in only small amounts in aqueous solutions of picropodophyllotoxin, is the form responsible for the inhibition of microtubule assembly by this drug.

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A Model for Thyroid Hormone–Receptor Interactions

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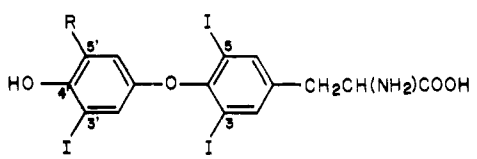
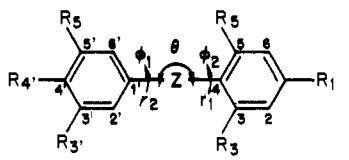
Theoretical electronic structure calculations on the thyroid hormones and analogues, as well as model hormone–receptor interactions, have been carried out. These studies (a) support the concept that the 4′-OH group is a H-bond donor to the in vivo nuclear receptor and suggest that at the receptor this OH group is trans to the 3′ (distal) substituent; (b) indicate that there is an important intramolecular interaction between 3′ and 4′ substituents, and those 3′ substituents that most favor both 4′ OH orientation trans to the 3′ group and a more acidic OH group substantially increase binding and biological activity; and (c) support the concept that there is a direct correlation between the conformational free energy of the aromatic rings and biological activity.

The two thyroid hormones, thyroxine (T_4: I, R = I) and



I, R = H or I

triiodothyronine (T_3: I, R = H), elicit a multitude of biological responses and are essential for normal growth and development.[1,2] A number of hypotheses[2] have been proposed to relate the various structural features of the thyroid hormones and their analogues (II) to the expression of their biological effects.



II

Biological activities measured in whole animals are influenced by metabolism, distribution, and the sequence of events between binding and the expression of their biological effect. Thus, an important recent advance in the study of the thyroid hormones and their analogues has been the development of suitable in vitro assays which appear to correlate well with in vivo thyromimetic activity.

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These assays measure the binding affinities of thyroid hormones and their analogues to isolated intact rat hepatic nuclei[3-8] and to solubilized rat hepatic nuclear nonhistone proteins.[9-11] In addition, the binding affinity of the hormones and their analogues to various purified plasma proteins has been determined.[12-14] Results of these studies which are especially pertinent for this paper are the following: (a) in binding of analogues to both rat hepatic intact nuclei and solubilized nuclear protein, there is a near quantitative 1:1 correlation between the binding constant and the in vivo rat antigoiter activity, once adjustments are made for the well-established in vivo metabolism of certain analogues;[3-5,8,9] and (b) there are similarities, as well as differences, between the structure–binding affinity relationships to the nuclear and plasma proteins.[5,12-14] It thus appears that these in vitro test systems are among the best currently available for the application of physicochemical approaches to understanding biological activity.

In particular, it now becomes useful to attempt to relate the free energies of association of thyroxine analogues to proteins to the conformational and H-bonding properties which come from theoretical calculations, and this is what we attempt to do in this paper. A fundamental assumption in this approach is that the most tightly bound analogue "sees" protein atoms which are complementary to it (e.g., form H bonds to available groups) but that less well-bound analogues are unable to form all the favorable contacts. One can assume that the protein can change its conformation to better accomodate less tightly bound analogues but only at a net cost in the free energy of binding.

Methods

(A) Analysis of Substituent Contributions. A formal theory for the analysis of group contributions has been presented elsewhere.[14] Here we present a very simplified version of this theory. The apparent standard free energy of binding ($\Delta G°$) of an analogue to a macromolecule is

$$\Delta G° = -RT \ln K \tag{1}$$

where R is the gas constant, T = 310 K (experimental temperature), and K is the binding constant. ΔG can be expressed as

$$\Delta G° = \Delta G°_{ref} + \sum_{i=1}^{n} \Delta G^1(X_i) + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \Delta G^2(X_i,Y_j) \tag{2}$$

where $\Delta G°_{ref}$ corresponds to the apparent standard free energy of binding of a reference compound, and n is the total number of (X) groups. $\Delta G^1(X)$ is the contribution to the standard free energy of binding of group X relative to the contribution of a reference group present at the same position. If the reference group is a hydrogen atom, $\Delta G°(X)$ is given by

