Conformational Properties of Central Nervous System Active Thyrotropin Releasing Hormone Analogues: Probing Structure–Activity Relationships at the Molecular Level[1]

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Crystal structure determinations have been carried out for the following seven thyrotropin releasing hormone analogues: I, (3*R*,6*R*)-6-methyl-5-oxothiomorpholin-3-ylcarbonyl-L-histidinyl-L-proline amide; II, (3*R*,6*S*)-6-methyl-5-oxothiomorpholin-3-ylcarbonyl-L-histidinyl-L-proline amide; III, (4*R*)-2-oxothiazolidin-4-ylcarbonyl-D-histidinyl-L-proline amide; IV, 5-ethylorotyl-L-histidinyl-L-proline amide; V, 5-*n*-propylorotyl-L-histidinyl-L-proline amide; VI, 5-bromoorotyl-L-histidinyl-L-proline amide; and VII, Phe^2-TRH. A surprising degree of conformational similarity has been observed for the peptide backbone. All peptide bonds are found to be trans. A composite hydrogen-bonding environment has been constructed for the TRH analogue system and examined for its inference with respect to receptor binding. A comparison of the conformations of these analogues with those displayed by Leu^5-enkephalin has also been made, and unexpected similarities have been revealed.

The tripeptide amide L-pyroglutamyl-L-histidinyl-L-proline amide is commonly known as TRH (thyrotropin releasing hormone). Since the characterization[2,3] of the chemical structure of TRH in 1969, the tripeptide amide has been the subject of extensive study, ranging from clinical application to conformational analysis. The availability of synthetic TRH and the applicability of radioimmunoassay, among other techniques, have led to the recognition that TRH possesses a broad spectrum of biological activity, in addition to governing the release of thyroid stimulating hormone (TSH, thyrotropin).

The fact that TRH has been shown to be present in animals in which it cannot function as a thyrotropin releasing hormone has led to the suggestion[4] that the TSH regulation may be a recent evolutionary development in which a preexisting compound was incorporated in a new physiological process. It appears that the primitive function of TRH is that of a neural peptide. This proposal is consistent with the wide distribution of TRH in areas of the body other than the hypothalamus. These aspects of TRH biochemistry have been discussed in detail in recent review articles.[4,5]

The full spectrum of biological activity of TRH is, most likely, not yet known. In addition to its thyrotropin releasing activity, it has been reported (see ref 4) to release prolactin in some species. TRH has also been found to display complex interaction with the central nervous system (CNS). Extensive recent review articles[6-11] have discussed the effects of TRH on the behavior of animals and man and on the CNS in general. Schwertner et al.,[12] guided by a working hypothesis that the endocyclic amide group of the p-Glu residue might be the necessary and sufficient moiety of this residue for psychostimulating activity, prepared a series of TRH analogues with appropriate heterocyclic substituents replacing the ring system of pGlu. It was hoped that such derivatives would have an extended biological half life compared with TRH, would be psychostimulants with little or no TSH releasing activity, and, consequently, be of use in the treatment of affective disorders. Though these expectations have met with only partial success,[13] we felt that crystal structure determinations for a number of the peptide analogues in this series would be of value toward achieving an understanding at the molecular level of their CNS activity.

We report here the crystal structure determinations for the following seven TRH analogues (Chart I): I, (3*R*,6*R*)-6-methyl-5-oxothiomorpholin-3-ylcarbonyl-L-histidinyl-L-proline amide; II, (3*R*,6*S*)-6-methyl-5-oxothiomorpholin-3-ylcarbonyl-L-histidinyl-L-proline amide; III, (4*R*)-2-oxothiazolidin-4-ylcarbonyl-L-histidinyl-L-proline amide; IV, 5-ethylorotyl-L-histidinyl-L-proline amide; V, 5-*n*-propylorotyl-L-histidinyl-L-proline amide; VI, 5-bromoorotyl-L-histidinyl-L-proline amide; VII, Phe^2-TRH.[14] Compound VII is of interest because it represents a TRH analogue in which substitution for a different residue, histidine, has been carried out.

We have examined the crystal structures with the following questions in mind: Is there a preferred conformation? What intra- and intermolecular interactions are displayed? Can the structures be interpreted in terms of a possible receptor binding model? We have also included the results from crystal structures of two examples, VIII[15]

(1) This work was presented in part at a symposium held from August 26 to 28, 1981, in Buffalo, NY. Stezowski, J. J., Eckle, E. In "Molecular Structure and Biological Activity"; Griffin, J. F.; Duax, W. L., Eds.; Elsevier, New York, 1982, pp 243–258. The center stereoscopic pair in Figure 4, page 249, displays a computer-generated conformation displaying an intramolecular hydrogen bond between the unprotonated imidazole ring and the proline amide NH_2 moiety instead of the conformation found in the crystal structure determination.

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(6) Horita, A.; Carino, M. A.; La Hann, T. R. In ref 4; pp 65–74.

(7) Prange, Jr., A. J.; Nemeroff, C. B.; Loosen, P. T.; Bissette, G.; Ostbohr III, A.; Wilson, I. C.; Lipton, M. S. In ref 4; pp 75–96.

(8) Collu, R.; Taché, Y. In ref 4; pp 97–121.

(9) Rastogi, R. B. In ref 4; pp 123–140.

(10) Horst, W. D.; Spirt, N.; Bautz, G. In ref 4; pp 141–143.

(11) Renaud, L. P.; Pittmann, Q. J.; Blume, H. W.; Lamour, Y.; Arnauld, E.; In ref 4; pp 147–161.

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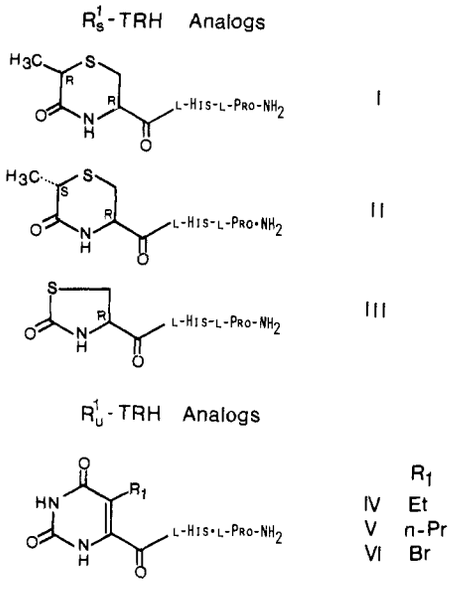
0022-2623/85/1828-0125$01.50/0

Chart I



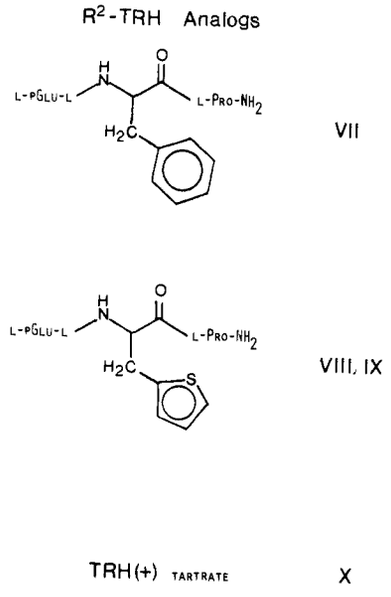
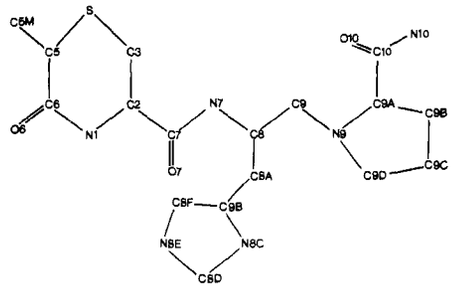


Chart II



and IX,[16] of Thi^2-TRH [Thi = β-(2-thienyl-L-alanyl] and one example for the tartrate salt of TRH, X,[17] Chart I, in our analysis. Also, we have examined the results of structure determinations for two crystal modifications of Leu^5-enkephalin, XI[18] and XII.[19]

There is no evidence indicating that any of the analogues I to IX have a higher affinity for CNS receptors than the natural hormone; however, it is known[13] that they have longer biological half lives. Consequently, we have not attempted to correlate the relative CNS activity data with conformation.

Experimental Section

All crystals used in this investigation were grown from aqueous solution and were found to contain water of hydration. The crystallographic properties for each are characterized in Table I. Diffraction intensities were measured with Syntex P$\bar{1}$ autodiffractometer operating in an ω-scan mode. Data were corrected for Lorentz and polarization effects but not for absorption.

The initial crystal structure models were determined either by direct methods,[20] Patterson analysis, or isomorphous replacement. They were developed[21] by difference Fourier techniques and variable-block block-diagonal least-squares refinement. Except for analogue VI, for which crystal quality was rather poor, all non-hydrogen atoms were refined with anisotropic temperature factors. In appropriate cases, hydrogen atoms were refined with isotropic temperature factors. The refinements are characterized in Table I.

Results and Discussion

Fractional atomic coordinates for non-hydrogen atoms are given in Table II. An example of the atom labeling is presented in Chart II. The illustrated scheme is used for all derivatives. Where a five-atom R^1 residue is present, the atom labels for the endocyclic amide group are unchanged. The unconventional (though consistent) labeling in the chart was used to facilitate computerized data analysis.

Stereoscopic projections[22] of a molecular unit for each analogue are presented, with the applicable atom labeling from Chart II, in Figures 1–3. For bond distances and bond angles for each molecule (10 examples), see supplementary material. The molecular conformations are characterized in Figure 4 with a graphical presentation of selected torsion angles. The relative CNS activity reported by Friderichs et al.[23] is displayed in Table III.

