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THYROTROPIN RELEASING HORMONE ANALOGS: A BUILDING BLOCK APPROACH TO THE CONSTRUCTION OF TETRACYCLIC PEPTIDOMIMETICS

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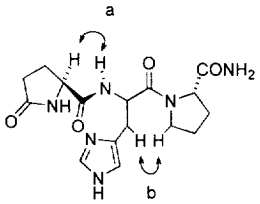
Abstract: A building block based approach was used to synthesize a pair of tetracyclic peptidomimetics that constrain all but one of the rotational degrees of freedom of the hypothalamic tripeptide hormone thyroliberin. One of the analogs bound to the thyroliberin endocrine receptor (TRH-R) with an affinity greater than that of an analog without constraints. The tetracyclic peptidomimetics were found to be partial agonists for the TRH-R receptor.

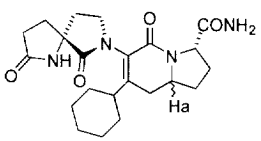
Thyroliberin (TRH) is the hypothalamic tripeptide that controls the release of thyroid stimulating hormone from the pituitary gland.[1] In addition to this endocrine activity, TRH has been found to have profound CNS effects.[2] Because of its biological relevance and the fact that TRH has only six rotatable bonds, TRH is an ideal model system for testing the validity of computer-based methods for predicting the receptor bound conformation of peptides. For example, Marshall and Font used a molecular modeling approach to identify conformations common to all of the active analogs of TRH.[3,4] This information was used to suggest a pair of potential conformations for the binding of TRH to its endocrine receptor TRH-R.[5] One of these suggested conformations is illustrated by structure **1**. This particular conformation is intriguing because of its similarity to the receptor bound conformation of TRH proposed by Gershengorn and coworkers using receptor mutants,[6] but does it really represent a receptor bound conformation of TRH? We report herein the synthesis and initial biological testing of a pair of "fully constrained" peptidomimetics (**2a,b**) designed to probe this question.

In order to evaluate the biological relevance of the TRH conformation represented in structure **1**, Marshall and Font proposed the synthesis of a series of lactam-based analogs for TRH.[5b,7] These analogs were designed by replacing spatially close hydrogens in **1** with carbon bridges,[8] as exemplified by **2a** and **2b**. While initial modeling studies could not differentiate the binding potential of **2a** and **2b**, recent work using partially constrained TRH analogs has refined the conformational model of TRH to a point where **2a** was expected to bind much more tightly to

Figure 1



1. Thyroliberin (TRH)



2a. Ha = β
2b. Ha = α

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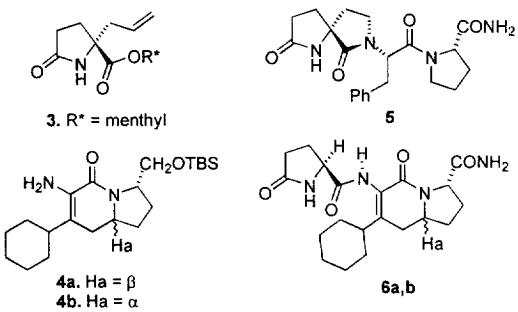
TRH-R than **2b**.[9] In addition to the added constraints, two additional changes were proposed for **2a** and **2b**. These changes were made in order to simplify the synthesis of the analogs. First, a double bond was placed into the six-membered ring lactam. This change removed the need to control the stereochemistry of two additional stereogenic centers in the bicyclic skeleton. Second, the imidazole ring was replaced by a cyclohexyl group. This change removed from the synthesis potential complications caused by the reactivity of the imidazole ring. While the change from the imidazole to a cyclohexyl group was known to reduce the binding and potency of a TRH analog by a factor of approximately 10^2 to 10^3,[9] cyclohexylAla^2-TRH does completely displace TRH from TRH-R and is still a full agonist for the receptor.

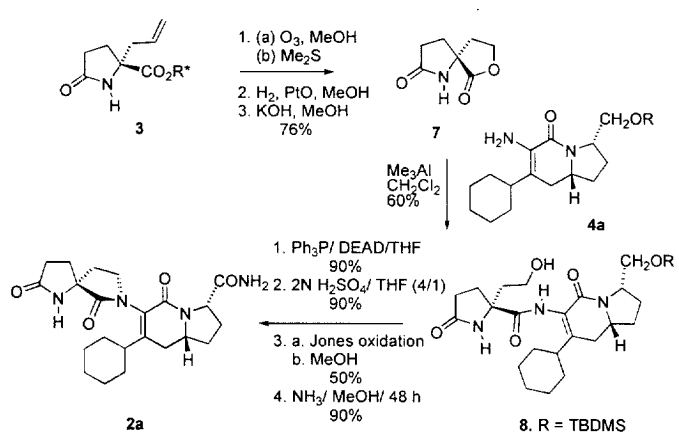
Even with the changes made to simplify the synthesis of analogs **2a** and **2b**, they proved to be very difficult to synthesize.[5b] For this reason we undertook an effort to develop new synthetic methodology for converting readily available amino acid derivatives into lactam based peptidomimetics.[10] This work was initially directed toward the construction of building blocks **3**,[11a] **4a**,[9] and **4b**,[9] which were then used to construct the partially restricted TRH analogs **5**,[11b] and **6a**,[9] and **6b**.[9] The partially constrained analogs were synthesized in order to evaluate how the individual constraints to be used in **2a** and **2b** affected the ability of the analogs to bind and activate TRH-R. In both cases, the added constraints proved to be compatible with both the binding and activation of TRH-R.

