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Communications to the Editor

Conformationally Restricted TRH Analogs: A Probe for the Pyroglutamate Region

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In principle, the development of the active analog approach to computer-assisted drug design can offer a unique opportunity for dramatically increasing the efficiency of designing new drug candidates by predicting the biologically active conformation of peptide ligands.[1] But how do we know that such molecular modeling predictions are accurate, and can a specific set of chemical probes be developed for answering this question? In the mid-1980s, Marshall and Font initiated an inquiry into this area by using the active analog approach to design a series of conformationally restricted thyrotropin-releasing hormone (thyroliberin, TRH) analogs represented by the general structure of **III** (Scheme 1).[2] From the start, it was realized that TRH would make an ideal test case for probing the effectiveness of the active analog approach because of the important role of TRH in regulating functions of the anterior pituitary gland and the nervous system,[3] the fact that TRH contains only six-rotatable bonds, and the existence of activity data for a large number of TRH analogs.[4,5] Due to the increased difficulty associated with the synthesis of imidazole-containing peptide analogs, the initial restricted analogs were designed to mimic the [Phe^2]TRH analog of TRH. Although the affinity and potency of this analog were significantly lower than TRH itself (see Table 1), at high concentrations [Phe^2]TRH completely displaced TRH from its G protein-coupled receptor (TRH-R) and led to the same maximal extent of TRH-R stimulation as TRH. For this reason, [Phe^2]TRH was judged to be a reasonable model for TRH, at least until it was determined if an added conformational constraint would still allow for binding of the analog to TRH-R.

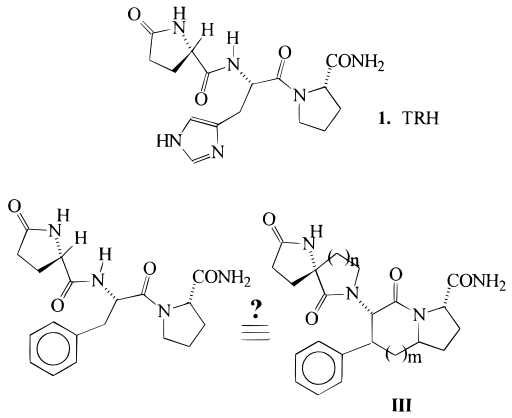
The constrained analogs were designed by replacing

Table 1. Binding and Activation of TRH-R Receptors by TRH Analogs

analog	K_i (nM)[a]	EC_{50} (nM)[b]
[N$^{\tau}$Me-His]TRH	1.2 (0.92−1.5)[c]	not determined
TRH	10 (ref. 9b)	0.68 (0.51−0.89)[c]
[Phe^2]TRH	1500 (1200−1900)[c]	110 (96−120)[c]
analog **3**	4400 (3600−5400)[c]	290 (260−330)[c]

[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 unlabeled TRH analogs for 1 h at 37 °C. The data are means of duplicate determinations in two or three experiments. [b] For activation, cells prelabeled 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. Experiments were performed with intact AtT-20 mouse pituitary tumor cells stably expressing TRH receptors.

Scheme 1



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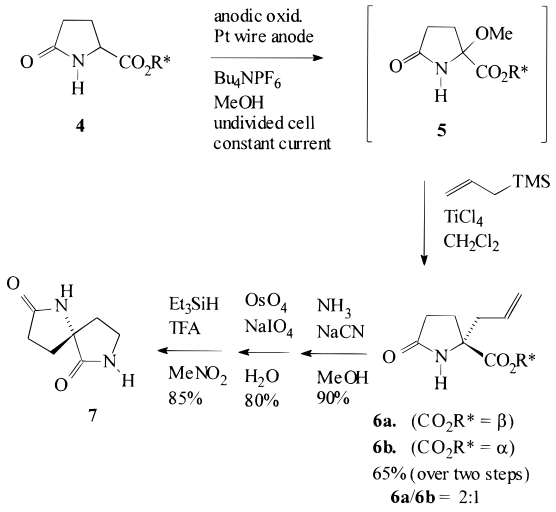
Scheme 2