The TRH analogues for which crystal structure determinations are available can be divided into two classes,

(16) Atomic coordinates were kindly supplied by B. Stensland.
(17) (a) Kamiya, K.; Takamoto, M.; Wada, Y.; Fujino, M.; Nishikawa, M.; *J. Chem. Soc., Chem. Commun.* **1980**, 438–439. (b) Atomic coordinates were obtained from the Cambridge Crystallographic Data File: Kennard, O.; Watson, D. G.; Allen, F. H.; Motherwell, W. D. S.; Town, W. G.; Roger, J. *Chem. Br.* **1975**, *11*, 213–216.
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(22) Johnson, C. K.; "ORTEP-II, a Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations", Oak Ridge National Laboratory: Oak Ridge, TN; Report No. ORNL-5138, 1971.
(23) Friderichs, E.; Schwertner, E.; Herrling, S.; Günzler, W.-A.; Ötting, F.; Flohé, L. "Structure and Activity of Natural Peptides"; Voelter, W.; Weitzel, G., Eds.; W. de Gruyter: New York, 1981; pp 461–481.

Table I. Characterization of the Crystallographic Problem and Data Set

	I	II	III	IV	V	VI	VII
space group	$P1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P2_1$	$C2$	$P2_12_12_1$
Z	2	4	4	4	4	4	4
T, K	120	120	297 (2)	297 (2)	120	120	120
a, Å	8.030 (2)	8.510 (1)	8.3870 (4)	10.7228 (8)	10.8145 (8)	25.428 (4)	14.831 (4)
b, Å	12.228 (3)	11.507 (1)	9.6645 (4)	14.098 (1)	14.122 (1)	11.212 (1)	11.440 (6)
c, Å	12.698 (2)	23.738 (3)	24.933 (1)	14.672 (2)	14.541 (2)	7.078 (2)	11.448 (7)
α, deg	91.93 (2)	90	90	90	90	90	90
β, deg	106.68 (2)	90	90	95.146 (6)	95.399 (9)	88.52 (2)	90
γ, deg	104.38 (2)	90	90	90	90	90	90
no. of 2θ values	43	30	41	30	40	43	35
angular range, deg	35.79–44.85	20.30–31.90	35.38–59.86	40.29–59.72	30.40–39.41	30.16–39.44	30.21–43.55
radiation	Mo $K\alpha$	Mo $K\alpha$	Cu $K\alpha$	Cu $K\alpha$	Mo Kα	Mo Kα	Mo $K\alpha$
formula/asymmetric unit	2-$C_{17}H_{24}N_6O_4S$)·$8H_2O$	$C_{17}H_{24}N_6O_4S·4H_2O$	$C_{15}H_{20}N_6O_4S·3H_2O$	2-($C_{18}H_{23}N_7O_5$)·$3H_2O$	$2(C_{19}H_{25}N_7O_5)·3H_2O$	$C_{16}H_{18}N_7O_5Br·2H_2O$	$C_{18}H_{21}N_4O_4·H_2O$
			Intensity Data				
no. of unique data	10061	5416	2104	3681	10076	4023	4734
no. obsd	7019	4082	1910	3565	6487	3341	3162
$I > n\sigma(I)$; n =	2	2	2	2	2	3	3
resolution = d_{min}, Å	0.619	0.619	0.834	0.869	0.619	0.652	0.768
R[a]	0.064	0.050	0.048	0.064	0.059	0.079	0.044
R_w[2b]	0.080	0.052	0.061	0.096	0.063	0.097	0.054
σ[c]	1.31	1.14	1.11	1.12	1.41	1.38	1.14
no. of variables	779	417	366	576	806	239	358
no. of contributing reflections[d]	8603	4936	2104	3641	7876	3657	3162
H atoms[e]	B, F, R	A, F, R	A, F, R	B, C, I	B, F, R	C, I	B, F, R
disorder[f]	H_2O, Pro			Pro	Pro		Pro

[a] The number of automatically centered 2θ (Bragg angle) values used to refine lattice parameters. [b] The weighted R value. The weighting scheme employed was a polynomial including $\sigma(F_o)$. The specific weighting scheme for each structure determination has been deposited with the structure factor tables. [c] The estimated standard deviation of an observation of unit weight. [d] Reflections classified as unobserved for which the calculated intensity was greater than the arbitrary cutoff value were used in the refinement. [e] Treatment of H atoms in the structure determination: A = all H atoms; B = partial set; C = calculated from standard geometry; F = found by difference Fourier techniques; I = no refinement; R = refined with isotropic temperature factors. [f] Disorder in atomic positions was encountered in the indicated crystal structures either in the proline residues, some of the water of hydration molecules, or both.

Table II. Fractional Atomic Coordinates with Estimated Standard Deviations[a]

I

ATOM	X	Y	Z
S(A)	1.1150(1)	.58633(8)	.41882(7)
C(A3)	.8766(5)	.5656(3)	.3460(3)
C(A2)	.8578(4)	.6541(2)	.2664(2)
N(A1)	.9538(4)	.6470(2)	.1856(2)
C(A6)	1.0981(5)	.6088(2)	.1953(3)
O(A6)	1.1553(4)	.6046(3)	.1140(2)
C(A5)	1.1891(5)	.5625(3)	.2995(3)
C(A5M)	1.3935(6)	.6104(4)	.3342(3)
C(A7)	.9118(4)	.7725(2)	.3307(2)
O(A7)	.8394(3)	.7861(2)	.4021(2)
N(A7)	1.0343(4)	.8561(2)	.3069(2)
C(A8)	1.0865(4)	.9700(2)	.3659(2)
C(A8A)	1.2775(4)	1.0362(2)	.3650(2)
C(A8B)	1.4193(4)	.9835(2)	.4280(2)
N(A8C)	1.4633(4)	.9852(2)	.5424(2)
C(A8D)	1.5917(5)	.9323(3)	.5704(3)
N(A8E)	1.6312(4)	.8962(2)	.4809(2)
C(A8F)	1.5217(4)	.9281(3)	.3891(2)
C(A9)	.9524(4)	1.0355(2)	.3102(2)
O(A9)	.8797(3)	1.0194(2)	.2080(2)
N(A9)	.9217(4)	1.1138(2)	.3731(2)
C(A9A)	.8049(5)	1.1848(3)	.3209(3)
C(A9B)	.8227(7)	1.2701(3)	.4206(3)
C(A9C)	.8704(6)	1.2075(3)	.5210(3)
C(A9D)	.9946(5)	1.1418(3)	.4946(3)
C(A10)	.8673(7)	1.2471(3)	.2318(3)
O(A10)	1.0298(6)	1.2853(3)	.2433(4)
N(A10)	.7377(7)	1.2635(3)	.1471(3)
S(B)	1.040	1.124	.7988
C(B3)	.8832(5)	1.1480(3)	.8690(3)
C(B2)	.8704(5)	1.0631(3)	.9543(3)
N(B1)	1.0439(4)	1.0729(2)	1.0345(2)
C(B6)	1.2102(5)	1.1114(2)	1.0242(2)
O(B6)	1.3453(4)	1.1151(3)	1.1031(2)
C(B5)	1.2383(5)	1.1588(3)	.9190(3)
C(B5M)	1.3889(6)	1.1235(4)	.8882(3)
C(B7)	.7751(4)	.9431(2)	.8936(2)
O(B7)	.6256(3)	.9263(2)	.8251(2)
N(B7)	.8594(4)	.8614(2)	.9162(2)
C(B8)	.7751(4)	.7471(2)	.8585(2)
C(B8A)	.9181(4)	.6838(2)	.8591(2)
C(B8B)	1.0369(4)	.7372(2)	.7925(2)
N(B8C)	.9691(4)	.7321(2)	.6785(2)
C(B8D)	1.1072(5)	.7896(3)	.6473(3)
N(B8E)	1.2550(4)	.8306(3)	.7338(2)
C(B8F)	1.2147(5)	.7983(3)	.8279(3)
C(B9)	.6404(4)	.6819(2)	.9139(2)
O(B9)	.6710(3)	.6973(2)	1.0162(2)
N(B9)	.4933(4)	.6051(2)	.8509(2)
C(B9A)	.3620(5)	.5399(3)	.9014(3)
C(B9B)●	.199(1)	.4777(7)	.8075(7)
C(B91)●	.248(2)	.4412(9)	.7965(7)
C(B9C)●	.2932(9)	.4585(5)	.7207(5)
C(B92)●	.248(1)	.5029(8)	.6971(6)
C(B9D)	.4369(5)	.5764(3)	.7291(3)
C(B10)	.4489(6)	.4716(3)	.9895(3)
O(B10)	.5564(5)	.4220(2)	.9771(2)
N(B10)	.3811(6)	.4631(3)	1.0754(3)
O(W1)	.7352(7)	.5124(4)	.5833(4)
O(W2)	.1967(4)	.8617(2)	.1125(2)
O(W3A)◆	.423(2)	.3561(9)	.4856(6)
O(W3B)◇	.400(4)	.342(2)	.451(1)
O(W4)	.4752(5)	.2046(2)	.6431(3)
O(W5)	.8659(6)	.4120(3)	.9682(4)
O(W6A)▲	.3676(8)	.3069(5)	.2517(5)
O(W6B)△	.454(2)	.313(1)	.246(1)
O(W7)	.5427(5)	.8549(5)	.1137(4)
O(W8A)▼	.7843(8)	.3742(5)	.7327(4)
O(W8B)▽	.732(1)	.3872(5)	.7974(5)

