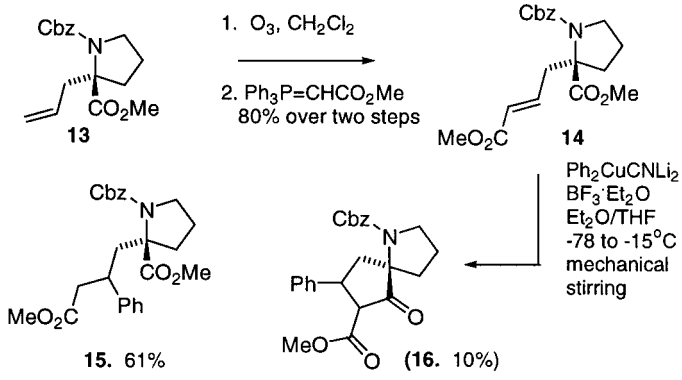
Scheme 4.



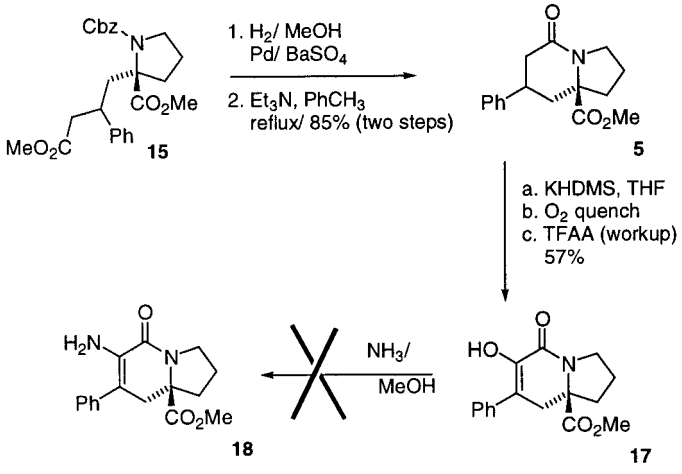
Scheme 5.

Fortunately, path b proved to be much more useful. As in the earlier syntheses of unsubstituted analogs,[13,14] the 2-allylproline starting material was treated under ozonolysis conditions and the resulting aldehyde subjected to a Wittig reaction to form an α,β-unsaturated ester (Scheme 6). At this point, the substituent needed for the central amino acid of the TRH derivative was added with the use of a higher order cuprate.[18] Two aspects of this reaction deserve comment. First, the cyclohexyl substituent utilized in **2a** was replaced with a phenyl ring in **3**, because the required phenyl cuprate could be generated more easily and because Phe^{2}TRH showed roughly the same biological activity as CyclohexylAla^{2}TRH. Both are a factor of 10^2–10^3 less active than the natural hormone in terms of binding and potency, both completely displace TRH from TRH-R_1 at high concentrations, and both are full agonists. In addition, conclusions about the compatibility of the bridges are made by comparing the constrained analog to *its* acyclic counterpart not having the constraint. Conclusions about **2a** were made by directly comparing the data obtained with that obtained using CyclohexylAla^{2}TRH.[7] Along these lines, conclusions about **3** would be made by directly comparing the data obtained for **3** with the data obtained for Phe^{2}TRH.[6] Second, the cuprate addition reaction on **14** needed to be stirred well and kept below −15°C. Above −15°C, the reaction led to substantial amounts of a byproduct tentatively assigned as **16**. Even when the reaction was optimized, a 10% yield (assuming **16**) of this product was obtained along with a 61% isolated yield of the desired Michael product.

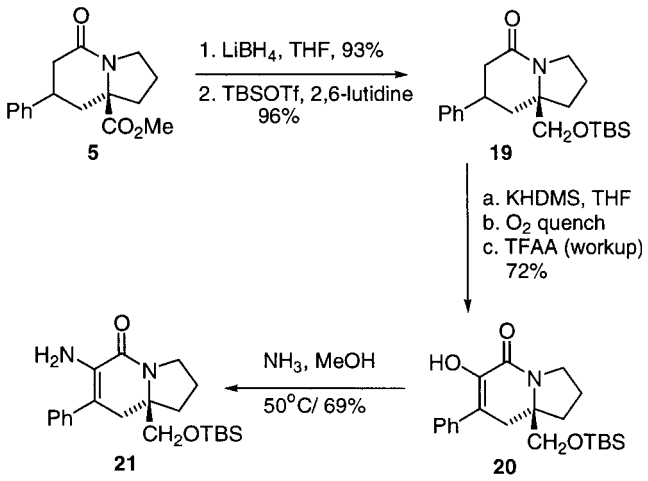
The conversion of **15** into desired bicyclic intermediate **5** was straightforward (Scheme 7). The Cbz protecting group was removed using hydrogen in methanol and Pd/$BaSO_4$ as the catalyst, and the six-member ring lactam was formed using triethylamine in toluene. An 85% yield of the bicyclic product was obtained over the two steps. The required carbonyl functionality alpha to the amide was then added by deprotonating the amide and quenching the resulting enolate with oxygen. Addition of trifluoroacetic anhydride to the workup ensured formation of the dicarbonyl equivalent.[19]



Scheme 6.



Scheme 7.



Scheme 8.

However, this initial pass at the synthesis failed when treatment of the α-ketoamide with ammonia in methanol failed to generate the desired product **18**. From the crude spectral data, it was clear that the presence of the methyl ester interfered with the reaction.

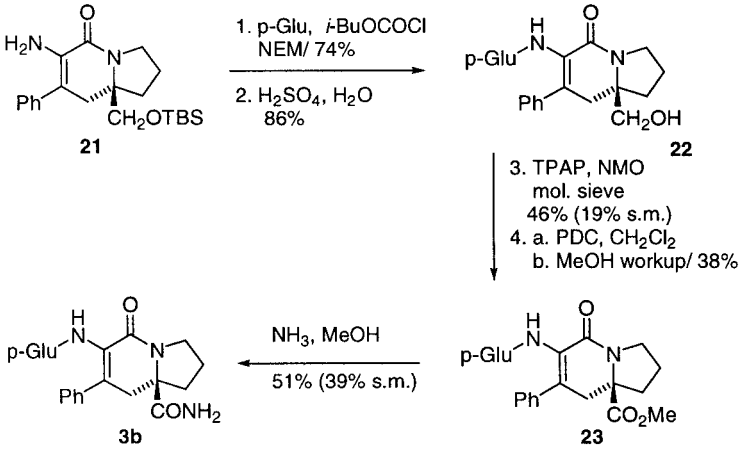
In order to avoid this problem, the methyl ester in **5** was selectively reduced with lithium borohydride and the resulting alcohol protected as the *t*-butyldimethylsilyl ether. This transformation both increased the yield obtained for functionalization of the carbon alpha to the amide carbonyl and allowed for conversion of the resulting dicarbonyl product **20** into enamine **21** (Scheme 8). As in earlier cases, the enamine group was surprisingly stable, and compound **21** was purified by chromatography through triethylamine treated silica gel.