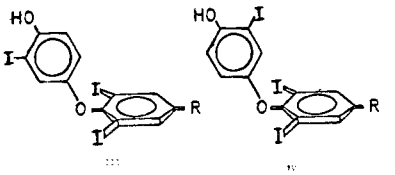
$$\Delta G°(X) = \Delta G°(AX) - \Delta G°(AH) = -RT \ln K_{AX}/K_{AH} \tag{3}$$

where $\Delta G°(AX)$ is the apparent standard free energy of binding of the hormone containing group X at a certain position and $\Delta G°(AH)$ is the standard free energy of binding of the hormone having a hydrogen atom in the same position. K_{AX} and K_{AH} are the corresponding binding constants. The $\Delta G^2(X,Y)$ term is defined by

$$\Delta G^2(X,Y) = [\Delta G°(AXY) - \Delta G°(AHH)] - [\Delta G°(AXH) - \Delta G°(AHH)] - [\Delta G°(AHY) - \Delta G°(AHH)] \tag{4}$$

This term corrects for the perturbing effect that groups X and Y have on each other when present together in the same molecule relative to their separate effects when present in different molecules.

(B) Determination of ΔG_{lock}. For compounds that have no bulky 2′ substituents it is not possible to determine whether the biologically active conformation is distal (III)



or proximal (IV) on the basis of physical studies (experimental or theoretical) on the hormone. This is due to the observation that for such compounds the proximal and distal energies are almost equal.[15] Thus, it appears that the distinction between these two conformations is a function of the receptor. It is possible, however, to test the concept that the hormone acts in a perpendicular conformation without specifying whether such conformation is proximal or distal. This is accomplished by evaluating the free energy required to "lock" analogues (ΔG_{lock}) into the perpendicular ring conformation.[16]

To determine ΔG_{lock}, the energy was calculated as a function of ϕ_1 and ϕ_2 by the CNDO/2 molecular orbital procedure,[18] and the resulting potential-energy surface was used to obtain a classical Boltzmann distribution of conformers.[20] The justification for using a classical instead of a quantum mechanical partition function is that $kT > 20\Delta E$, where k is the Boltzmann constant, T = 310 K, and ΔE is the separation between the vibrational energy levels (based on the force constants obtained from the curvature of the potential energy well). The relative population of the mutually perpendicular ring conformation is given by

$$K = \frac{4 \exp(-E(90,0)/RT)}{\int_0^{360} \int_0^{360} \exp[-E(\phi_1,\phi_2)/RT] d\phi_1 d\phi_2} \tag{5}$$

and

$$\Delta G_{lock} = -RT \ln K \tag{6}$$

where R is the gas constant, T = 310 K (the temperature of the experiment), and the integral in the denominator is the partition function for the ϕ_1 and ϕ_2 degrees of freedom. The factor of 4 in the numerator of eq 5 arises from the presence of four perpendicular conformations: 90,0, 90,180, 270,0, and 270,180, whose energies are approximately equal.

If we used a factor of 2 instead of 4 in eq 5, we would be evaluating the free energy needed to lock the molecule into *just* the distal (III) or just the proximal conformation (IV). Our analysis of the biological activities uses the *relative* ΔG_{lock} (see Results and Discussion); thus, it is irrelevant which factor we use. Because of this "indeterminacy", our analysis allows us only to test the hypothesis of perpendicularity of the thyroxine conformation but not whether they are proximal or distal.

The energy was calculated at 30° intervals of ϕ_1 and ϕ_2. To demonstrate the feasibility of using such a crude grid, the potential energies of 2,6,3′-triiodo-4′-hydroxydiphenyl ether and of its 2,6-dichloro analogue were calculated at 10° intervals of ϕ_1 and ϕ_2, and the results from the two grids were compared. It was found that the minimum energy conformations, rotational barriers, and ΔG_{lock} are in excellent agreement in the two grids.

Although we have derived ΔG_{lock} (eq 6) for the special case of the diphenyl ether linkage, it is a more general concept and can be applied to test conformational preferences for any receptor model. In the general form it may

be applied to any number of rotational degrees of freedom.