II

ATOM	X	Y	Z
S	.20658(9)	.81974(6)	.45876(3)
N(1)	.1698(2)	.8371(2)	.32810(8)
C(2)	.0344(3)	.8672(2)	.36246(9)
C(3)	.0324(3)	.7947(2)	.4164(1)
C(5)	.3499(3)	.8706(2)	.4067(1)
C(5M)	.5130(4)	.8364(3)	.4277(1)
C(6)	.3166(3)	.8271(2)	.34740(9)
O(6)	.4236(2)	.7895(2)	.31689(8)
C(7)	.0187(3)	.9969(2)	.37569(9)
O(7)	−.0772(2)	1.0300(2)	.41183(7)
N(7)	.1069(3)	1.0712(2)	.34618(8)
C(8)	.1037(3)	1.1945(2)	.35894(9)
C(8A)	.2575(3)	1.2523(2)	.3397(1)
C(8B)	.3959(3)	1.2073(2)	.37121(9)
N(8C)	.4113(3)	1.2232(2)	.42906(8)
C(8D)	.5460(3)	1.1724(3)	.4425(1)
N(8E)	.6167(3)	1.1254(2)	.3973(1)
C(8F)	.5224(3)	1.1462(2)	.3514(1)
C(9)	−.0323(3)	1.2556(2)	.32926(9)
O(9)	−.0668(2)	1.2328(1)	.27975(7)
N(9)	−.1061(2)	1.3400(2)	.35834(8)
C(9A)	−.2363(3)	1.4053(2)	.3332(1)
C(9B)	−.3204(3)	1.4574(2)	.3847(1)
C(9C)	−.1900(3)	1.4715(2)	.4284(1)
C(9D)	−.0861(3)	1.3652(2)	.41928(9)
C(10)	−.1848(3)	1.4988(2)	.29180(9)
O(10)	−.2822(2)	1.5336(2)	.25646(7)
N(10)	−.0405(3)	1.5416(2)	.29534(9)
O(W1)	.2913(2)	.4570(2)	.87713(8)
O(W2)	.0933(3)	.4390(2)	.78381(8)
O(W3)	.8112(3)	.0623(2)	.52516(8)
O(W4)	.2801(3)	.4016(2)	.2032(1)

III

ATOM	X	Y	Z
S	.0857(1)	−.0918(1)	.99300(3)
N(1)	.0539(4)	−.1421(3)	.8930(1)
C(2)	−.1072(5)	−.1106(3)	.9094(1)
C(3)	−.1098(5)	−.1405(4)	.9699(2)
C(6)	.1703(5)	−.1251(4)	.9296(1)
O(6)	.3151(4)	−.1297(4)	.9211(1)
C(7)	−.1546(4)	.0394(3)	.8994(1)
O(7)	−.2842(3)	.0809(3)	.9161(1)
N(7)	−.0533(4)	.1214(3)	.8735(1)
C(8)	−.0823(4)	.2685(3)	.8675(1)
C(8A)	.0721(5)	.3425(4)	.8512(2)
C(8B)	.2090(4)	.3083(3)	.8867(1)
N(8C)	.2088(4)	.3413(4)	.9408(1)
C(8D)	.3457(6)	.2913(5)	.9591(2)
N(8E)	.4308(4)	.2298(4)	.9211(1)
C(8F)	.3442(5)	.2395(4)	.8744(2)
C(9)	−.2096(4)	.2950(3)	.8247(1)
O(9)	−.2319(3)	.2105(2)	.78756(9)
N(9)	−.2879(3)	.4138(3)	.82680(9)
C(9A)	−.4013(4)	.4498(3)	.7839(1)
C(9B)	−.4630(6)	.5929(4)	.8010(1)
C(9C)	−.4464(5)	.5915(4)	.8621(1)
C(9D)	−.2902(5)	.5156(4)	.8710(1)
C(10)	−.3239(4)	.4542(3)	.7291(1)
O(10)	−.1810(3)	.4859(3)	.7225(1)
N(10)	−.4215(4)	.4278(3)	.6888(1)
O(W1)	.2440(4)	.4134(3)	.7174(1)
O(W2)	.4796(4)	−.1919(4)	.2129(1)
O(W3)	.9639(5)	−.4805(4)	.9764(1)

Table II (*Continued*)

IV

ATOM	X	Y	Z
N(A1)	.0297(4)	.5629	.8072(3)
C(A2)	.1206(5)	.6318(4)	.8010(4)
C(A3)	.0989(5)	.7252(4)	.8060(4)
C(A3A)	.1952(5)	.8016(5)	.7955(5)
C(A3B)	.2469(7)	.8426(6)	.8881(6)
C(A4)	-.0283(5)	.7543(5)	.8179(4)
O(A4)	-.0618(4)	.8377(3)	.8260(4)
N(A5)	-.1157(4)	.6829(4)	.8204(3)
C(A6)	-.0921(5)	.5864(4)	.8201(4)
O(A6)	-.1715(4)	.5280(3)	.8305(4)
C(A7)	.2477(4)	.5930(4)	.7798(4)
O(A7)	.2584(4)	.5563(4)	.7040(3)
N(A7)	.3406(4)	.6023(3)	.8450(3)
C(A8)	.4688(5)	.5783(4)	.8265(4)
C(A8A)	.5570(5)	.5926(4)	.9127(4)
C(A8B)	.5814(5)	.6955(4)	.9335(4)
N(A8C)	.6397(4)	.7497(4)	.8705(3)
C(A8D)	.6482(6)	.8357(5)	.9082(5)
N(A8E)	.6012(5)	.8382(4)	.9892(4)
C(A8F)	.5576(5)	.7482(5)	1.0052(4)
C(A9)	.4764(5)	.4745(4)	.7967(3)
O(A9)	.4248(4)	.4116(3)	.8370(3)
N(A9)	.5521(4)	.4538(4)	.7314(3)
C(A9A)	.5740(5)	.3546(5)	.7091(4)
C(A9B)	.6592(8)	.3610(7)	.6308(5)
C(A9C)■	.631(1)	.460(1)	.5876(8)
C(A91)□	.705(2)	.446(2)	.626(2)
C(A9D)	.6047(6)	.5197(6)	.6672(4)
C(A10)	.6285(5)	.2967(4)	.7893(4)
O(A10)	.6014(4)	.2116(3)	.7979(3)
N(A10)	.7133(5)	.3389(4)	.8494(4)
N(B1)	.5108(4)	1.0538(4)	.6962(3)
C(B2)	.6005(5)	.9865(4)	.6804(4)
C(B3)	.5709(5)	.8991(4)	.6479(4)
C(B3A)	.6656(6)	.8226(5)	.6333(5)
C(B3B)	.6835(9)	.8071(8)	.5335(8)
C(B4)	.4385(5)	.8755(5)	.6314(4)
O(B4)	.3973(4)	.7979(3)	.6030(3)
N(B5)	.3554(4)	.9464(4)	.6478(3)
C(B6)	.3859(5)	1.0373(5)	.6791(4)
O(B6)	.3068(4)	1.0968(4)	.6901(4)
C(B7)	.7350(5)	1.0169(4)	.7084(4)
O(B7)	.7725(4)	1.0174(3)	.7885(3)
N(B7)	.8043(4)	1.0365(4)	.6401(3)
C(B8)	.9376(5)	1.0561(4)	.6620(4)
C(B8A)	1.0010(5)	1.0690(5)	.5726(4)
C(B8B)	1.0217(5)	.9770(5)	.5272(4)
N(B8C)	1.1018(4)	.9101(4)	.5690(4)
C(B8D)	1.0979(6)	.8385(6)	.5105(5)
N(B8E)	1.0218(5)	.8542(5)	.4368(4)
C(B8F)	.9725(6)	.9433(6)	.4445(4)
C(B9)	.9551(5)	1.1468(4)	.7161(3)
O(B9)	.9033(4)	1.2217(3)	.6896(3)
N(B9)	1.0338(4)	1.1448(3)	.7935(3)
C(B9A)	1.0440(5)	1.2282(4)	.8522(4)
C(B9B)	1.0879(8)	1.1861(6)	.9477(5)
C(B9C)	1.1639(8)	1.0979(6)	.9225(6)
C(B9D)	1.0935(5)	1.0594(4)	.8375(4)
C(B10)	1.1322(6)	1.3029(4)	.8230(4)
O(B10)	1.1223(5)	1.3857(3)	.8529(3)
N(B10)	1.2196(5)	1.2793(4)	.7698(5)
O(W1)	.7342(6)	.1276(5)	.9685(3)
O(W2)	.1007(6)	.4739(4)	.0183(3)
O(W3)	.6795(6)	.1122(5)	.4696(3)