Conversion of enamine **21** into the *cis*-peptide bond TRH analog **3** proceeded as outlined in Scheme 9. The enamine functionality was coupled to the N-terminal pyroglutamate group by means of the mixed anhydride intermediate using isobutylchloroformate. It should be noted that other coupling procedures using the standard HOBT/EDCI, HOBT/TBTU, and BOP-Cl/Et_3N conditions were not effective. Following the coupling reaction, the silyl protecting group was removed and the resulting alcohol oxidized using a two step procedure. Attempts to oxidize the alcohol directly to an acid using either a Jones oxidation or PDC in DMF met with failure. For the two step procedure, a methanol workup was used in order to directly form methyl ester **23**. The yield of this procedure was not optimized. Finally, treatment of methyl ester **23** with ammonia in methanol afforded analog **3**.

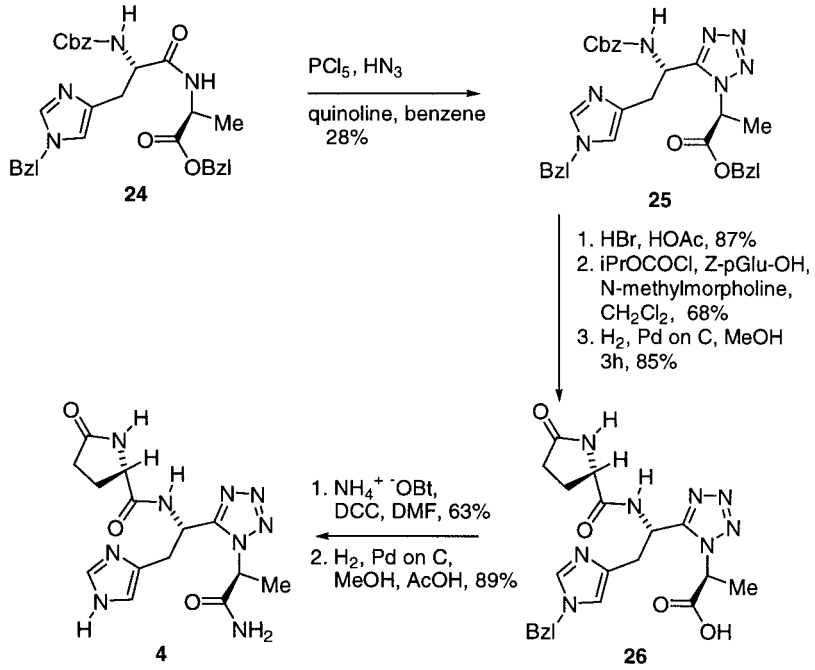
Analog **3** was evaluated both as a potential agonist and a potential antagonist for TRH-R_1. For agonist behavior, monkey kidney COS-1 cells transiently expressing TRH receptors and pre-labelled with [^{3}H]-inositol were incubated with various doses (1.0–1000 μM) of **3**.[20] Inositol phosphate formation was measured. In no case did the presence of **3** lead to an increase in the amount of inositol phosphate generated, and it was concluded that **3** was not an agonist for TRH-R_1. For antagonist behavior, the cells were incubated with both **3** and varying concentrations of TRH itself. In no case did the presence of **3** alter the extent of inositol phosphate released. Based on this result, it was concluded that **3** was also not an antagonist for TRH-R_1.

A similar observation was made for the analog using a tetrazole ring to constrain the HisPro region of the hormone (**4**). As illustrated in Scheme 10, the tetrazole ring was introduced into the molecule by treating a Cbz-HisAla-OBz dipeptide derivative with PCl_5, quinoline, and HN_3. The N-terminal Cbz group was then removed from the constrained building block with the use of HBr in acetic acid and the resulting amine coupled to a Cbz protected pyroglutamic acid using a mixed anhydride as the activating group. Cleavage of the N-terminal Cbz group and the C-terminal benzyl ester afforded tripeptide **26**. The desired TRH analog was completed by converting the C-terminal acid to a primary amide with the use of freshly prepared ammonium salt of HOBt and DCC and then deprotecting the imidazole benzyl protecting group. As in the case of **3**, analog **4** did not show any affinity for TRH-R_1.

The observation that *neither* **3** nor **4** showed any affinity for TRH-R_1 suggested that the conformation of TRH having a *cis*-peptide in the HisPro region does not play a role in the binding of TRH to its endocrine receptor, TRH-R_1. In this case, the data for the two analogs worked together to make the argument more compelling. Typically, a lack of activity



Scheme 9.



Scheme 10.

for a single constrained analog can not be used to draw conclusions about the biological relevance of the conformation being mimicked. The conformational constraints added to the analog simply make other changes in the molecule. For example, the methylene bridge used in **3** to constrain the *cis*-peptide bond also added steric bulk to the central portion of the analog and fixed the orientation of the side chain of residue two. In **4**, the tetrazole ring used to constrain the *cis*-peptide bond both added steric bulk to the amide region of the molecule and caused a loss in the hydrogen-bonding capacity of the analog. However, when taken together the lack of activity for multiple analogs can shed light on the biological relevance of a proposed conformation. In the current example, both analogs **3** and **4** may have had multiple differences relative to the natural hormone, but only the *cis*-peptide bond was common to both. The fact that the analogs behaved similarly implied that it was the presence of this *cis*-peptide bond that interfered with TRH/TRH-R_1 binding. The synthesis and testing of a third analog ([AzPro[3]]-TRH) has recently added further support to this conclusion (Zhang, Gershengorn and Marshall, unpublished). [AzPro[3]]-TRH proved to be 35-fold less active than the natural hormone. Azaproline (AzPro) has been proposed by Zouikri et al.[21] to enhance the stability of the *cis*-amide conformation and has been shown using both theory and NMR (Berglund, Zhang and Marshall, unpublished) to do so in the TRH analog.

The observations made using all three TRH analogs combined with the fact that the closely related *trans*-peptide mimetic **2a** bound TRH-R_1 more tightly and was more potent than its unconstrained counterpart confirmed that the initial modelling efforts were correct to assume a *trans*-amide bond conformation for the HisPro region of TRH. It appears clear that the design of both agonists and potential antagonists for TRH-R_1 in the future will need to incorporate a *trans*-peptide bond in the HisPro region of the hormone.

