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DESIGN AND SYNTHESIS OF HYPERTREHALOSEMIC HORMONE MIMETICS

Michito Sato[†,§], Michael Kahn[†,§,*], ZhaoLan Lin[¶], Michael E. Johnson[¶,*] and Timothy K. Hayes[‡]
[†]Department of Chemistry, [¶]Center for Pharmaceutical Biotechnology and [¶]Department of Medicinal Chemistry & Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60680, and [‡]Laboratories for Invertebrate Neuroendocrine Research, Department of Entomology, Institute for Biosciences and Technology, Texas Agricultural Experiment Station, Texas A&M University, College Station, TX 77843

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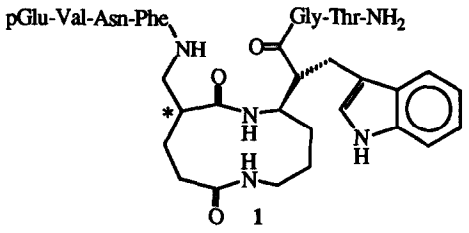
Abstract: Hypertrehalosemic hormone (HTH) is an insect neurohormone isolated from the tropical cockroach, *Blaberus discoidalis*. To examine the hypothesis that the bioactive conformation of HTH contains a β-turn, we have designed, synthesized and evaluated a first generation HTH analog incorporating a conformationally restricted β-turn mimetic. Conformational analysis and biological evaluation indicates that an isomer closely approximating a type II β-turn exhibits limited biological activity, while isomers with extended conformations exhibit no activity, providing evidence for the turn hypothesis.

Hypertrehalosemic hormone (HTH) is a blocked neutral decapeptide (pGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr-NH_2), isolated by Hayes et al. from the tropical cockroach,[1] that exerts control over energy metabolism by regulating hemolymph trehalose. Based upon the results of bioassays with HTH analogs and correlation with bend frequency data, Hayes et al. proposed that the bioactive conformation of HTH includes a β-turn spanning residues Ser5-Trp8.[2,3] To examine this hypothesis, we have synthesized conformationally restricted HTH analogs. The design, synthesis and evaluation of the first generation analog **1** is described here.



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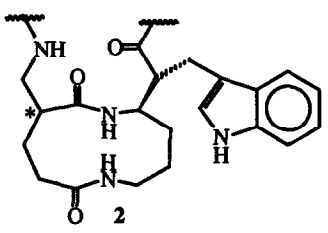

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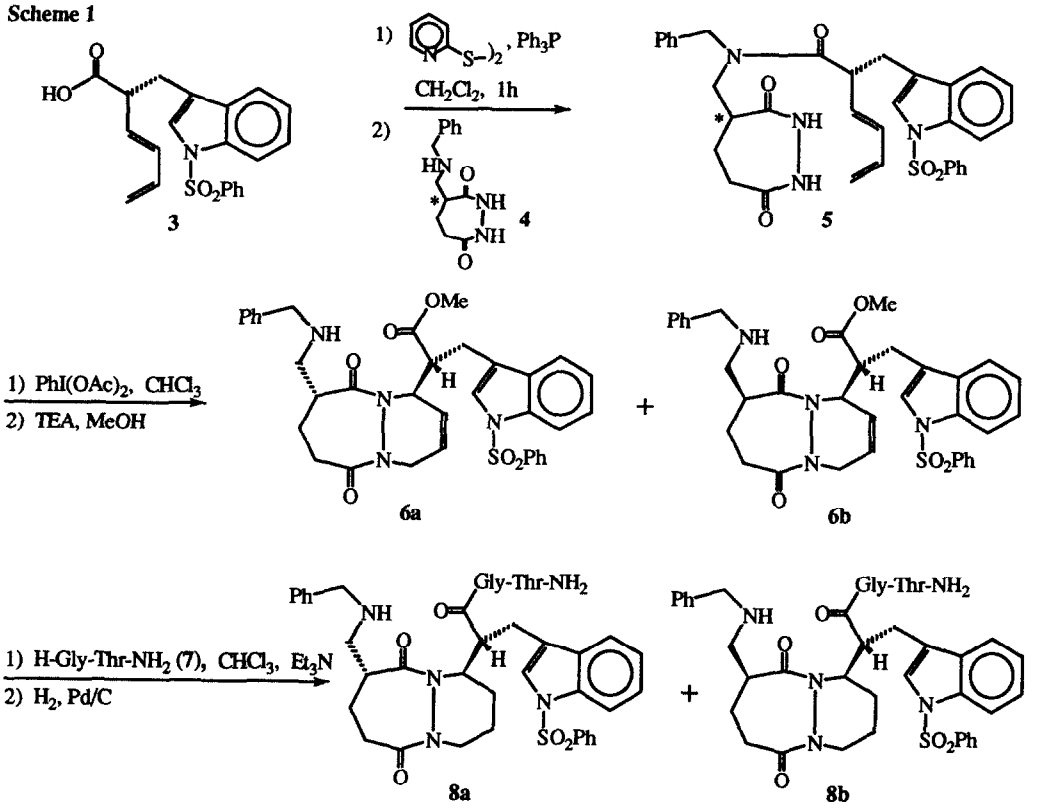
[§]Present Addresses: M.S., Lion Akzo Co. Ltd., No. 13-12, 7-Chome, Hirai, Edogawa-Ku, Tokyo 132, Japan; M.K., Department of Pathobiology, SC38, University of Washington, Seattle, WA 98195.