V

ATOM	X	Y	Z
N(A1)	.0230(3)	.5629	.8066(2)
C(A2)	.1141(3)	.6304(2)	.7997(2)
C(A3)	.0901(3)	.7248(3)	.8038(2)
C(A3A)	.1869(3)	.8010(3)	.7975(2)
C(A3B)	.2363(3)	.8393(3)	.8933(2)
C(A3C)	.3463(3)	.9057(3)	.8876(3)
C(A4)	-.0370(3)	.7538(3)	.8153(2)
O(A4)	-.0707(2)	.8369(2)	.8224(2)
N(A5)	-.1233(3)	.6808(2)	.8177(2)
C(A6)	-.0975(3)	.5853(2)	.8195(2)
O(A6)	-.1765(2)	.5254(2)	.8312(2)
C(A7)	.2395(3)	.5926(2)	.7789(2)
O(A7)	.2508(2)	.5577(2)	.7027(2)
N(A7)	.3326(3)	.6029(2)	.8449(2)
C(A8)	.4591(3)	.5791(2)	.8258(2)
C(A8A)	.5490(3)	.5907(2)	.9131(2)
C(A8B)	.5807(3)	.6924(2)	.9349(2)
N(A8C)	.6406(3)	.7463(2)	.8723(2)
C(A8D)	.6608(3)	.8300(3)	.9136(3)
N(A8E)	.6185(3)	.8311(2)	.9976(2)
C(A8F)	.5659(3)	.7442(3)	1.0118(2)
C(A9)	.4674(3)	.4757(2)	.7941(2)
O(A9)	.4130(2)	.4122(2)	.8325(2)
N(A9)	.5459(3)	.4556(2)	.7302(2)
C(A9A)	.5671(3)	.3555(2)	.7070(2)
C(A9B)	.6536(5)	.3625(3)	.6293(3)
C(A9C)▲	.6266(6)	.4628(5)	.5856(4)
C(A91)△	.705(1)	.4510(7)	.6313(6)
C(A9D)	.6028(4)	.5220(3)	.6672(3)
C(A10)	.6233(3)	.2973(2)	.7890(2)
O(A10)	.5935(3)	.2132(2)	.7964(2)
N(A10)	.7046(3)	.3395(2)	.8509(2)
N(B1)	.4991(3)	1.0561(2)	.6952(2)
C(B2)	.5876(3)	.9889(2)	.6813(2)
C(B3)	.5574(3)	.8997(2)	.6512(2)
C(B3A)	.6522(3)	.8236(3)	.6384(3)
C(B3B)	.6735(4)	.8058(3)	.5367(3)
C(B3C)	.7722(6)	.7307(4)	.5268(5)
C(B4)	.4262(3)	.8773(3)	.6329(2)
O(B4)	.3855(2)	.7991(2)	.6064(2)
N(B5)	.3436(3)	.9504(2)	.6465(2)
C(B6)	.3741(3)	1.0402(3)	.6783(2)
O(B6)	.2949(2)	1.1002(2)	.6901(2)
C(B7)	.7216(3)	1.0174(2)	.7088(2)
O(B7)	.7612(2)	1.0176(2)	.7911(2)
N(B7)	.7897(3)	1.0367(2)	.6392(2)
C(B8)	.9224(3)	1.0563(2)	.6602(2)
C(B8A)	.9870(3)	1.0678(3)	.5708(2)
C(B8B)	1.0116(3)	.9761(3)	.5249(2)
N(B8C)	1.0952(3)	.9121(2)	.5682(2)
C(B8D)	1.0993(3)	.8404(3)	.5098(3)
N(B8E)	1.0245(3)	.8552(3)	.4320(2)
C(B8F)	.9675(4)	.9420(3)	.4403(2)
C(B9)	.9413(3)	1.1482(3)	.7159(2)
O(B9)	.8882(2)	1.2216(2)	.6880(2)
N(B9)	1.0215(3)	1.1462(2)	.7922(2)
C(B9A)	1.0329(3)	1.2294(2)	.8519(2)
C(B9B)	1.0761(4)	1.1874(3)	.9479(3)
C(B9C)	1.1517(4)	1.1001(3)	.9241(3)
C(B9D)	1.0824(3)	1.0611(3)	.8357(2)
C(B10)	1.1241(3)	1.3035(3)	.8230(2)
O(B10)	1.1164(3)	1.3860(2)	.8539(2)
N(B10)	1.2115(3)	1.2786(2)	.7700(2)
O(W1)	-.7337(3)	.6288(3)	.0342(2)
O(W2)	-.1008(3)	.9731(2)	.9785(2)
O(W3)	-.6672(4)	.6106(3)	.5325(2)

Table II (*Continued*)

VI				VII			
ATOM	X	Y	Z	ATOM	X	Y	Z
BR	.87618(2)	0	.77613(9)	N(1)	.3224(1)	.4297(2)	.9537(1)
N(1)	1.0147(2)	.1907(4)	.7428(7)	C(2)	.3472(1)	.4963(2)	1.0558(2)
C(2)	.9643(2)	.1585(5)	.7544(7)	C(3)	.4427(2)	.4524(2)	1.0831(2)
C(3)	.9472(2)	.0402(6)	.7604(7)	C(45)	.4765(2)	.4041(2)	.9681(3)
C(4)	.9857(2)	-.0531(5)	.7580(9)	C(6)	.3911(2)	.3801(2)	.8979(2)
O(4)	.9757(2)	-.1615(6)	.7619(7)	O(6)	.3845(2)	.3250(2)	.8065(2)
N(5)	1.0370(2)	-.0122(5)	.7514(6)	C(7)	.3480(1)	.6279(2)	1.0316(2)
C(6)	1.0524(2)	.1050(5)	.7343(8)	O(7)	.3758(1)	.6686(1)	.9392(1)
O(6)	1.0999(2)	.1280(4)	.7105(7)	N(7)	.3199(1)	.6940(1)	1.1204(1)
C(7)	.9261(2)	.2623(5)	.7640(7)	C(8)	.3246(1)	.8211(2)	1.1146(2)
O(7)	.9260(2)	.3297(4)	.8987(7)	C(8A)	.2804(2)	.8696(2)	1.2261(2)
N(7)	.8971(2)	.2755(5)	.6097(6)	C(8B)	.2818(1)	1.0009(2)	1.2340(2)
C(8)	.8610(2)	.3740(5)	.5831(8)	C(8C)	.2376(2)	1.0702(2)	1.1527(2)
C(8A)	.8666(2)	.4199(5)	.3789(8)	C(8D)	.2385(2)	1.1917(2)	1.1640(3)
C(8B)	.9162(2)	.4862(4)	.3369(8)	C(8E)	.2809(2)	1.2438(2)	1.2574(3)
N(8C)	.9630(2)	.4359(5)	.2777(6)	C(8F)	.3246(2)	1.1761(2)	1.3388(2)
C(8D)	.9983(2)	.5201(5)	.2435(8)	C(8G)	.3256(1)	1.0554(2)	1.3269(2)
N(8E)	.9760(2)	.6241(5)	.2778(7)	C(9)	.4237(1)	.8601(1)	1.1107(2)
C(8F)	.9247(2)	.6064(5)	.3374(9)	O(9)	.47669(9)	.8246(1)	1.1851(1)
C(9)	.8047(2)	.3276(5)	.6137(8)	N(9)	.4486(1)	.9389(1)	1.0301(1)
O(9)	.7844(2)	.2684(5)	.4874(7)	C(9A)	.5436(1)	.9758(2)	1.0260(2)
N(9)	.7795(2)	.3550(4)	.7760(7)	C(9B)	.5526(2)	1.0293(3)	.9028(2)
C(9A)	.7278(2)	.3011(5)	.8227(9)	C(9C)●	.4636(3)	.9911(4)	.8310(4)
C(9B)	.7279(3)	.2989(8)	1.040(1)	C(9D)	.3985(2)	.9722(2)	.9232(2)
C(9C)	.7557(3)	.4150(9)	1.0855(9)	C(10)	.5678(1)	1.0652(2)	1.1189(2)
C(9D)	.8016(3)	.4177(8)	.9407(9)	O(10)	.64819(9)	1.0781(1)	1.1469(1)
C(10)	.6811(2)	.3743(5)	.7555(9)	N(10)	.5024(1)	1.1307(1)	1.1618(1)
O(10)	.6362(2)	.3347(4)	.7804(8)	C(91)●	.4691(4)	1.0579(4)	.8690(4)
N(10)	.6915(2)	.4812(4)	.6811(9)	O(W1)	.9343(1)	.2965(2)	.3999(2)
O(W1)	.6999(2)	.1210(5)	.4103(9)				
O(W2)	.8924(2)	.6783(5)	.8523(9)				

[a] Population parameters for positionally disordered atoms are indicated symbolically as follows: (●) 0.5, (♦) 0.7, (◇) 0.3, (▪) 0.66, (▫) 0.33, (▲) 0.6, (△) 0.3, (▼) 0.55, and (▽) 0.45.

those in which substitution has replaced the pyroglutamate ring (R^1-TRH analogues, I–VI) and those in which the imidazole ring of the histidine residue has been replaced (R^2-TRH analogues, VII–IX). Compounds I–VI are R^1-TRH analogues that can be appropriately divided into two subclasses defined by the stereochemistry of the α carbon atom. R_s^1-TRH analogues (I–III) have an sp^3-hybridized α carbon atom, whereas R_u^1-TRH analogues (IV–VI) have an sp^2-hybridized one. The difference in stereochemistry of the R_s^1 and R_u^1 analogues precludes a high degree of conformational similarity between members of the two classes.

Examination of the stereoscopic projections in Figures 1–3 and the distribution of torsion angles in Figure 4 provides insight into the similarities and differences of the conformations displayed by these TRH analogues. Because several crystals have two molecules per asymmetric unit, there are more observations of conformation (15) than crystal structure determinations (10).

In all 15 observations, the peptide bonds are all trans. The torsion angle ϕ_1' is indicative more of chemical structure than of conformation, since its value is limited by the presence of a ring. The average value for the 10 examples in which the ring juncture contains a sp^3-hybridized carbon is 99 ± 6° and that for the sp^2 hybridization is −177 ± 1°. The similarity of all values for the former set demonstates that all the R_s^1 analogues have maintained the configuration of L-pyroglutamine.