Experimental

(2*R*)-*N*-(Benzyloxycarbonyl)-2-[(*E*)-3′-(carbomethoxy)-prop-2′-enyl]proline methyl ester (14). In a 250 mL flask, 10.2 g (0.0336 mol) of the Cbz protected 2-allylproline derivative **13** was dissolved in 70 mL of dichloromethane. Ozone was bubbled though the solution at −78°C. The solution turned blue 70 min later. After an additional 5 min, the bubbling of ozone was stopped and nitrogen was bubbled through the solution until the blue color disappeared. To the −78°C solution was added 25.8 g (0.0773 mol, 2.3 equiv.) of methyl (triphenylphosphoranylidene)acetate and the reaction mixture was warmed to room temperature. After 20 h of stirring, 100 mL of water and 150 mL of dichloromethane were added to the reaction mixture. The aqueous layer was separated and washed twice with 100 mL of dichloromethane. The combined organic layers were dried over $MgSO_4$ and concentrated. The crude product was chromatographed through 300 g of silica gel using ether/hexane (8:2) as the eluant to afford 9.69 g (80%) of pure product as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) A 1:1 mixture of rotamers was observed. δ 7.36–7.27 (m, 5H), 6.89 (ddd, 1H, J=7.0, 15.2, 15.2 Hz), 5.92 (d, 0.5H, J=15.7 Hz), 5.83 (d, 0.5H, J=15.7 Hz), 5.22 (a of ab, 0.5H, J_{ab}=12.2 Hz), 5.13 (a′ of a′b′, 0.5H, $J_{a'b'}$=12.5 Hz), 5.10 (b′ of a′b′, 0.5H, $J_{a'b'}$=12.6 Hz), 5.01 (b of ab, 0.5H, J_{ab}=12.1 Hz), 3.80–3.65 (m, 6H, two s at 3.73 and 3.72 representing 5H), 3.56–3.46 (m, 2H, one s at 3.46 representing 1H), 3.24 (dd, 0.5H, J=7.1, 14.5 Hz), 3.00 (a of abx, 0.5H, J_{ax}=7.0 Hz, J_{ab}=14.5 Hz), 2.92–2.76 (m, 1H), 2.16–1.78 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.9, 166.6, 154.4, 143.8, 143.4, 136.7, 128.4, 128.3, 127.9, 127.6, 124.6, 67.8, 67.3, 66.8, 52.5, 52.3, 51.5, 49.0, 48.2, 38.2, 37.4, 36.8, 35.9, 23.1, 22.6; IR (neat/NaCl) 2960, 2882, 1707, 1659, 1406, 1349, 1244, 1180, 1124, 1026, 998, 744, 703 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 362 (MH^+, 100), 302 ($M^+-C_2H_3O_2$, 12), 258 (24), 226

($M^+-C_8H_7O_2$, 41), 154 (50); HRFAB calcd for $C_{19}H_{24}N_1O_6$ (M+1) 362.1603, found 362.1583; Anal. calcd for $C_{19}H_{23}N_1O_6$: C, 63.15, H, 6.41, found: C, 63.12, H, 6.57.

(2*R*)-*N*-(Benzyloxycarbonyl)-2-[2′-(phenyl)-3′-(carbomethoxy)-propyl]proline methyl ester (15). Copper (I) cyanide was dried in an Abderhalden apparatus (acetone refluxing at 56°C) overnight. To a flamed dried 250 mL flask was added 2.938 g (0.0328 mol, 2 equiv.) of copper cyanide, which was gently flame-dried again under high vacuum. The flask was then connected to a mechanical stirrer and protected by an argon balloon. To a suspension of copper cyanide in 20 mL of dry ether and 20 mL of THF was added 36.4 mL (0.0656 mol, 4 equiv.) of 1.8 M (cyclohexane/ether) phenyllithium solution at −78°C. The flask was transferred into a ice bath and cooled back to −78°C 10 min later. To the reaction mixture was added 4.03 mL (0.0328 mol, 2 equiv.) of $BF_3{\cdot}Et_2O$. After 10 min, compound **14** (5.9 g/16.4 mmol) in 20 mL of ether was added to the flask. The reaction temperature was raised to about −45°C (CH_3CN/dry ice bath) 30 min later and further warmed up to about −20°C (CCl_4/dry ice bath) after an additional 100 min. The temperature was kept at −20°C for 40 min before the reaction was quenched by 150 mL of 10% NH_4OH/NH_4Cl. The mixture was then stirred at room temperature for 30 min and was diluted with 160 mL of ether afterwards. The organic layer was separated, and the aqueous layer was extracted by 160 mL of ether two more times. The combined organic layers were washed with 10% NH_4OH/NH_4Cl and 5% $NaHCO_3$ and dried over $MgSO_4$ before being concentrated. The crude product was chromatographed through 550 g of silica gel using ether/hexane (8:2) as the eluant to afford 4.40 g (61%) of pure product as a yellow oil along with 670 mg of byproduct tentatively assigned as **16** (10% if **16**). ^{1}H NMR (300 MHz, $CDCl_3$) A mixture of rotamers and stereochemical isomers was observed. δ 7.41–7.16 (m, 10H), 5.26–5.09 (m, 2H), 3.77–3.63 (m, 3H, one s at 3.65 representing 1H), 3.49, 3.46, 3.40 (3 s, 5H), 3.22–3.15 (m, 1H), 2.78 (dd, 0.5H, J=4.2, 14.7 Hz), 2.66–2.34 (m, 3.5H), 1.84–1.61 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) isomers observed δ 174.9, 174.5, 172.2, 171.8, 154.7, 144.9, 144.4, 136.9, 136.3, 129.0, 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.6, 126.7, 126.5, 68.8, 67.9, 67.2, 66.7, 52.3, 52.2, 51.3, 49.4, 48.5, 43.2, 43.0, 39.8, 38.6, 38.0, 37.9, 37.0, 35.6, 22.6, 22.1; IR (neat/NaCl) 3065, 3030, 2953, 2882, 1736, 1701, 1602, 1497, 1447, 1406, 1342, 1244, 1167, 1124, 1019, 913, 857, 772, 695 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 440, (MH^+, 100), 380 ($M^+-C_2H_3O_2$, 49), 336 (66), 304 ($M^+-C_8H_7O_2$, 54), 246 (27); HRFAB calcd for $C_{25}H_{30}N_1O_6$ (M+1) 440.2073, found 440.2076; Anal. calcd for $C_{25}H_{29}N_1O_6$: C, 68.32, H, 6.65, N, 3.19, found: C, 68.38, H, 6.78, N, 3.06.

(2*R*)-2-[2′-(Phenyl)-3′-(carbomethoxy)-propyl]proline methyl ester. To a solution of 4.57 g (0.0104 mol) of **15** in 100 mL of methanol was added 1.14 g of 5% palladium in barium sulfate. The reaction mixture was hydrogenated with a hydrogen balloon. After being stirred overnight, the mixture was filtered through celite. The filtrate was then concentrated to give 3.2 g of crude product as a pale yellow oil, which was used in the following step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) A mixture of two isomers was observed. δ 7.37–7.17 (m, 5H), 3.55 (s, 2.2H), 3.51 (s, 2.2H), 3.44 (s, 1.6H), 3.27–3.12 (m, 1H), 2.95–2.76 (m, 1H), 2.68–2.37 (m, 3H), 2.28–2.11 (m, 2H), 1.98–1.63 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.0, 169.4, 142.1, 128.5, 127.2, 126.8, 67.5, 52.5, 44.8, 39.2, 38.6, 37.4, 36.5, 20.6. The crude product was carried on to the next step without further characterization.