(C) Computational Details. The CNDO/2 molecular orbital method[18] was used in the majority of the calculations. Except for the halogens, the standard atomic parameters were used.[18] For comparison with the CNDO/2 results, ab initio molecular orbital calculations using the Gaussian 70 quantum chemistry program[21] with an STO-3G basis set[22] were carried out in selected cases. Unless specifically noted,[23] standard geometrical parameters (selected as suitable average values from available experimental data)[24,25] were those previously used in our study of the intramolecular interactions of ortho-substituted phenols.[19] The integral in the denominator of eq 5 was evaluated numerically. Our use of CNDO/2 in these studies is supported by the good qualitative agreement with experiment found in ref 15 for the diphenyl ether conformational analysis. For the outer-ring H-bonding studies, the choice of this method is supported by the fact that CNDO/2 appears to be among the best semiempirical methods for H-bonding studies.[18] We also used ab initio calculations where appropriate below to check (refine) the CNDO/2 predictions.

Results and Discussion

There are three key areas in thyroid hormone analogues. These are the outer ring (3′, 4′, and 5′) substituents, the interring linkage and 3,5 substituents, and the amino acid side chain. In each of the following sections, we will describe our theoretical calculations and show how the theoretical calculations and the qualitative structure–activity relationships can be combined to suggest the nature of the hormone–receptor interaction.

Role of Outer Ring Substituents in Determining Activity. (a) Structure–Activity Relationships. The structure–activity relationships pertaining to the 3′, 4′, and 5′ substituents for the in vivo rat antigoiter assay[2] and in vitro binding to *nuclear receptors*[3-9,26] can be briefly summarized as follows: Maximal activity results from monosubstitution ortho to the 4′-OH by a moderately lipophilic alkyl or halogen 3′ group, and it is the distal conformation (III) and not the proximal conformation (IV) which is the active form of analogues monosubstituted ortho to the 4′-OH. Disubstitution (3′ and 5′) ortho to the 4′-OH decreases activity (as compared to monosubstitution) in direct proportion to the size of the second ortho substituent, and a 4′-OH group imparts maximal activity, with 4′-NH_2, 4′-OCH_3, and 4′-H groups decreasing activity by 10- to 100-fold.[2,26]

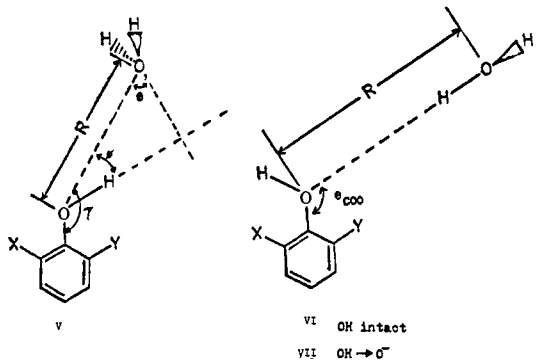
Similarly, the structure–activity relationships pertaining to the 3′, 4′, and 5′ substitutents for in vitro binding of analogues to the human *plasma protein*, thyroxine binding globulin (TBG),[12-14] can be briefly summarized as follows: maximal binding results from disubstitution ortho to the 4′-OH by moderately lipophilic, electron-withdrawing 3′ and 5′ substituents with compounds monosubstituted ortho to the 4′-OH and compounds with electron-donating 3′,5′ substituents having very low binding affinities. 4′-OCH_3, 4′-NH_2, and 4′-H substituents cause a 30- to 50-fold decrease in binding, as compared to the 4′-OH.

On the basis of the in vivo rat antigoiter activities of analogues,[2] the binding of analogues to intact rat hepatic nuclei[3,4] and to solubilized nuclear protein,[9,26] it appears that the un-ionized 4′-phenolic hydroxyl is forming an intermolecular hydrogen bond with some appropriate functional group on the "receptor". In contrast, studies of the relative binding affinities of analogues to TBG[12,13] suggest that it is the 4′-phenoxide ion that binds to this plasma protein. The human plasma protein prealbumin has also been found to strongly bind the thyroid hormones and analogues with the binding affinity higher for T_4 than for T_3.[14,27-30] In addition, X-ray crystallographic studies[31,32] of prealbumin show that the vicinity of the binding site where the 4′-O^- apparently binds contains no charged amino acid side chains but rather a number of aliphatic hydroxyl groups. On the basis of these X-ray studies and the similarity of TBG and prealbumin binding, we have assumed that the 4′-O^- binds in both proteins via a hydrogen bond.

Therefore, we decided to use theoretically calculated inter- and intramolecular hydrogen bond strengths of ortho-substituted phenols and phenoxides in order to investigate (a) likely orientations for possible nuclear receptor and plasma protein proton donor and proton acceptor groups, and (b) the reasons for the observed order of biological activities for compounds with different 3′, 4′, and 5′ substituents.