Table III. Relative[a] CNS Activity (TRH = 1.0)

compd	rel act.	no.	rel act.
I	83	IV	0.09
IB[b]	17	V	0.8
III	0.8	VI	0.7

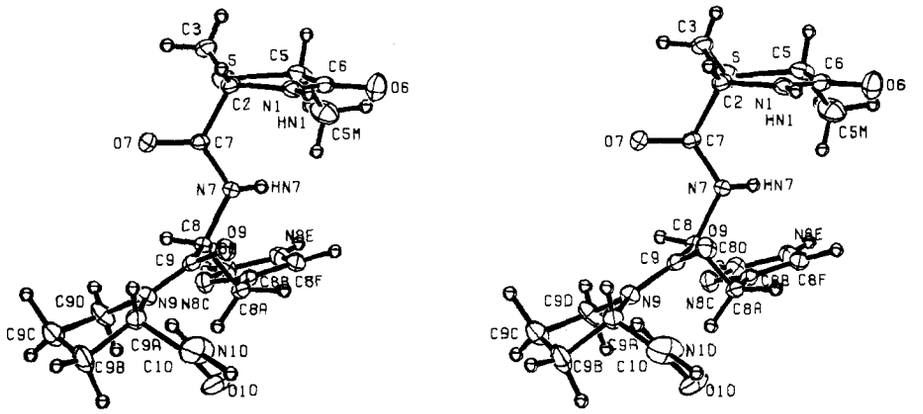
[a] The values tabulated are based upon studies of antagonism against reserpine hypothermia in mice. For details, see Friderichs et al.[23] [b] The absolute stereochemistry of I and II were determined in the course of the crystal structure analyses. Assignment was made with respect to the known peptide configurations.

The orientation of the R^1 ring with respect to the first peptide bond is given by ψ_1. All R^1 analogues and the four examples of Thi^2-TRH display similar ψ_1 values (orientation R_s^1-A). In contrast, the crystal structures of Phe^2-TRH and TRH(+) present a second orientation (R_s^1-B) for the L-pGlu ring. There are also two orientations observed for the heterocyclic ring of the R_u^1 analogues (R_u^1-A and R_u^1-B). The respective average ψ_1 angles are 8 ± 5, 145 ± 1, −113 ± 4, and 111°.

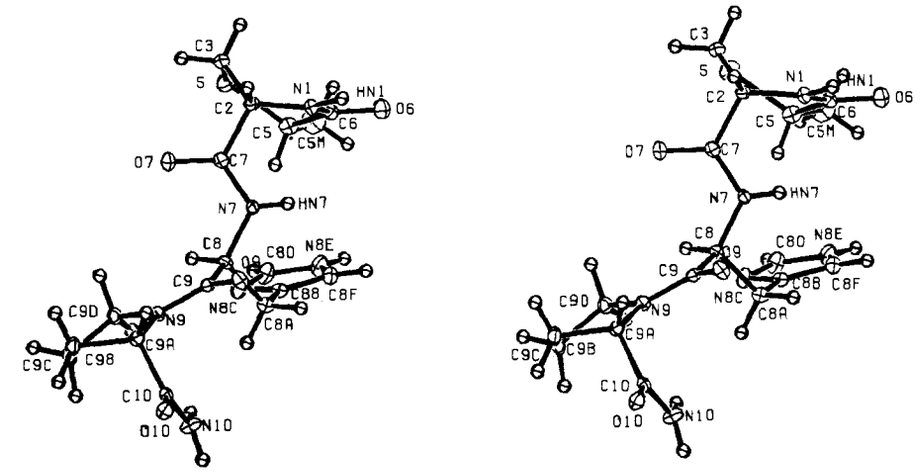
It is clear from Figure 4 that the sets of dihedral angles ϕ_2, ψ_2, and ϕ_3 are indicative of a preferred conformation for the remainder of the peptide backbone. There are two preferred orientations (ψ_3), differing by a rotation of ca. 180° about the C_α–C′ proline bond, for the primary amide.

The orientation of the R^2 substituents is defined by two dihedral angles, χ_1 and χ_2. Angle χ_1 can be regarded as defining the position of the side chain relative to the

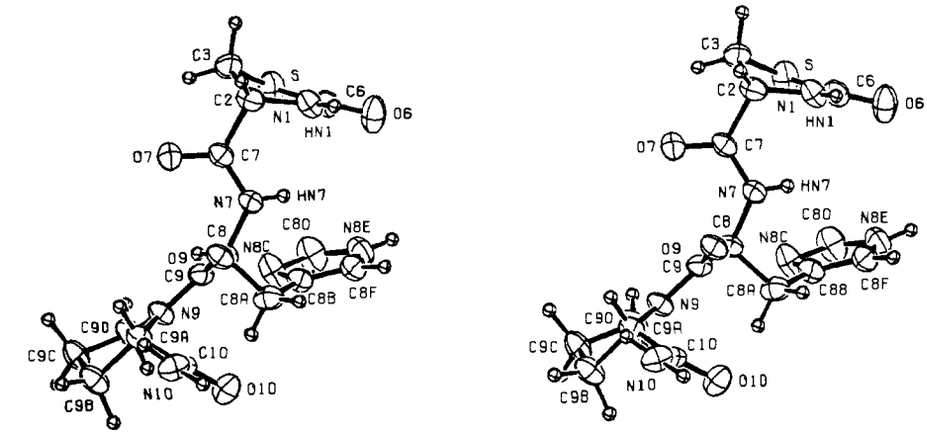
(24) Metcalf, G. in "Thyrotropin Releasing Hormone"; Griffiths and Bennett, Eds.; Raven Press: New York; 1983; pp 315–326.



I



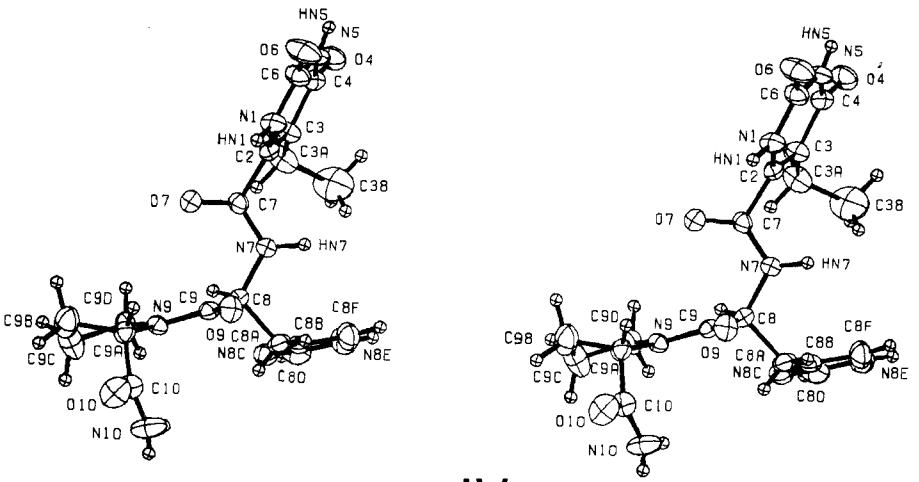
II



III

R_s^1 – Analogs

Figure 1. Stereoscopic projections for one molecule each of TRH analogues I to III. Atom labels are displayed for each derivative. Carbon, nitrogen, oxygen, and sulfur atoms are presented with thermal ellipsoids consistent with their refined thermal parameters. Hydrogen atoms are depicted with arbitrarily small isotropic temperature factors in order to minimize clutter.



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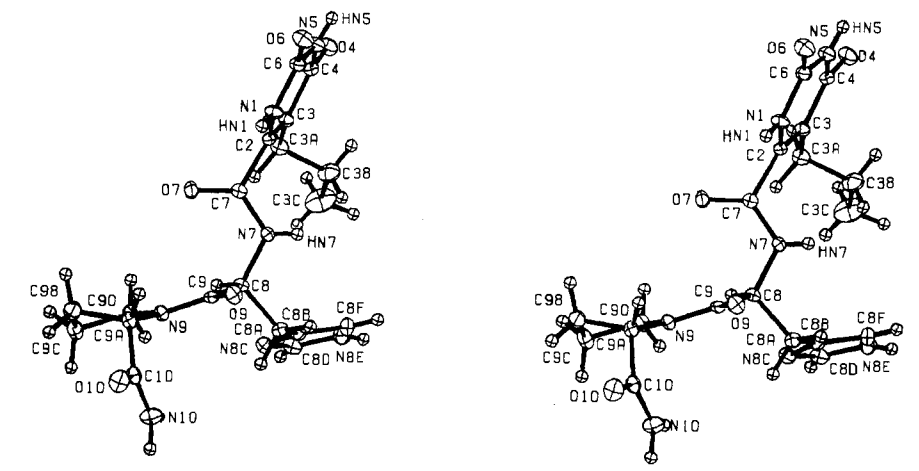