Synthesis. Based on the proposed location of the β-turn, we have replaced residues 5-8 with our previously described 11-membered ring β-turn mimetic system **2**.[4,5] As Trp[8] has been identified as being critical for bioactivity, the 3-indolylmethyl group was attached at the carbon corresponding to the C_α of residue 8. Synthesis of the requisite β-turn mimetic system **2** is outlined in Scheme 1. Coupling[6] of the previously described diene acid **3**[5], which was prepared enantiospecifically using the Evans oxazolidone protocol,[7] and diacyl hydrazide **4**,[5] provided the diene amide **5** in 65% yield. Intramolecular oxidative cyclization[4,8] in the presence of iodobenzene diacetate provided **6a** and **6b** as a 50:50 diastereomeric mixture. The bulky (1-benzenesulfonylindolyl)-3-yl-methyl group generates enantiospecific cyclization to form the S-epimer at the new chiral center, giving only the two diastereomers **6a** and **6b**, out of the four possible diastereomers. The two diastereomers were separated by flash chromatography. Subsequent reaction with dipeptide **7** and hydrogenation provided **8a** and **8b**. Completion of synthesis on the separated diastereomers is outlined in Scheme 2. **1a** and

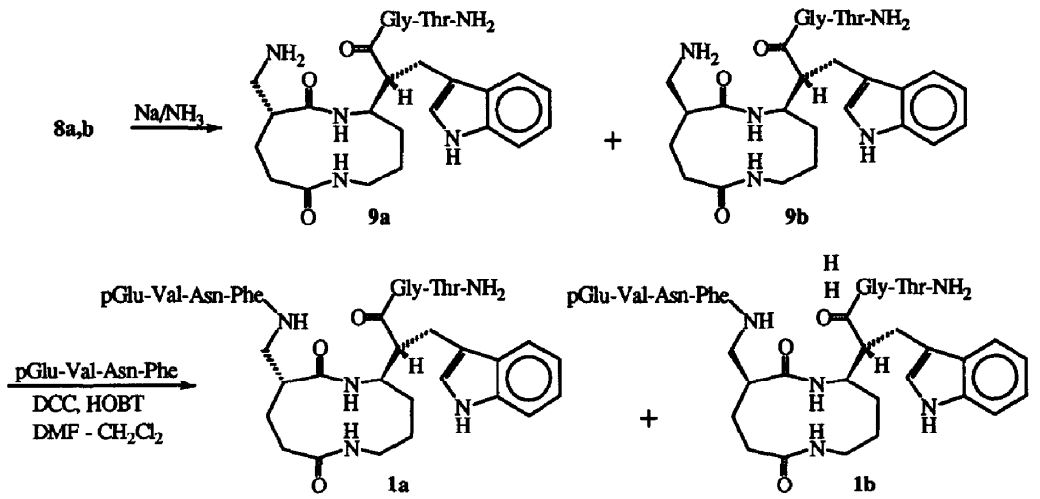
Scheme 1



1b were characterized using NMR (400 MHz) and MS.

Structural and Conformational Analysis. The separation of the ^1H chemical shift of the diastereotopic benzyl protons of **6a** ($\Delta\delta \sim 0.4$ ppm) suggested that they reside in significantly different chemical environments.

Scheme 2



In contrast, the difference in the ^1H chemical shifts of these protons in **6b** is quite small ($\Delta\delta < 0.1$ ppm) as shown in Fig. 1. This suggested that in **6a**, movement of the mimetic chain extension with the phenyl group is restricted, whereas the chain extension is mobile in **6b**.