(b) The Models Used. As noted above, the in vivo and in vitro structure–activity relationships indicate that the "distal" conformation is the biologically active one. For the purposes of modeling the interaction of the 3′, 4′, and 5′ substituents with receptor, we have assumed that the outer ring is in the correct location on the receptor with the 3′ group oriented in a "distal" conformation (III).

We have already conducted an extensive theoretical CNDO/2 and ab initio examination of the intramolecular hydrogen bonding of ortho-substituted phenols.[19] In this study, we examine the intermolecular hydrogen bonding of ortho-substituted phenols as a model system for the un-ionized phenolic ring binding to nuclear receptors and the intermolecular hydrogen bonding of ortho-substituted phenoxides as a model system for the ionized phenolic ring binding to TBG. H_2O was used both (a) as a model proton acceptor in the nuclear receptor when the un-ionized phenol is functioning as a proton donor (V) and (b) as a



model proton donor in the nuclear receptor when the un-ionized phenol is functioning as a proton acceptor (VIa) or in TBG when the ionized phenol is functioning as a proton acceptor (VIb). Water is an appropriate model for the H-bond donor/acceptor for a number of reasons: (a) aliphatic hydroxyl groups on prealbumin have been implicated in the H bonding to outer-ring groups in the thyroxine–prealbumin complex;[31,32] (b) the H-bonding donating and accepting properties of H_2O are very similar to those of CH_3OH,[34] which is a very appropriate model to the threonine and serine OH groups on prealbumin; (c) calculations using water as a H-bond donor and acceptor are simpler to carry out because of the fewer degrees of freedom to vary; and (d) water as the H-bond model has the least steric bulk of any possible H-bond donor or acceptor; thus, use of it as a model allows us to focus on steric effects due to thyroxine ring substituents.

The geometry of the model system for proton donation of a phenol to H_2O (V) was defined as follows. The two monomeric units lie in perpendicular planes with the

Table I. CNDO/2 and Ab Initio Hydrogen Bond Energies (ΔE in kcal/mol) of Phenol as Proton Donor to H_2O (V: X = Y = H)

	ϕ (degrees)					
R (Å)	0	10	20	30	50	70
ab initio						
2.63[a] – ΔE	8.93	8.70	7.36	5.62	2.87	1.89
θ[b]	47	58	67	77	98	126
CNDO/2						
2.63[a] – ΔE	6.05	5.64	4.72	3.49	1.51	1.06
θ[b]	47	58	67	77	98	126
2.54[c] – ΔE	6.63	6.42	5.52	4.18	1.69	1.52
θ[d]	12	11	17	24	73	157

[a] Minimum energy (ab initio) value for phenol at $\phi = 0°$. [b] Minimum energy (ab initio) values for phenol at $R = 2.63$ Å. [c] Minimum energy (CNDO/2) value for phenol at $\phi = 0°$. [d] Minimum energy (CNDO/2) values for phenol at $R = 2.54$ Å.

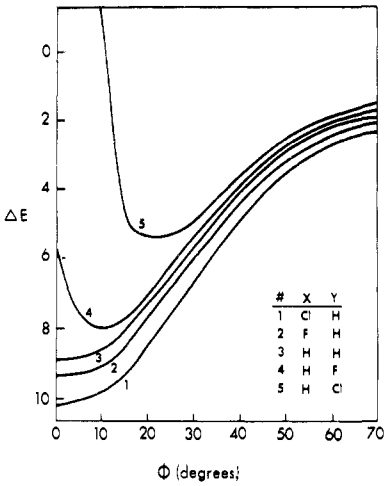


Figure 1. Ab initio hydrogen bond energies (ΔE, in kcal/mol) of various phenols as proton donors to H_2O (V). The θ and R values used are listed in Table I.