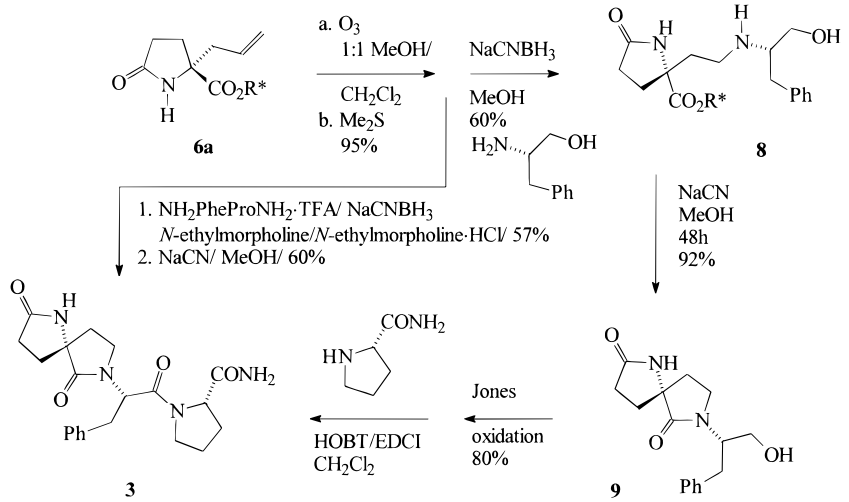


spatially close hydrogens in the conformation predicted to be active with carbon bridges (Scheme 1). This transformation had the net effect of imbedding the peptide backbone into a lactam-based framework.[6] If an analog were unable to bind or activate TRH-R, then one of two conclusions could be reached. Either the predicted conformation was wrong or the bridge itself interfered with binding or activation. In this case, the chemical probe would fail in its attempt to answer questions concerning the validity of the molecular-modeling predictions and a different conformational constraint would need to be identified. However, if an analog were found to be a potent agonist for the receptor site, then we would know that neither the bridge nor the locked conformation interfered with binding or activation to a significant extent, and therefore, the analog would be useful for making inferences regarding the active conformation of TRH. We herein report that the use of a two-carbon bridge to restrict the pyroglutamate region of [Phe^2]TRH (analog **3**) does not interfere with the binding, potency, or efficacy of the analog.

We reported the synthesis of a spirocyclic building block for use in the construction of conformationally restricted pyroglutamate analogs (Scheme 2).[7] The key to this synthesis was the electrochemical functionalization and subsequent alkylation of pyroglutamate derivative **4** to form **6a**.[8] Although we had initially intended to use the final building block **7** in order to construct TRH analogs, it became clear that a simpler approach might involve treating **6a** directly with the primary amine of an amino acid. Alternatively, the double bond of **6a** could be cleaved to an aldehyde, an amino acid derivative introduced by reductive amination, and then the molecule cyclized to form the spirocyclic ring system. The later approach proved to be most effective (Scheme 3). Two variations of this route were explored. In the first, the reductive amination step was used to introduce the amino alcohol derived from (−)-phenylalanine. The amino alcohol was used instead of the amino acid because it simplified isolation of the product. Following a sodium cyanide-catalyzed cyclization to form the spirocyclic ring, the alcohol **9** was oxidized to the acid and then the acid coupled to prolinamide to form the desired TRH analog **3**. The last step was not optimized due to the success of the second route. In this approach, the aldehyde obtained from ozonolysis of **6a** was treated directly with the desired dipeptide during the reductive amination step. The resulting product was then cyclized with the use of sodium cyanide as a catalyst. In this way, the desired conformationally restricted TRH analog could be synthesized from pyroglutamic acid in just six steps. Both routes have advantages. Although the second route is much faster, it relies on the fact that the C-terminus of TRH is an amide. Esters are not compatible with the cyclization reaction. Therefore, the short route that is so effective here is most likely not generally applicable to a wide variety of peptide analogs. The amino-alcohol route should be more general.

TRH analog **3** was tested for its ability to activate TRH-R and was compared to the unrestricted [Phe^2]-TRH (Table 1). 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.[9] The data revealed that the affinity and potency of restricted analog **3** were only 3-fold lower than that of the unrestricted [Phe^2]-TRH. The maximal extents of stimulation of IP formation were similar for TRH, [Phe^2]TRH, and **3**. A comparison between **3** and [Phe^2]TRH indicated that the added bridge in **3** did not seriously interfere with either binding or activation. Since the pyroglutamate carbonyl

Scheme 3



is critical for high-affinity binding to TRH-R (substitution with CH_2 results in a 100000-fold decrease in affinity for TRH),[9a] it is tempting to suggest that the restricted molecule **3** was still able to adopt a conformation that maintained this contact point with the recepter along with the other contact sites required for high-affinity binding.[3] However, the low affinities for both [Phe^2]TRH and **3** for TRH-R relative to TRH itself render such a conclusion premature. Work aimed at verifying these conclusions by constructing the conformationally restricted TRH analog analogous to **3** (i.e., His instead of Phe) is underway.

In summary, we have shown that restricting the pyroglutamate region of [Phe^2]TRH with a spirocyclic peptide analog does not stop the molecule from binding and activating TRH-R. This result indicated that the overall approach of using an electrolysis reaction to introduce bridges into the pyroglutamate region of TRH analogs can lead to compounds that bind and activate TRH-R and suggests that this approach may provide a means for "mapping" the conformational requirements of the TRH-R receptor. Many questions remain. In addition to the question raised above concerning the relationship between the results obtained using analogs of [Phe^2]TRH and results from similar studies using analogs of TRH itself, questions concerning the reasons for the 3-fold loss in affinity and potency and the effect of additional bridges constraining the HisPro region of TRH need to be addressed.

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Supporting Information Available: Data for the binding of TRH-R by TRH analogs, data for the activation of TRH-R by TRH analogs, and characterization data (^{1}H NMR, ^{13}C NMR, 2D-NMR experiments, LRMS, and HRMS) for analog **3** (10 pages). See any current masthead page for ordering information.

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