(6*R*)-1-Aza-6-carbomethoxy-4-phenyl-2-oxobicyclo[4.3.0]-nonane (5). To a solution of 3.2 g of crude deprotected material made in the previous step in 135 mL of toluene was added 8.7 mL of Et_3N. The solution was heated to reflux for 21 h. The reaction mixture was then concentrated. The crude product (3.6 g) was chromatographed through 180 g of silica gel using ether/dichloromethane/methanol (8:1:1) as the eluant to afford 2.4 g (85%, two steps combined) of pure product as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.18 (m, 5H), 3.86–3.79 (m, 1H), 3.55 (ddd, 1H, J=2.7, 9.4, 9.4 Hz), 3.44 (s, 3H), 3.28–3.23 (m, 1H), 2.71–2.62 (m, 2H), 2.43–2.37 (m, 1H), 2.20 (a of abx, 1H, J_{ax}=6.1, J_{ab}=14.0 Hz), 1.98–1.68 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.9, 169.5, 142.1, 128.4, 127.2, 126.8, 67.4, 52.5, 44.8, 39.1, 38.6, 37.3, 36.5, 20.5; IR (neat/NaCl) 3023, 2953, 2889, 1736, 1652, 1497, 1434, 1349, 1216, 1173, 1118, 1019, 759, 703 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 274 (MH^+, 100), 214 ($M^+-C_2H_3O_2$, 23), 131 (10); HRFAB calcd for $C_{16}H_{20}N_1O_3$ (M+1) 274.1443, found 274.1431; Anal. calcd for $C_{16}H_{19}N_1O_3$: C, 70.31, H, 7.01, found: C, 69.88, H, 7.27.

(6*R*)-1-Aza-6-hydroxymethyl-4-phenyl-2-oxobicyclo[4.3.0]-nonane. To a 0°C solution of 2.423 g (8.864 mmol) of **5** in 35 mL of THF was added 6.65 mL (0.0133 mol, 1.5 equiv.) of 2 M $LiBH_4$. The reaction was warmed to room temperature and stirred overnight. The reaction was then recooled to 0°C, and 35 mL of 1N HCl, 10 mL of brine, and 35 mL of ethyl acetate added to the reaction solution. After separation, the aqueous layer was extracted twice with 35 mL of ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was chromatographed through 100 g of silica gel using ether/dichloromethane/methanol (8:1:1) as the eluant to afford 2.024 g (93%) of pure product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) A mixture of two isomers was observed. δ 7.34–7.20 (m, 5H), 4.54–4.47 (m, 0.1H), 4.06–3.94 (m, 1.9H), 3.61(d, br,1H, J=10.9 Hz), 3.40–3.25 (m, 2H), 2.89 (tt, 1H, J=4.4, 12.5 Hz), 2.65–2.27 (m, 3H), 2.06 (ddd, 1H, J=2.5, 5.0, 11.1 Hz), 1.97–1.76 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.5, 143.2, 128.6, 126.8, 126.7, 66.2, 65.6, 43.1, 39.4, 37.0, 36.7, 34.8, 20.2; IR (neat/NaCl) 3340 br, 3023, 2945, 1645, 1462, 1420, 1237, 1180, 1069, 1040, 998, 913,765, 695 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 246 (MH^+, 49), 214 (M^+-CH_3O, 18), 154 (100); HRFAB calcd for $C_{15}H_{20}N_1O_2$ (M+1) 246.1494, found 246.1487; Anal. calcd for $C_{15}H_{19}N_1O_2$: C, 73.44, H, 7.81, N, 5.71, found: C, 73.56, H, 8.04, N, 5.68.

(6*R*)-1-Aza-6-[(*t*-butyldimethylsiloxy)methyl]-4-phenyl-2-oxobicyclo[4.3.0]nonane (19). To a 0°C solution of 6.4 g (0.026 mol) of the alcohol made above in 110 mL of dichloromethane was added 7.2 mL (0.030 mol, 1.2 equiv.) of TBDMSOTf and 6.1 mL (0.052 mol, 2 equiv.) of 2,6-lutidine. The reaction was kept at 0°C for

1 h and then diluted with 100 mL of dichloromethane. The organic solution was washed two times with 100 mL of 5% aqueous citric acid, 100 mL of 5% $NaHCO_3$ and once with brine. The organic layer was then dried and concentrated. The crude product was chromatographed through 400 g of silica gel using ether/dichloromethane/methanol (8:1:1) as the eluant to afford 9.0 g (96%) of pure product as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.19 (m, 5H), 3.98 (ddd, 1H, J=8.4, 12.2, 12.2 Hz), 3.43 (a of ab, 1H, J_{ab}=9.9 Hz), 3.36 (b of ab, 1H, J_{ab}=9.9 Hz), 3.36–3.29 (m, 1H), 2.95–2.88 (m, 1H), 2.53–2.50 (m, 2H), 2.23–1.74 (m, 6H), 0.90 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.8, 143.6, 128.7, 126.7, 67.2, 64.9, 43.4, 39.4, 37.8, 37.0, 34.9, 25.8, 20.2, 18.2, −5.5, −5.6; IR (neat/NaCl) 3459, 3030, 2945, 2882, 1652, 1455, 1426, 1335, 1258, 1208, 1103, 998, 843, 772, 703, 660 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 360 (MH^+, 100), 214 ($M^+-C_7H_{17}OSi$, 43)156 (8), 131 (23); HRFAB calcd for $C_{21}H_{34}N_1O_2Si$ (M+1) 360.2359, found 360.2350; Anal. calcd for $C_{21}H_{33}N_1O_2Si$: C, 70.15, H, 9.25, found: C, 70.21, H, 9.53.