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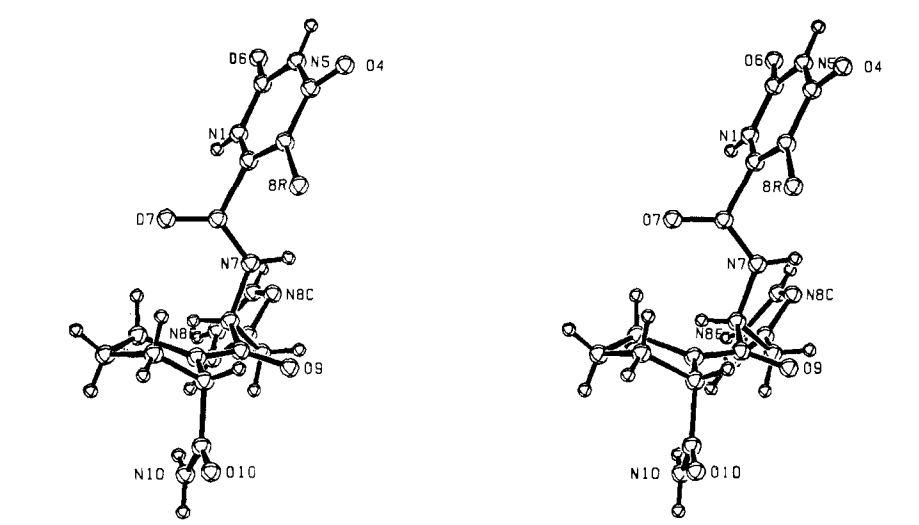
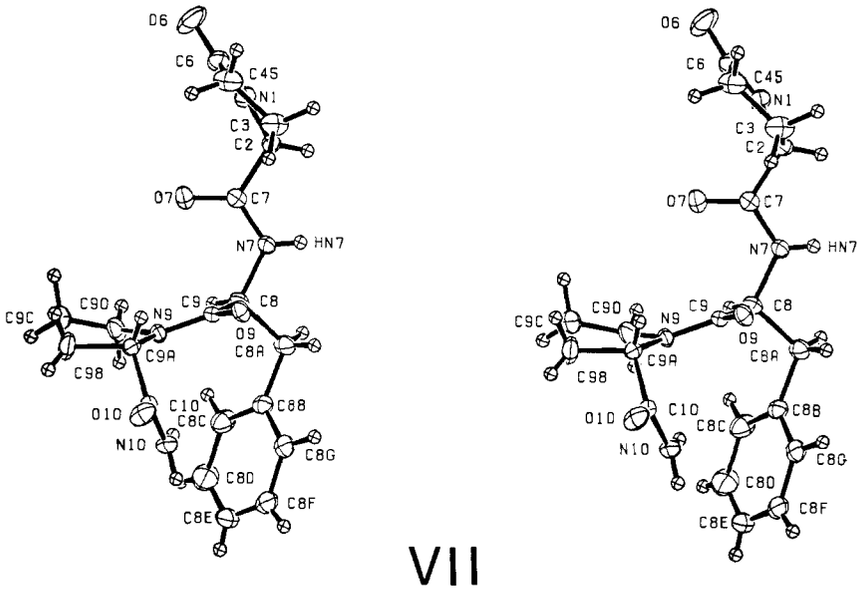


VI

R^1_u – Analogs

Figure 2. Stereoscopic projections for one molecule each of TRH analogues IV to VI. See caption to Figure 1 for description.



VII

Phe^2-TRH

Figure 3. Stereoscopic projections for one molecule each of TRH analogue VII. See caption to Figure 1 for description.

peptide backbone, whereas χ_2 characterizes the orientation of the planar ring. As illustrated in Figure 4, there is a preferred value, $-69 \pm 6°$, for χ_1. The only compound in which the histidine side chain is not in this position is the example in which the imidazole ring is protonated and involved in an intramolecular hydrogen bond. The phenyl ring of Phe^2-TRH also has been found to be in a similar position (though different orientation) as the protonated imidazole ring, so that hydrogen bonding is not required to stabilize a conformation with χ_1 ca. 180°. With only one exception, VI, the χ_2 values for the imidazole ring are very similar, $\langle\chi_2\rangle = -65 \pm 2°$. The imidazole ring of VI is an orientation very similar to that of the 2-thienyl rings in the four examples of Thi^2-TRH.

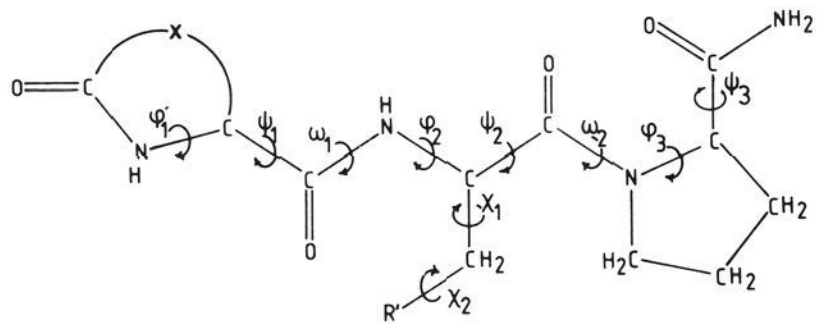
Figure 5 illustrates the conformational similarities and differences described above for the series of TRH analogues by presenting a least-squares superposition[25] of selected molecules. It also displays a composite "hydrogen bonding environment" constructed with all the donor and acceptor nitrogen and oxygen atoms involved in intermolecular hydrogen bonds with each symmetry-independent molecule (15 examples). Symbols associated with the nature of the interaction (e.g., + is a donor to carbonyl atom O9) are used for each atom. Though complex, the stereoscopic projection presents a number of features that may be relevant to the CNS receptor binding of TRH analogues.

As might be expected, there is some clustering of donors and/or acceptors around appropriate functional groups for those portions of the chemical structure for which similar conformations are observed. The spatial distribution of the participating atoms is influenced by the substituent environment presented by the conformation of the TRH analogue. For example, there are four clusters of donors and/or acceptors (depending on substituent orientation) associated with the proline amide; there is a fairly diffuse spatial distribution of donors to the histidine carbonyl oxygen atom, whereas the distribution around the peptide carbonyl oxygen atom of R^1 is fairly narrow.

Examination of the hydrogen bonding environment of the R^1 substituents should be carried out with two points in mind. First, the CNS activity[24] of DN 1417, which is the lactone analogue of pGlu, indicates that the endocyclic NH moiety is not necessary for acceptor binding. Consequently, a hydrogen-bonding receptor role for the endocyclic carbonyl moiety is strongly indicated. Second, the chemical structure of the R_u^1 analogues for which we have determined crystal structures present two spatially equivalent endocyclic carbonyl groups, only one of which is expected to hydrogen bond with the receptor. This observation implies near equivalence for the orientations R_u^1-A and R_u^1-B described above with respect to their suitability as hydrogen-bond acceptors. The distribution of hydrogen bond donors to the endocyclic carbonyl groups is, of course, diffuse. Nonetheless, two regions in space are of interest in terms of a receptor model. The first consists of a series of four donor atoms (site A, connected by a dotted line in the figure) that define one region in which a receptor donor could interact with either R_s^1-A or R_u^1 endocyclic carbonyl groups. The second region involves the potential interaction of a donor with the carbonyl groups in orientation R_s^1-B or the alternative carbonyl group of the R_u^1 analogues (site B, indicated by a blacked out symbol in the figure).

There is some indication of possible "hydrophobic interaction" that can be discerned from Figure 5. For example, the orientations of the phenyl ring in Phe^2-TRH and of the imidazole ring in TRH(+) place these substituents in a hydrophilic region associated with hydrogen bonding to the proline amide in all other examples. Equally interesting, the orientations of the unprotonated imidazole rings give rise to a zone of hydrogen-bonding interactions that interfaces smoothly with those of other parts of the molecule to give rise to a "hydrophilic side"

(25) Liebman, M. N. In "Molecular Structure and Biological Activity"; Griffin, J. F.; Duax, W. L., Eds.; Elsevier: New York, 1982; pp 193–212.