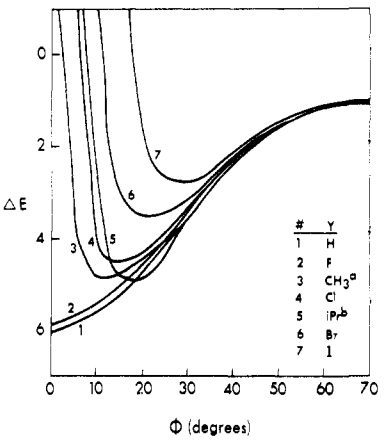


Figure 2. CNDO/2 hydrogen bond energies (ΔE, in kcal/mol) of various phenols as proton donors to H_2O (V, X = H): for number **3**, the superscript a indicates that the CH_3 group is staggered; for number **5**, the superscript b indicates that the *i*-Pr CH_3 groups are pointed away from OH and staggered. The R and θ values used are listed in Table I.

phenolic OH and O of the H_2O coplanar with the aromatic ring and with the aromatic ring plane bisecting the internal angle of the H_2O. We then evaluated the hydrogen bond energies (ΔE)[33] with respect to variations in R (the O···O distance), ϕ (the angle between the phenol O–H bond and the O···O line), and θ [the angle between the O···O line and the bisector of the HOH (water) angle] for phenol itself

Table II. CNDO/2 and Ab Initio Hydrogen Bond Energies (ΔE in kcal/mol) of H_2O as Proton Donor to Phenol (VIa: X = Y = H)

	ϕ_{COO}						
	110	120	125	130	140	160	180
ab initio							
$-\Delta E$	3.33	4.14	4.31	4.30	4.05	2.53	0.46
R, Å[a]	2.83	2.80	2.79	2.79	2.80	2.90	3.37
CNDO/2							
$-\Delta E$	5.94	6.16	6.12	6.06	5.83	4.77	2.83
R, Å[b]	2.57	2.56	2.54	2.56	2.56	2.60	2.74

[a] Minimum energy (ab initio) values for phenol at each ϕ_{COO}. [b] Minimum energy (CNDO/2) values for phenol at each ϕ_{COO}.

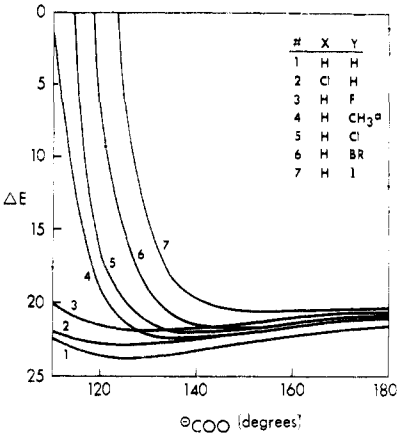


Figure 3. Ab initio hydrogen bond energies (ΔE, in kcal/mol) of H_2O as proton donor to various phenols (VIa).

(V: X = Y = H; Table I and Figures 1 and 2). With $\phi = 0$, R and θ were simultaneously varied to yield a minimum energy at $R = 2.63$ Å and $\theta = 47°$ (ab initio) and $R = 2.54$ Å and $\theta = 12°$ (CNDO/2). The interaction energies at these geometries (ΔE) were 8.93 kcal/mol for the ab initio calculations and 6.63 kcal/mol for CNDO/2. With R fixed at these values, ΔE was minimized as a function of θ for different values of θ The results of these searches are summarized in Table I.

As can be seen in Table I, the energy changes for variations in θ and ϕ are more reasonable for the ab initio (formation of a bifurcated hydrogen bond as ϕ increases) calculations than for CNDO/2 (H_2O protons directed at the phenolic O at $\phi = 70°$).[33] Hence, all further CNDO/2 and ab initio calculations were performed using the ab initio minimum energy values of R and of θ as a function of ϕ. Although the ab initio energies are more attractive than the CNDO/2 ones, the shapes of the ab initio and CNDO/2 ΔE vs. ϕ curves for phenol are approximately the same and all minimize at $\phi = 0°$ (O–H--O colinear) (Figures 1 and 2).

The geometry of the model system for H_2O as proton donor to a phenol (VIa) was defined as follows. The two monomeric units lie in perpendicular planes with the phenolic OH and the HO of the H_2O involved in the hydrogen bond coplanar with the aromatic ring. The O--H–O involved in the hydrogen bond are colinear, since this geometry should give near maximal hydrogen-bond strength.[34] The second O–H bond of the H_2O lies in a plane perpendicular to the aromatic ring plane in order to minimize any interactions of this second H_2O proton with the phenol (see VIa).