(6*R*)-1-Aza-6-[(*t*-butyldimethylsiloxy)methyl]-3-hydroxy-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene (20). To a −78°C solution of 0.331 g (0.921 mmol) of **19** in 18 mL of dichloromethane was added 5.52 mL (2.76 mmol, 3 equiv.) of 0.5 M KHMDS in toluene. After 1 h, oxygen was bubbled through the solution for 75 s and the reaction mixture was then quenched with 0.21 mL (1.5 mmol) of TFAA. Oxygen flow was stopped 50 seconds later, and the reaction was protected under argon. The reaction was then warmed up to room temperature over 45 min. To the mixture was added 20 mL of $NaHSO_3$, and the solution was then extracted three times with 20 mL of ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product (0.67 g) was chromatographed through 30 g of silica gel using ether/hexane (8:2) as the eluant to afford 0.247 g (72%) of pure product as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.77 (d, 2H, J=7.1 Hz), 7.48–7.43 (m, 2H), 7.35–7.31 (m, 1H), 7.00 (s, 1H), 3.79–3.70 (m, 2H), 3.74 (a of ab, 1H, J_{ab}=8.4 Hz), 3.48 (b of ab, 1H, J_{ab}=9.0 Hz), 3.20 (a of ab, 1H, J_{ab}=16.2 Hz), 2.78 (b of ab, 1H, J_{ab}=16.1 Hz), 2.52 (ddd, 1H, J=3.5, 6.0, 12.4 Hz), 2.17–2.08 (m, 2H), 1.83 (dd, 1H, J=8.8, 11.1 Hz), 0.92 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) A mixture of tautomers was observed. δ 179.7, 161.4, 157.9, 145.1, 140.0, 138.2, 136.8, 133.9, 128.7, 128.4, 128.3, 128.1, 127.6, 127.2, 111.3, 66.8, 65.6, 64.0, 62.8, 45.1, 44.3, 35.8, 33.7, 31.5, 25.7, 21.7, 21.0, 18.1, −5.6; IR (neat/NaCl) 3318 br, 3058, 2960, 2861, 1645, 1462, 1349, 1258, 1208, 1103, 998, 842, 772, 695 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 374 (MH^+, 100), 284 (12), 228 ($M^+-C_7H_{17}OSi$, 89), 136 (14); HRFAB calcd for $C_{21}H_{32}N_1O_3Si$ (M+1) 374.2151, found 374.2149; Anal. calcd for $C_{21}H_{31}N_1O_3Si$: C, 67.52, H, 8.36, N, 3.75, found: C, 67.55, H, 8.70, N, 3.93.

(6*R*)-1-Aza-3-amino-6-[(*t*-butyldimethylsiloxy)methyl]-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene (21). Ammonia gas was bubbled through a solution of 0.423 g (1.13 mol) of **20** in 32 mL of methanol at 50°C for 20 min and then at room temperature for 10 min. The gas inlet and outlet needles were removed from the septum, and the mixture was stirred at 50°C for 6 days. If during the 6 days, the ammonia pressure inside the flask decreased (determined based on the appearance of the septum), ammonia gas was bubbled through the solution again. The reaction solution was then concentrated. The crude product was chromatographed through 25 g of silica gel (slurry neutralized with 4 drops of Et_3N) using ether/hexane as the eluant to afford 0.290 g (69%) of pure product as a yellow resin. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.27 (m, 5H), 4.14 (s, br, 2H), 3.79 (a of ab, 1H, J_{ab}=9.6 Hz), 3.76–3.67 (m, 2H), 3.45 (b of ab, 1H, J_{ab}=9.3 hz), 2.97 (d, 1H, J=16.1 Hz), 2.75 (d, 1H, J=16.0 Hz), 2.48 (ddd, 1H, J=3.1, 6.4, 12.4 Hz), 2.10–2.02 (m, 2H), 1.79–1.72 (m, 1H), 0.90 (s, 9H), 0.08 (s, 2.3H), 0.06 (s, 3.7H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.7, 140.0, 131.0, 128.6, 127.2, 126.6, 110.5, 63.6, 62.4, 45.3, 35.8, 35.7, 25.7, 21.4, 18.1, −5.6, −5.7; IR (neat/NaCl) 3445 br, 3346, br, 3057, 2945, 2868, 1630, 1567, 1455, 1349, 1258, 1103, 1005, 843, 722, 703 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 373 (MH^+, 67), 227 ($M^+-C_7H_{17}OSi$, 100), 156 (23); HRFAB calcd for $C_{21}H_{33}N_2O_2Si$ (M+1) 373.2311, found 373.2278.

(6*R*,12*S*)-1-Aza-3-(pyroglutamylamino)-6-[(*t*-butyldimethylsiloxy)methyl]-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene. To a −20°C (CCl_4/dry ice bath) solution of 0.078 g (0.60 mmol, 1.1 equiv.) of pyroglutamic acid in 9 mL of THF was added 0.076 mL (0.60 mmol, 1.1 equiv.) of NEM and 0.078 mL (0.60 mmol, 1.1 equiv.) of isobuytlchloroformate. After the reaction mixture was stirred at −20°C for 40 min, compound **21** (0.204 g, 0.548 mmol, dissolved in 5 mL of THF) was added to the flask. The reaction was warmed to room temperature and 3 h later the solution was concentrated. The residue was then dissolved in 40 mL of chloroform and washed two times with 25 mL of 5% citric acid and once with brine solution. The organic layer was dried over Na_2SO_4 and concentrated. The crude product (0.41 g) was chromatographed through 20 g of silica gel using ether/dichloromethane/methanol (70:15:15) as the eluant to afford 0.196 g (74%) of pure product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.70 (s, 1H), 7.36–7.27 (m, 5H), 4.06–4.02 (m, 1H), 3.98 (a of ab, 1H, J_{ab}=9.9 Hz), 3.62–3.57 (m, 2H), 3.42 (b of ab, 1H, J_{ab}=9.9 Hz), 3.27 (d, 1H, J=17.7 Hz), 2.59 (d, 1H, J=17.6 Hz), 2.46–2.15 (m, 4H), 2.03–1.95 (m, 2H), 1.79–1.64 (m,2H), 0.92 (s, 9H), 0.10 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.6, 170.8, 161.8, 142.1, 138.2, 128.3, 128.2, 127.4, 123.1, 63.8, 62.0, 57.7, 45.3, 37.4, 35.5, 29.2, 25.8, 25.0, 21.6, 18.1, −5.5, −5.6; IR (neat/NaCl) 3241 br, 2960, 2882, 1701, 1645, 1511, 1447, 1244, 1103, 1004, 836 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 506 (MNa^+, 18), 484 (MH^+, 100), 373 ($MH^+-C_5H_5NO_2$, 96), 338 ($M^+-C_7H_{17}OSi$, 39), 241 (64), 227 (85); HRFAB calcd for $C_{26}H_{38}N_3O_4Si$ (M+1) 484.2631, found 484.2644.