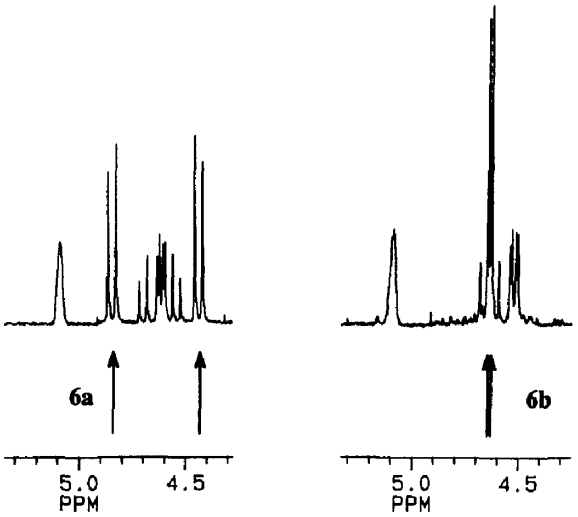


Fig. 1. 400 MHz ^1H NMR spectra, showing the diastereotopic benzyl protons for **6a** and **6b**.

The stereochemistry of **6a,b** was assigned through molecular dynamics simulation of these compounds using the Macromodel program[9], which showed that the R,S,R diastereomer exhibited a stable H-bond between the ring carbonyl and the amide hydrogen of the peptide extension, restricting diastereotopic proton rotational

mobility, whereas the R,S,S diastereomer exhibited an unstable H-bond. Based on this analysis, we have assigned **6a** as being the R,S,R diastereomer, and **6b** as being the R,S,S diastereomer. Conformational analysis of the final mimetic ring structures in **1a** and **1b** indicates that the low energy conformer for **1b** closely approximates the anticipated type II β-turn, with a dihedral angle of 5° for the chain extension bonds, compared to 15° for an idealized type II β-turn. In contrast, the dihedral angle for **1a** is approximately 119°, providing very poor geometry for a reverse turn. Fig. 2 shows the overlay of **1b** with an idealized β-II turn formed from the sequence Ser-Pro-Gly-Trp, demonstrating the close conformational mimicry.

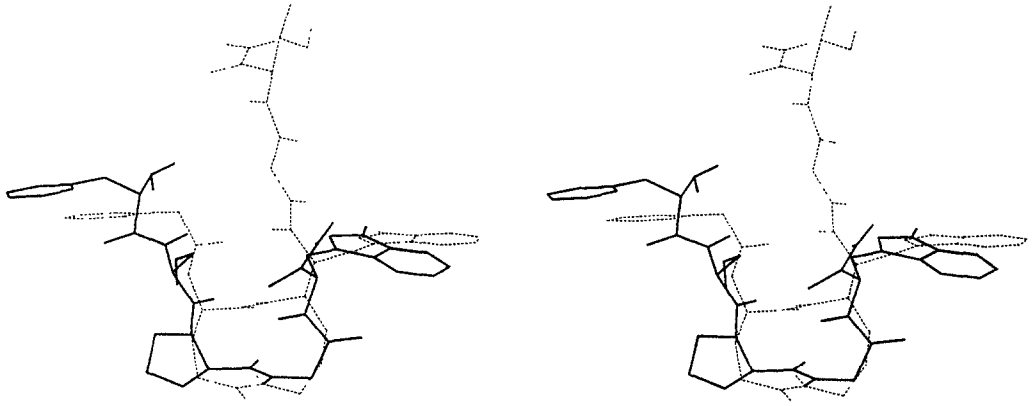


Fig. 2. Overlay of low energy conformer for **1b** (dashed line) with an ideal type II β-turn (solid line) with sequence Ser-Pro-Gly-Trp. The six-atom rms deviation between the mimetic and the peptide is approximately 0.64 Å.