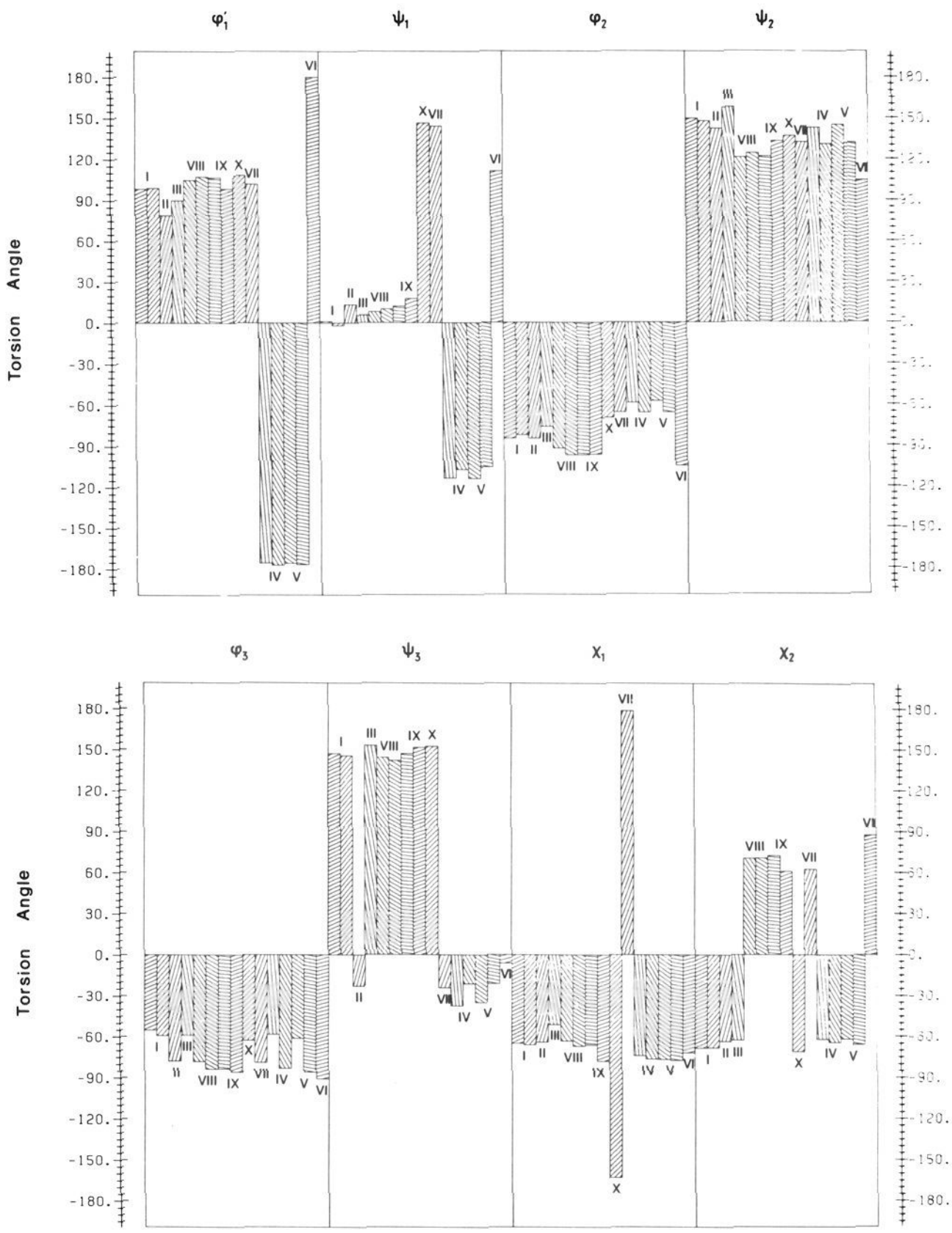


Figure 4. A graphical presentation of selected torsion angles for compounds I–X. The values for all 15 observations are presented. Derivatives of type R_s^1-TRH are grouped together, followed by Thi^2-TRH examples, TRH(+), Phe^2-TRH, and then the R_u^1 analogues. Where a compound label (e.g., I) is centered over two bars, there are two molecules per crystallographic asymmetric unit. *Note*! Convention dictates that torsion angles lie between −180 and 180°. This results in values of ϕ_1' and χ_1 being depicted in the computer-generated bar graphs appearing to be greatly different in magnitude when, in fact, they represent very similar conformations.

of the molecule. Orientation R_s^1-A provides better interaction of the endocyclic carbonyl ring with this hydrophilic region than does R_s^1-B. Furthermore, with the orientations R_s^1-A and R_u^1-A there is a clearly hydrophobic side of the molecule in which the only hydrophilic moiety present is the peptide carbonyl group of pGlu. Hydrogen bonding of this moiety to a largely hydrophobic donor from the receptor seems probable.

In the above discussion, we have sought to analyze the crystal structures of a series of CNS active TRH analogues of dramatically different chemical structures in an effort to gain insight into the spatial aspects of their receptor binding. We have established that there is a very satisfying degree of conformational similarity in those portions of the molecules where the chemical structure allows access to the same conformation. By constructing a composite hy-

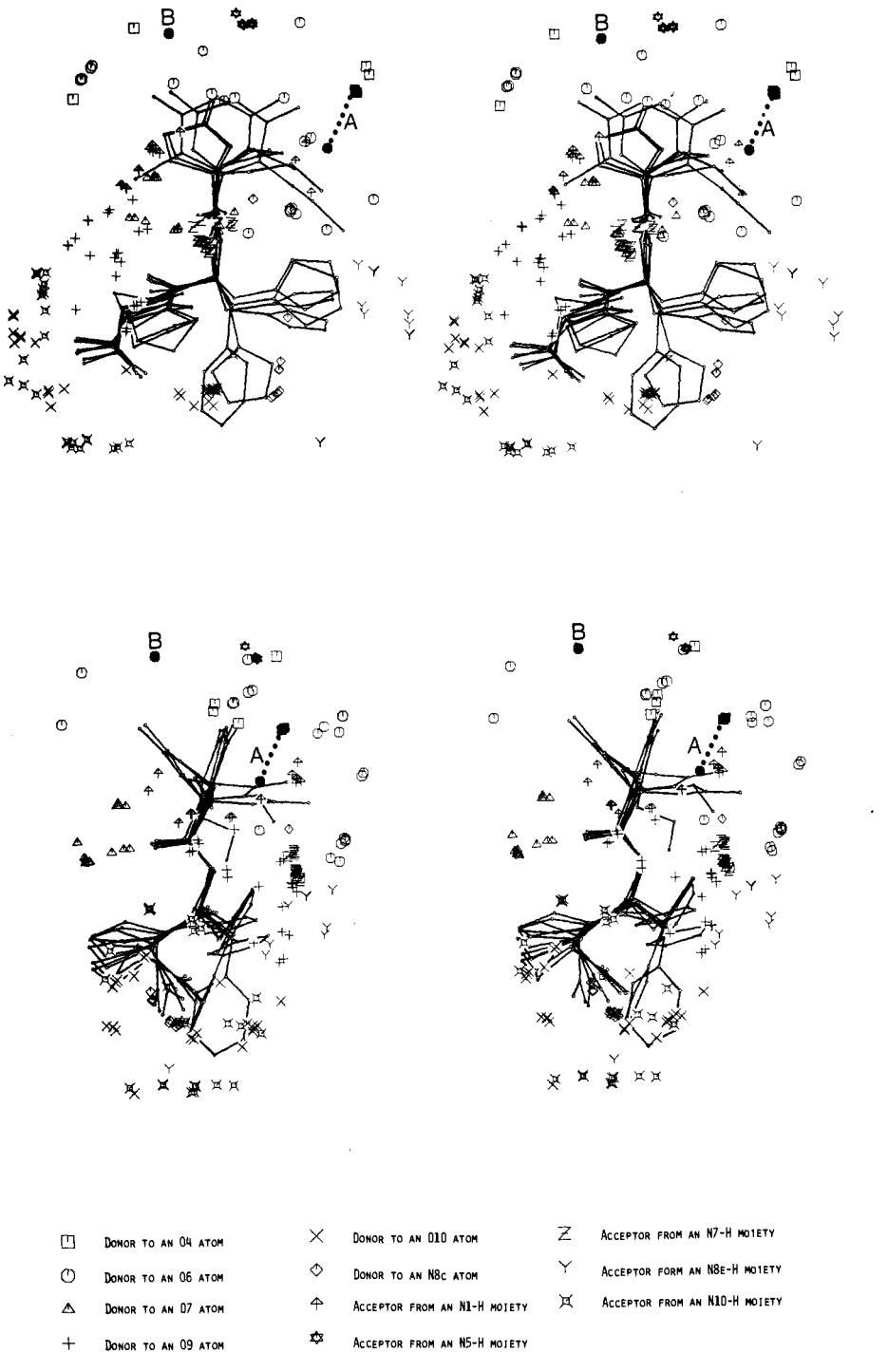


Figure 5. A stereoscopic projection of a least-squares superposition of selected TRH analogues. One molecule each of V, VI, VII, IX, and X has been least-squares fit by using coordinates of atoms C2, C7, O7, N7, C8, C9, O9, N9, C9A, and C10 to the structure of one molecule of I. The same fitting procedure has been used to construct the composite hydrogen-bonding environment that is depicted therein. All potential hydrogen-bonding interactions (contact distances to 3.2 Å) for all TRH analogue molecules have been included, even though not all of the TRH analogue molecules are drawn.

drogen-bonding environment, we have detected regions in space where intermolecular hydrogen bonding interactions are common. Based on these observations, we suggest that CNS receptor–TRH analogue interactions may conform, at least in part, to the model shown schematically in Figure 6.

The receptor interacts via a common hydrogen bond donor, D1, with either an R_s^1 or R_u^1 carbonyl oxygen atom. The conformation (R_s^1-A) for I has been chosen because it places the endocyclic carbonyl group in a more hydrophilic environment than does R_s^1-B. An acceptor, A1, interacts with the His peptide NH moiety a little further "down" the hydrophilic side of the molecule. Either a donor, D2, or an acceptor, A2, hydrogen bonds with the proline amide moiety still further down the molecule. The other side of the molecule is largely hydrophobic, with the exception that there is one hydrogen bond acceptor present, the carbonyl oxygen atom of the pGlu peptide residue. Interaction of this moiety with a His or Tyr hydrogen bond donor would be particularly suitable, since these residues are compatible with hydrophobic environments.

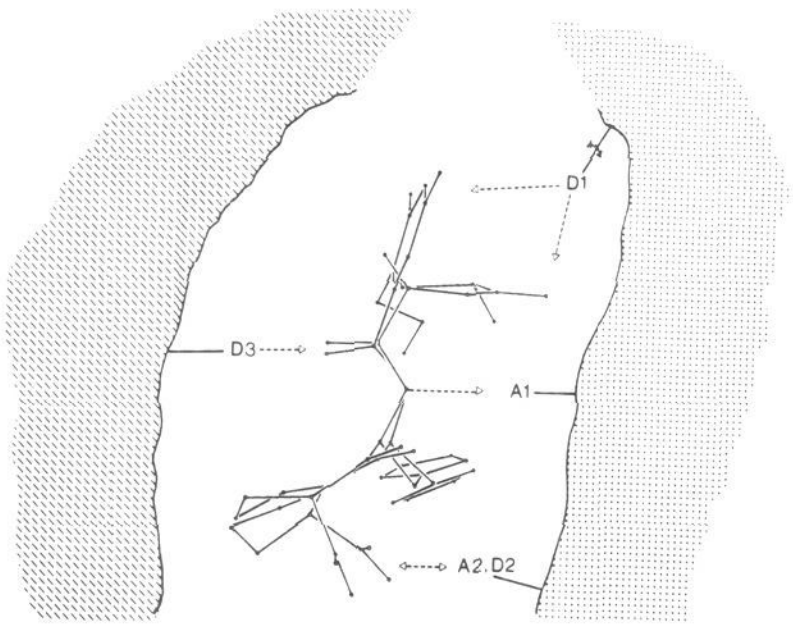


Figure 6. A schematic representation of possible CNS receptor–TRH analogue binding. The concept reflects the interpretation of interactions within the composite hydrogen-bonding environment and the conformations observed for TRH analogues. Depicted are superimposed examples of an R_s^1-A conformer, I, and an R_u^1-A conformer, V, interacting with hydrogen-bond donors, D, to carbonyl oxygen atoms or acceptors, A, from amide moieties. The carbonyl groups involved are the endocyclic carbonyl of residue 1, the pGlu peptide carbonyl, and depending on orientation, the carbonyl of the proline amide. The acceptors interact with the His NH and, possibly, the Proline amide. The area shaded with dots is considered to be hydrophilic, and the area shaded with broken lines is considered hydrophobic. The arrow on the D1–receptor "bond" indicates reorientation of this receptor moiety to accommodate either an R_s^1 or R_u^1 TRH analogue.