(6*R*,12*S*)-1-Aza-3-(pyroglutamylamino)-6-hydroxymethyl-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene (22). The coupled product synthesized in the previous step (0.926 g, 1.92 mmol) was dissolved in 8.2 mL of 2N H_2SO_4 and 25 mL of THF. The mixture was stirred overnight. To the mixture was then added saturated Na_2SO_4 solution until the pH of the reaction mixture reached 9 to 10. The solution was evaporated in vacuo and the residue was dissolved in

methanol. The solid material was filtered, and the filtrate was dried over Na_2SO_4 and concentrated. The crude product was chromatographed through 50 g of silica gel using ether/dichloromethane/methanol (2:1:1) as the eluant to afford 0.610 g (86%) of pure product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.60 (s, 1H), 7.36–7.27 (m, 5H), 4.05–4.01 (m, 1H), 3.90 (a of ab, 1H, J_{ab}=12.5 Hz), 3.77–3.71 (m, 1H), 3.74 (b of ab, 1H, J_{ab}=11.6 Hz), 3.65–3.58 (m, 1H), 2.96 (a of ab, 1H, J_{ab}=17.7 Hz), 2.81 (b of ab, 1H, J_{ab}=18.0 Hz), 2.37–1.64 (m, 8H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.1, 172.8, 161.6, 143.7, 137.7, 128.6, 128.3, 127.3, 123.3, 66.6, 64.1, 57.4, 45.8, 38.8, 36.7, 29.1, 25.2, 22.4; IR (neat/NaCl) 3276 br, 2953, 2882, 1694, 1659, 1518, 1462, 1335, 1258, 1075, 1047, 913 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 370 (MH^+, 100), 338 (M^+-CH_3O, 10), 259 ($MH^+-C_5H_5NO_2$, 26), 227 ($M^+-C_6H_8NO_3$, 13), 185 (22), 154 (16), 93 (43); HRFAB calcd for $C_{20}H_{24}N_3O_4$ (M+1) 370.1767, found 370.1749.

(6*R*,12*S*)-1-Aza-3-(pyroglutamylamino)-6-formyl-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene. To a solution of 0.281 g (0.761 mmol) of **22** in 2 mL of dichloromethane was added 0.134 g (1.14 mmol, 1.5 equiv.) of NMO, 0.38 g of ground and dried 4 Å molecular sieves, and 0.0214 g (0.0609 mmol, 8 mol%) of TPAP. The mixture was stirred for 3 h and then diluted with 10 mL of dichloromethane. The suspension was passed through a funnel packed with glass wool and the filtrate was concentrated. The crude product was chromatographed through 40 g of silica gel using ether/dichloromethane/methanol (50:35:15) as the eluant to afford 0.129 g (46%) of product and acetal byproduct (formed in the column). The acetal compound could be converted back to the desired aldehyde by treatment with Amberlyst 15 resin in acetone and water. This oxidation reaction also resulted in 0.0521 g (19%) of recovered starting material. ^{1}H NMR (300 MHz, $CDCl_3$) A mixture of rotamers was observed. δ 9.84 (s, 1H), 8.42 (s, 1H), 7.37–7.27 (m, 6H), 4.09–4.00 (m, 1H), 3.86–3.71 (m, 2H), 3.33 (d, 0.7H, *J*=17.6 Hz), 3.24 (d, 0.3H, *J*=17.1 Hz), 3.04 (d, 0.3H, *J*=17.5 Hz), 2.88 (d, 0.7H, *J*=17.6 Hz), 2.42–1.67 (m, 8H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 201.5, 179.2, 170.9, 161.7, 140.7, 137.7, 128.8, 128.4, 127.1, 126.7, 124.5, 70.1, 57.4, 45.8, 36.6, 34.9, 29.1, 25.2, 22.2; IR (neat/NaCl) 3261 br, 3058, 2960, 2889, 1701, 1659, 1511, 1441, 1279, 1075, 921, 772, 723 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 368 (MH^+, 4), 307 (21), 279 (9), 154 (100); HRFAB calcd for $C_{20}H_{22}N_3O_4$ (M+1) 368.1610, found 368.1604.

(6*R*,12*S*)-1-Aza-3-(pyroglutamylamino)-6-carbomethoxy-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene (23). To a solution of 0.010 g (0.027 mmol) of the aldehyde made above in 0.8 mL of DMF was added 0.021 g (0.054 mmol, 2 equiv.) of pyridinium dichromate (PDC). The reaction mixture was stirred for 21 h. Methanol (2 mL) was then added to the reaction and the mixture stirred for 15 min. The solution was concentrated at approximately 40°C under high vacuum. The residue was then dissolved in 2 mL of dichloromethane and 2 mL of methanol, and the solution was concentrated again. The crude product was chromatographed through silica gel using ether/dichloromethane/methanol (2:1:1) as the eluant to afford 4 mg (38%) of pure product as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) A mixture of rotamers was observed. δ 8.51 (s, 1H), 7.40–7.26 (m, 6H), 4.03–3.99 (m, 1H), 3.85–3.77 (m, 4H), 3.72–3.62 (m, 1H), 3.56 (d, 0.9H, *J*=17.2 Hz), 3.45 (d, 0.1H, *J*=16.9 Hz), 2.93 (d, 0.1H, *J*=17.1 Hz), 2.81 (d, 0.9H, *J*=15.8 Hz), 2.46–2.41 (m, 1H), 2.38–1.92 (m, 6H), 1.72–1.67 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.6, 174.2, 170.1, 162.4, 142.1, 137.5, 128.6, 128.3, 127.4, 126.9, 123.9, 67.7, 57.6, 53.3, 45.3, 39.4, 37.6, 29.1, 25.1, 22.1; IR (neat/NaCl) 3255 br, 2953, 1701, 1659, 1511, 1441, 1279, 1230, 1110, 921, 766, 731 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 398 (MH^+, 27), 246 (27), 185 (100); HRFAB calcd for $C_{21}H_{24}N_3O_5$ (M+1) 398.1716, found 398.1703.

(6*R*,12*S*)-1-Aza-3-(pyroglutamylamino)-6-carboxamoyl-4-phenyl-2-oxobicyclo[4.3.0]non-3-ene (3). Ammonia gas was bubbled through a solution of 0.054 g (0.14 mmol) of **23** in 6 mL of dry methanol for 20 min at room temperature. The ammonia inlet and outlet needles were then removed from the septum, and the reaction mixture was then stirred at 40°C for 4 days. The solution was concentrated and the crude material was chromatographed through 7 g of silica gel using ether/dichloromethane/methanol (2:1:1) as the eluant to afford 0.027 g (51%) of pure product as a white solid and 0.021 g (39%) of recovered starting material. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.75 (s, 1H), 7.56 (s, 1H), 7.40–7.25 (m, 5H), 5.89 (s, 1H), 4.14–4.09 (m, 1H), 3.88–3.83 (m, 1H), 3.71–3.64 (m, 1H), 3.59 (d, 1H, *J*=16.9 Hz), 2.81 (d, 1H, *J*=17.0 Hz), 2.70–2.65 (m, 1H), 2.35–1.72 (m, 7H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.0, 175.6, 173.8, 161.6, 143.2, 136.6, 129.0, 128.5, 127.3, 127.1, 124.8, 67.6, 57.4, 46.1, 39.3, 38.8, 28.9, 25.7, 22.3; IR (neat/NaCl) 3248 br, 2974, 1680, 1511, 1434, 1328, 1272, 1103, 1019, 766, 695 cm^{-1}; LRFAB MS *m/e* (rel. intensity) 383 (MH^+, 64), 339 (MH^+-CH_2NO, 39), 246 (25), 185 (100); HRFAB calcd for $C_{20}H_{23}N_4O_4$ (M+1) 383.1719, found 383.1726.