Biological Activity. Fig. 3 indicates that **1b** has limited, but significant hyperglycemic activity in the bioassay system.[10] The observed response doubled and was significantly different from the response of either the Ringer control or the R diastereomer. Doubling the standard error range of **1a** failed to overlap with the mean response or double the standard error range of the negative control or of **1b**. However, the response at this high dose (10 nmole) of **1b** was only about one third that of HTH at 10 pmole. **1b** is structurally more related to the [Gly^5]-HTH analog which also lacks a hydroxyl side chain at position 5, and has a lowered maximum activity.[3] That analog has a maximum response approximately two thirds that of HTH. Thus, some of the lower activity observed for **1b** relative to HTH would be expected

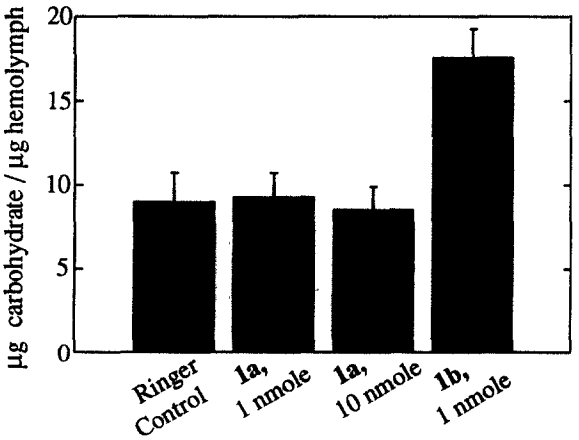


Fig. 3. Assay results for **1a** and **1b**.

because of its similarity to [Gly^5]-HTH. Neither mimetic isomer (at an injected dose of 10 nmoles) would act as an antagonist to HTH (injected at 35 pmole). The unnatural relative alignment of the N- and C-terminal peptide fragments for **1a** is a likely reason for the observed inactivity of this analog. The detection of activity for **1b**, containing the correct turn mimetic, is consistent with the proposal of a turn in the receptor recognized conformation of HTH.[2,3] Further studies utilizing our recently developed β-turn mimetic system[11] are in progress, and will be reported in due course.

Acknowledgements

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9. The 11-member ring moieties for **6a** and **6b**, **1a** and **1b**, with three methyl groups attached at the positions of chain attachment, were built in a computer using the MACROMODEL program [Mohamadi, F.; Richards, N.G.J.; Guida, W.C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W.C. *J. Comput. Chem.* **1990** *11*, 440, and references therein]. Monte Carlo conformational searches were carried out to find the global minimum and the distribution of the conformers by using BATCHMIN with the MM2/MACROMODEL force field by randomly rotating torsional angles in the ring systems. Conformational analyses were judged to be complete with an acceptance ratio of 50-60%, and a minimal

duplication rate of approximately 250, using a total of 1000 Monte Carlo steps, with a 50 kJ/mol energy window. For the dynamics simulations of **6**, the full structures were built, with the simulation starting from the low energy ring conformations. Stochastic molecular dynamics calculations were carried out using the BATCHMIN program for compounds **6a** and **6b** with 1 fs time steps for 1000 ps at temperatures of both 300 and 350 K in vacuo as an approximation to the $CHCl_3$ solvent in which the NMR measurements were made. Monte Carlo conformational analysis of **1a,b** followed the same procedures, but utilized the volume based continuum solvation model to simulate the relevant aqueous environment.

10. Adult male *Blaberus discoidalis* Serville cockroaches were used as bioassay specimens. Animals were cultured at 27 °C in wood shavings with a 12 hr light/12 hr dark photoperiod and provided food and water *ad libitum*. Test insects were decapitated at six days of adult age to remove the cephalic endocrine system and provide an organism that was maximally responsive to endocrine agents [Hayes, T.K.; Keeley, L.L. *General and Comparative Endocrinology* **1985** *57*, 246]. Decapitated insects were tested after 24 hours. Aliquots of hemolymph (5 µL) were withdrawn by puncture of a dorsal abdominal intersegmental fold immediately before the test injection of the sample, and two hours after the injection. Each analog was dissolved in 10 µL of Euphrussi Beadle Ringer (EBR) and injected into the abdominal hemocoel. The hemolymph was diluted with 95 µL of Ringer, vortexed and centrifuged to remove cellular components. The carbohydrate concentrations of the 0 and 2-hr hemolymph samples were determined with the anthrone method [Roe, J.H. *J. Biol. Chem.* **1955** *212*, 335]. Only 2-hr measurements are reported here. Eight replicates were run for each data point. Both negative (animals injected with EBR) and positive (animals injected with HTH) controls were used to validate the assay. The positive control injection of 35 pmole of HTH gave a response of 38.9 ± 2.8 µg carbohydrate/µL hemolymph. This controlled response ensured that the assay was functioning to detect HTH on the day of each experiment. The negative control is illustrated for comparison in Fig. 3, and was used to find baseline carbohydrate levels in the hemolymph.
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