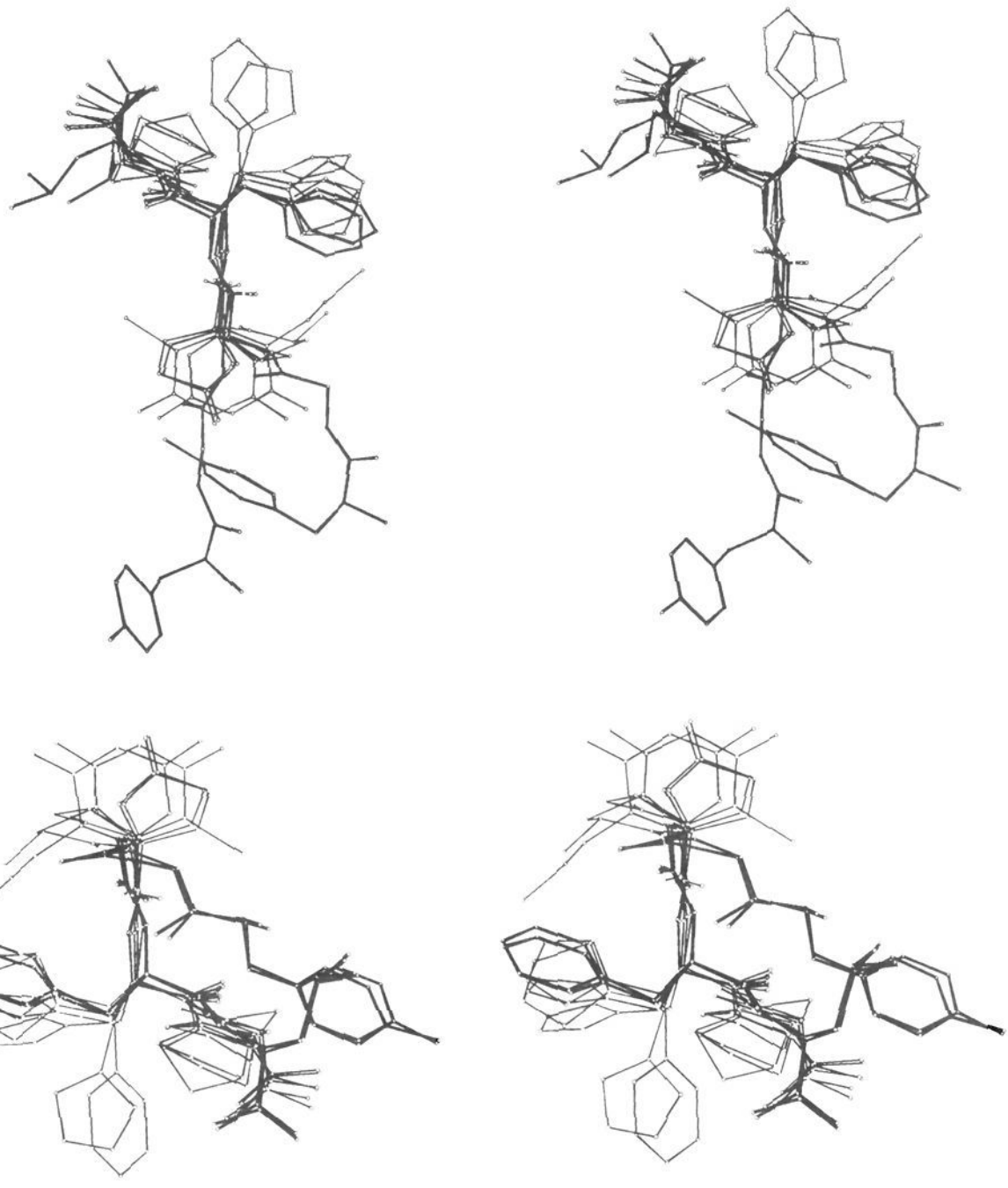


Figure 7. A stereoscopic projection derived from a least-squares fit of the four observed conformations of Leu^5-enkephalin (XI and XII) to one molecule of I. The coordinates of the atoms analogous to atoms C2, C7, O7, N7, C8, C9, O9, N9, C9A, and C10 of the TRH analogues were employed to determine the superpositions. All eight examples of XI and XII were fit. For clarity, only the "best" and "worst" fits are presented for each structure determination. The range of mean deviation for XI (upper figure) and XII (lower figure) compared with I are 0.514 to 0.567 and 0.422 to 0.693 Å, respectively. The representative set of TRH analogues used for Figure 3 is displayed to give a basis for assessing the quality of the fit.

As stated in the introduction, we have compared the conformations of the TRH analogues with those observed in two crystal structure determinations[18,19] for Leu[5]-enkephalin. Literature reports[6,24] indicate that TRH, opiates (such as morphine), and opiate antagonists (such as naloxone) interact but apparently not by binding to the same receptors. We are unaware of any direct comparisons of TRH and enkephalin receptor binding but felt that since both are CNS active and interact with some of the same drugs, a comparison of conformational properties for the two peptide systems could be of interest.

We have detected a surprising degree of similarity between the conformations of the TRH analogues and those of the last three residues of the enkephalin. There are eight independent observations for the latter system (four in XI and four in XII). We have compared the conformations directly,[26] by least-squares superposition methods, with the conformation of I. Figure 7 displays the best and worst fit conformations from each crystal structure determination plotted, together with representative examples of the TRH analogues. The similarities are sufficient to suggest that TRH might bind to some enkephalin receptors or vice versa. If TRH does bind to enkephalin receptors, the smaller molecule will very likely only interact with part of the binding site, which implies that different physiological effects might result. We hope that these observations will provide incentive for suitable biological studies for the determination of the presence or absence of TRH–enkephalin receptor interactions.

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Supplementary Material Available: Tables of atomic coordinates, bond distances, bond angles, torsion angles, temperature factors, and observed and calculated structure factors for compounds I–VII (169 pages). Ordering information is given on any current masthead page.

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Notes

The Covalent Linking of Two Nucleotide Analogues to Antibodies

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Two anticancer drugs, antagonists of nucleic acids, were covalently linked to antibodies specifically reactive with B leukemia cells and thus with a potential possibility of drug targeting to the tumor site. The drugs cytosine 1-β-D-arabinoside and 5-fluorouridine, competitive inhibitors of enzymes involved in DNA synthesis, were linked to antibodies via a dextran bridge. Cytosine 1-β-D-arabinoside was linked to periodate-oxidized dextran and fluorouridine to dextran hydrazide. The dextran derivatives were in turn linked to antibodies that recognized a specific membrane IgM on B leukemia cells. The drug–antibody conjugates maintained most of the original antigen-binding capacity of the antibody and the full pharmacological activity of the drugs.

The major drawback of cancer chemotherapy is its toxicity to normal cells. One possible way to increase the effectiveness of antitumor drugs would be to find methods of altering their distribution in the body so as to increase their local concentration at the tumor site while maintaining lower systemic concentrations. This could be achieved by chemical coupling of drugs to carriers such as antibodies with preferential affinity toward tumor cells. The linking of antineoplastic agents such as drugs or toxins to antibodies specifically or preferentially reactive with tumor cells has gained wide interest in recent years. Antibodies with the capacity to specifically recognizing tumor cells and tissues have by now been developed and some of them were shown to be able to concentrate the tumors sites in vivo.[1] We have previously bound the antineoplastic-reactive antibiotics daunomycin and adriamycin to antibodies and used these conjugates as therapeutic agents in vitro and in vivo in several tumor systems.[2] Although these two drugs are effective against many types of neoplasia, some tumors are almost unresponsive to them yet are sensitive to other drugs such as 5-fluorouracil. A malignant growth is usually made up of more than one type of cell.[3] The development of this variability is a continuous process often furthered by drug treatments that can lead to the development of resistant cell clones. For that reason chemotherapeutic treatment regimes often involve mixtures of drugs with different mechanisms of action. We have, therefore, extended our studies to the linking of two additional drugs of a different nature, antimetabolites of nucleic acids, to antibodies. Both these drugs inhibit competitively enzymes involved in DNA synthesis. 5-Fluorouridine (FU) inhibites thymidylate synthetase, while cytosine arabinoside (ARA-c) inhibits DNA polymerase.[4]

We describe the binding procedures of these drugs to antibodies through backbones of dextran derivatives and their inhibitory activity on culture tumor cell lines.

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(2) Arnon, R.; Sela, M. *Immunol. Rev.* **1982**, *62*, 5.
(3) Poste, G.; Fidler, I. J. *Nature (London)* **1980**, *283*, 139.
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