***Z*-His(Bzl)-ψ[CN_4]-Ala-*O*Bzl (24).** To a stirred suspension of PCl_5 (458 mg, 2.2 mmol) in chloroform (8 mL), quinoline (0.496 mL, 4.4 mmol) was added at 10–15°C. The mixture was stirred for 20 min at room temperature before the crystalline dipeptide *Z*-His(Bzl)-Ala-*O*Bzl (1.081 g, 2 mmol) was added in portions with stirring. After 30 min at room temperature a benzene solution of hydrazoic acid in benzene (6 mL) was added. The reaction mixture was stirred at room temperature for 2 h before being evaporated. The crude residue was partitioned between ethyl acetate and water (20 mL of each). The organic layer was washed with 1N $KHSO_4$ (2×20 mL), 1N $NaHCO_3$ (2×20 mL), H_2O (2×20 mL), and saturated NaCl solution (20 mL). When the dried (Na_2SO_4) ethyl acetate solution was evaporated and the residue purified by flash chromatography (pure ethyl acetate as an eluent), the tetrazole derivative (323 mg, 28.5%) was isolated: mp 125–126°C; $[\alpha]_D$=−38° (*c*=0.5, MeOH); TLC R_f=0.55 (ethyl acetate), R_f=0.3 (CH_2Cl_2: acetone=10:1), R_f=0.73 (butanol: acetic acid: water=4:1:1); FABMS *m/e* 568 (MH^+) calcd for $C_{31}H_{33}N_7O_4$ 567.

HBr×His(Bzl)-ψ[CN_4]-Ala-OBzl. A solution of *Z*-His(Bzl)-ψ[CN_4]-Ala-*O*Bzl (323 mg, 0.571 mmol) in 0.5 mL acetic acid was treated with 3.9 mL of 36% HBr in acetic acid while stirring. After 20 min at room temperature, the

solution was poured into 25 mL of the mixture ether:hexane= 1:1 (precooled to −10°C) with vigorous stirring. The oily hydrobromide precipitated, and the upper phase was discarded. The oil was washed with hexane (3×20 mL) and dried in high vacuo over KOH to give the dipeptide HBr salt (256 mg, 87%) as a very hygroscopic glass. The material was carried on without further characterization.

***Z*-Pyr-His(Bzl)-ψ[CN_4]-Ala-OBzl.** A solution of 145 mg (0.55 mmol) of *Z*-Pyr-OH in 8 mL of CH_2Cl_2 was cooled to −20°C and treated with 60 μL (0.55 mmol) of *N*-Methylmorpholine, followed by 74 μL (0.55 mmol) of isobutylchloroformate. After stirring for 10 min, solid HBr× His(Bzl)-ψ[CN_4]-Ala-*O*Bzl (256 mg, 49.8 mmol) was introduced, followed by the addition of *N*-methyl-morpholine (55 μL, 0.5 mmol) at a rate such that the temperature stayed below −10°C. After being stirred for 1 h at −10°C, the mixture was warmed slowly to room temperature and left with stirring overnight. The solvent was removed in vacuo. The crude residue was taken up in ethyl acetate (30 mL) and washed with 1N $KHSO_4$ (3×15 mL), 1N $NaHCO_3$ (3× 15 mL), water (2×15 mL), and saturated NaCl solution (20 mL). The ethyl acetate solution, dried over anhydrous Na_2SO_4, was evaporated to dryness. The crude crystalline material was washed with ether and filter off serving 230 mg (67.8%) of pure product: mp 96–97°C; HPLC purity 97%, t_R=13.5 min. The HPLC purification was run in 25 min linear gradient mode (30–70% solvent B in A^{22}); R_f=0.54 (ethyl acetate:MeOH=20:1), R_f=0.45 (CH_2Cl_2:acetone= 15:1), R_f=0.56 ($CHCl_3$:MeOH=10:1); FABMS *m/e* 679 (MH^+) calcd for $C_{36}H_{38}N_8O_6$ 678.

Pyr-His(Bzl)-ψ[CN_4]-Ala-OH (26). *Z*-Pyr-His(Bzl)-ψ[CN_4]-Ala-*O*Bzl (150 mg, 0.22 mmol) in methanol (15 mL) was hydrogenated in the presence of 10% palladized charcoal (40 mg) at a pressure of 4–5 kg/cm^2 on a Parr apparatus for 3 h (monitored by TLC). After evaporation of the filtered solution, the residual glassy solid was washed few times with hexane. Yield 86 mg (85%); R_f=0.48 (butanol:acetic acid:water=4:1:1); HPLC purity 98%, t_R=9.86 min (gradient 5–35% B in A^{22} in 25 min); FABMS *m/e* 455 (MH^+) calcd for $C_{21}H_{26}N_8O_4$ 454.

Pyr-His(Bzl)-ψ[CN_4]-Ala-NH_2. To the solution of Pyr-His(Bzl)-ψ[CN_4]-Ala-OH (57 mg, 0.126 mmol) in 1 mL of CH_2Cl_2 and 1.5 mL DMF, ammonium salt of HOBt was added followed by DCC (28.5 mg, 0.138 mmol). The reaction mixture was left with stirring overnight. Solvents were removed in vacuo and the crude material has been purified on preparative HPLC (gradient 5–35%B) giving 36 mg (63%) of the desired product. HPLC purity 97%, t_R=8.06 min (gradient 5–35% B in A^{22} in 25 min); FABMS *m/e* 454 calcd for $C_{21}H_{27}N_9O_3$ 453.

Pyr-His-ψ[CN_4]-Ala-NH_2 (4). Pyr-His(Bzl)-ψ[CN_4]-Ala-NH_2 (21 mg, 0.046 mmol) in MeOH (3 mL) and acetic acid (0.5 mL) was hydrogenated in the presence of 10% palladized charcoal (10 mg) at a pressure of 4–5 kg/cm^2 on a Parr apparatus for 48 h (monitored by TLC). After evaporation of the filtered solution, the residual crude product was purified on preparative HPLC (gradient 5–35% B in A^{22}) giving 15 mg of the desired analog. HPLC purity 98%, t_R=6.12 min (gradient 5–35% B in A in 25 min); FABMS *m/e* 364 (MH^+) calcd for $C_{14}H_{21}N_9O_3$ 363.

Acknowledgements

We thank the National Institutes of Health (RO1 GM5324001 and R01 DK43036) for their generous financial support. In addition, we thank the Washington University High Resolution NMR Facility, partially supported by NIH grants RR02004, RR05018, and RR07155, and the Washington University Mass Spectrometry Resource Center, partially supported by NIH RR00954, for their assistance.