Figure 2



With the synthesis of the building blocks complete, attention was focused on the synthesis of **2a** and **2b.** The key was to find a strategy that would allow for both the coupling of building block **3** to **4a** and **4b** and the generation of a spirocyclic lactam constraint for the pyroglutamate moiety without disturbing the potentially sensitive enamine group in building blocks **4a** and **4b**. This was a worrisome challenge since the previous route to **5** utilized a reductive amination reaction that was not compatible with the presence of the enamine group. Fortunately, any problems with the enamine were avoided with the approach outlined in Scheme 1 for the construction of **2a**. In this scheme, the olefin in **3** was cleaved with an ozonolysis reaction, the resulting aldehyde reduced to

Scheme 1



an alcohol, and the alcohol cyclized to form a very stable spirocyclic lactone. The lactone was then opened with **4a** and trimethylaluminum in order to form the alcohol amide **8**.[12] Compound **8** was cyclized to form the desired spirocyclic lactam derivative using standard Mitsunobu conditions.[13] At this point, the synthesis of the tetracyclic ring skeleton was complete. The fully restricted TRH analog **2a** was then completed by converting the TBDMS ether to a primary amide as in the earlier syntheses of TRH analogs **6a** and **6b**. The fully restricted TRH analog with the opposite bridgehead stereochemistry (**2b**) was synthesized in similar yield using an identical approach. In this case, building block **4b** was utilized in the trimethylaluminum reaction.

Once synthesized the analogs were examined for their ability to both bind and activate TRH-R using AtT-20 mouse pituitary tumor cells stably expressing TRH receptors (Table 1).[14] Both **2a** and **2b** were found to bind

Table 1 Binding and Activation of TRH-R Receptors by TRH analogs **2a** and **2b**

Analog	K_i (nM)[a]	EC_{50} (nM)[b]
TRH	23 (6–90)[c]	0.49 (0.32–0.74)[c]
CyclohexylAla^2-TRH[d]	6,500 (5,400–7,900)[c]	430 (380–480)[c]
Analog **2a**	1,500 (700–2,900)[c]	1,800[e] (900–3,600)[c]
Analog **2b**	36,000 (16,000–83,000)[c]	18,000[e] (3,900–81,000)[c]

Experiments were performed with intact AtT-20 mouse pituitary tumor cells stably expressing TRH receptors.

a. 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.
b. For activation, cells prelabelled with *myo*-[^{3}H]inositol 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.
c. 95% confidence intervals.
d. Data taken from ref 8.
e. Analogs **2a** and **2b** were partial agonists of TRH-R. **2a** reached a maximum potency of 47% (42–51%)[c] of TRH, while **2b** reached a maximum potency of only 28% (20–36%)[c] that of TRH.

to TRH-R. As expected, analog **2a** having an *R*-configuration at the bridgehead bound to TRH-R with an affinity 24 times greater than that obtained with **2b**. This result was consistent with the data for earlier experiments using analogs **6a** and **6b**.[9] Analog **2a** also had an affinity for TRH-R that was approximately four times greater than that observed with the unrestricted analog (cyclohexylAla^2-TRH). Clearly, the bridges in **2a** enhanced binding of the analog to TRH-R. Since the fully resticted analog **2a** was fixed into a conformation consistent with the TRH conformation represented by structure **1**, *this observation suggested that the conformation of TRH illustrated in 1 does represent a conformation of TRH capable of binding to TRH-R*. The active analog approach utilized by Marshall and coworkers successfully predicted a conformation of TRH consistent with TRH/TRH-R binding.

Finally, analogs **2a** and **2b** proved to be a partial agonists of TRH-R. Even at maximally effective concentrations, stimulation by **2a** and **2b** only reached levels of second messenger formation that were 47% and 28%, respectively, of that stimulated by the native hormone, TRH. To our knowledge, these fully restricted

analogs are the first partial agonists found for TRH-R (the unrestricted Cyclohexylala^2-TRH is a full agonist); an observation that suggests that it is possible to develop antagonists for TRH-R. Efforts to develop TRH-R antagonists and to explore methods of restoring full agonist behaviour to a fully restricted TRH analog are underway.

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