References

1. For reviews on the biology of TRH see: Thyrotropin-Releasing Hormone: Biomedical Significance; Metcalf Olson, G. L., Cheung, H. C., Voss, M. E., Hill, D. E., Kahn, M., Madison, V. S., Cook, C. M., Sepinwall, J., Eds.; *Proceedings of the Biotech USA 89 Meeting*, San Francisco, October 1989.

2. For recent examples see: (a) Olson, G. L.; Cheung, H.-C.; Chaing, E.; Madison, V. S.; Sepinwall, J.; Vincent, G. P.; Winokur, A.; Gary, K. A. *J. Med. Chem.* **1995**, *38*, 2866. (b) Coa, J.; O'Donnell, D.; Vu, H.; Payza, K.; Pou, C.; Godbout, C.; Jakob, A.; Pelletier, M.; Lembo, P.; Ahmad, S.; Walker, P. *J. Bio. Chem.* **1998**, *273*, 32281, and references therein.

3. For approaches to understanding TRH/TRH-R_1 binding see: (a) Hinkle, P. M.; Woroch, E. L.; Tashjian, G. H. *J. Biol. Chem.* **1974**, *249*, 3085. (b) Goren, H. J.; Bauce, L. G.; Vale, W. *Mol. Pharmacol.* **1977**, *13*, 606. (c) Kamiya, K.; Takamoto, M.; Wada, Y.; Fujino, M.; Nishikawa, M. *J. Chem. Soc., Chem. Commun.* **1980**, 438. (d) Sharif, N. A.; To, Z.; Whiting, R. L. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 1306. (*E*) Vonhof, S.; Feuerstein, G. Z.; Cohen, L. A.; Labroo, V. M. *Eur. J. Pharmacol.* **1990**, *180*, 1. (f) Perlman, J. H.; Laakkonen, L. J.; Guarnieri, F.; Osman, R.; Gershengorn, M. C. *Biochemistry* **1996**, *35*, 7643. (g) Laakkonen, L. J.; Guarnieri, F.; Perlman, J. H.; Gershengorn, M. C.; Osman, R. *Biochemistry* **1996**, *35*, 7651.

4. For an overview of the approach to active site modeling utilized see: (a) Beusen, D. D.; Shands, E. F. B.; Karasek, S. F.; Marshall, G. R.; Dammkoehler, R. A. *J. Mol. Struct. (Theochem)* **1996**, *370*, 157. (b) Buesen, D. D.; Shands, E. F. B. *Drug Discovery Today* **1996**, *1* (10), 429. (c) Dammkoehler, R. A.; Karasek, S. F.; Shands, E. F. B.; Marshall, G. R. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 491. (d) Dammkoehler, R. A.; Karasek, S. F.; Shands, E. F. B.; Marshall, G. R. *J. Comput.-Aided Mol. Des.* **1989**, *3*, 3.

5. For applications of the active analog approach to TRH see: (a) Marshall, G. R.; Gorin, F. A.; Moore, M. L. In *Annual Reports in Medicinal Chemistry*; Clarke, F. H., Ed.; Academic: New York, 1978; Vol. 13, p 227 and references therein. (b) Font, J. Ph.D. thesis, Washington University in St. Louis, 1986.

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

7. (a) Li, W.; Moeller, K. D. *J. Am. Chem. Soc.* **1996**, *118*, 10106. (b) Laakkonen, L.; Li, W.; Perlman, J. H.; Guarnieri, F.; Osman, R.; Moeller, K. D.; Gershengorn, M. C. *Mol. Pharmacol.* **1996**, *49*, 1092.

8. Chu, W.; Perlman, J. H.; Gershengorn, M. C.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3093.

9. Richardson, J.; Richardson, D. C. *Prediction of Protein*

Structure and Principles of Protein Conformation; Fasman, G., Ed.; Plenum: New York, 1989.
10. Liakopoulou-Kyriakides, M.; Galardy, R. E. *Biochemistry* **1979**, *18*, 1952.
11. (a) Marshall, G. R.; Humblet, C.,; Opdenbosch, N. V.; Zabrocki, J. In *Proceedings of the 7th American Peptide Symposium*, Rich, D. H., Gross, E., Eds.; Pierce Chemical Company: Rockford IL; 1989; p 669. (b) Zabrocki, J.; Dunbar, Jr., J. B.; Marshall, K. W.; Toth, M. V.; Marshall, G. R. *J. Org. Chem.* **1992**, *57*, 502.
12. For reviews of TRH analog activity data see: (a) Vale, W.; Rivier, C. *Handbook of Psychopharmacology*; Iverson, L. I. et al., Eds.; Plenum: New York, 1975; Vol. 5, 195. (b) Goren, H. J.; Bauce, L. G.; Vale, W. *Mol. Pharmacol.* **1977**, *13*, 606.
13. Seebach, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390.
14. Gramberg, D.; Robinson, J. A. *Tetrahedron Lett.* **1994**, *35*, 861.
15. Dumas, J.-P.; Germanas, J. P. *Tetrahedron Lett.* **1994**, *35*, 1493.
16. For the initial paper describing the application of RCM to a peptidomimetic see: (a) Miller, S. J.; Grubbs, R. H. *J. Am. Chem. Soc.* **1995**, *117*, 5855. For reviews of RCM reactions see: (b) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446 and (c) Blechert, S.; Schuster, M. *Angew. Chem., Int. Ed.* **1997**, *36*, 2036. For a route to fused nitrogen heterocycles see: (d) Martin, S. F.; Liao, Y.; Chen, H. J.; Pätzel, M.; Ramser, M. N. *Tetrahedron Lett.* **1994**, *35*, 6005.
17. (a) Curran, D. P.; Gu, X.; Zhang, W.; Dowd, P. *Tetrahedron* **1997**, *53*, 9023. (b) Molander, G. A.; Harris, C. R. *J. Am. Chem. Soc.* **1995**, *117*, 3705.
18. Lipshutz, B. H.; Sengupta, S. *Org. React. (NY)* **1992**, *41*, 135.
19. Li, W.; Hanau, C. E.; d'Avignon, A.; Moeller, K. D. *J. Org. Chem.* **1995**, *60*, 8155.
20. For published procedures see: (a) Perlman, J. H.; Thaw, C. N.; Laakkonen, L.; Bowers, C. Y.; Osman, R.; Gershengorn, M. C. *J. Biol. Chem.* **1994**, *269*, 1610. (b) Straub, R. E.; Frech, G. C.; Joho, R. H.; Gershengorn, M. C. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9514.
21. Zouikri, M.; Vicherat, A.; Aubry, A.; Marraud, M.; Boussard, G. *J. Peptide Res.* **1998**, *52*, 19.
22. HPLC has been performed using solvents system: A: 0.5% TFA in water; B: 0.038% TFA, 90% acetonitrile, 10% water.