In this case, ΔE was minimized with respect to R at different values of θ_{COO} for phenol itself (VIa, X = Y = H). Table II summarizes these studies. In these searches, the

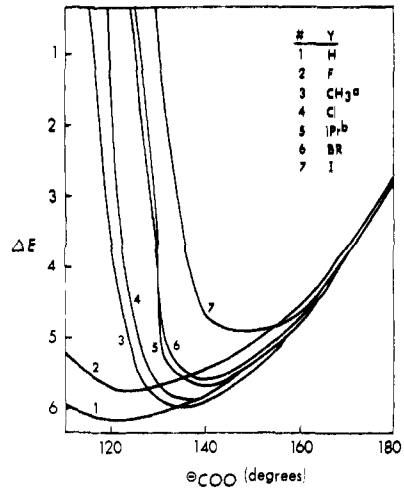


Figure 4. CNDO/2 hydrogen bond energies (ΔE, in kcal/mol) of H_2O as proton donor to various phenols (VIa, X = H): for number **3**, the superscript a indicates that the CH_3 group is staggered; for number **5**, the superscript b indicates that the *i*-Pr CH_3 is pointed away from OH and staggered.

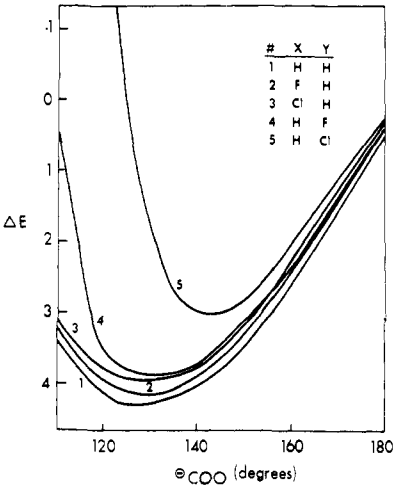


Figure 5. CNDO/2 hydrogen bond energies (ΔE, kcal/mol) of H_2O as proton donor to various phenoxides (VIb): for number **4**, the superscript a indicates that the CH_3 group is staggered.

interaction energies for CNDO/2 are greater than those found with ab initio. The shapes of the ab initio and CNDO/2 ΔE vs. θ_{COO} curves (Figures 3 and 4), however, are approximately the same, for both minimize at θ_{COO} ~125° (hydrogen bond approximately bisecting the COH angle of phenol). All further calculations were performed utilizing these minimum energy values of R as a function of θ_{COO} presented in Table II.

The geometry of the model system for H_2O as a proton donor to a phenoxide was defined as above for H_2O as a proton donor to a phenol (VIb), except the phenolic proton is left out and the phenoxide R(C–O) is shortened to 1.33 Å.[23] Here again, CNDO/2 ΔE values were determined for simultaneous variations of R and θ_{COO} for phenoxide (VIb: X = Y = H). The minimum-energy R values are essentially invariant (and are considerably smaller than those found for phenol) as θ_{COO} is varied. Figure 5 shows that as θ_{COO} is varied ΔE varies very little, minimizing at θ_{COO} ~125° and is about four times ΔE for the corresponding phenol–HOH dimer. All further calculations were performed utilizing these minimum-energy values of R as a function of θ_{COO}.

(c) The Effect of Variations of Substituents on the Interaction of Phenol and Phenoxide with Water. Utilizing the reference geometries (V, VIa, and VIb) as defined above for X = Y = H and minimum energy, R, ϕ, ϕ, and θ_{COO} values, ΔE values were calculated for a number of variations of X, Y, and geometrical parameters and are described below.

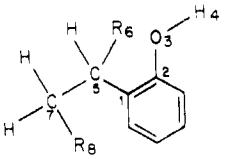
When the phenolic OH group acts as a proton acceptor (VIa), the variation in the CNDO/2 H-bond energies upon substitution trans to the approaching H_2O (Y = H and X = H, F, Cl, Br, I, CH_3, *i*-Pr or OH) is very small (±0.2 kcal/mol). In contrast, the ab initio calculations suggest a larger and chemically more reasonable decrease in the H-bond acceptor ability of the phenol upon o-halogen substitution (Y = H, X = F, Cl). In almost all cases, the trend is as follows: electron-withdrawing X substituents reduce the electron density on the phenolic O, decreasing ΔE for VIa and VIb and increasing it for V; electron-donating X substituents, of course, have the opposite effect.[19]

The situation is quite different when the Y substituent is varied, since it is capable of sterically interacting with the H_2O molecule. Figures 1 and 2 show that strong repulsive interactions result between Y and the H_2O molecule for small values of ϕ for V (phenols functioning as proton donors). These repulsive interactions decrease as ϕ increases, until the ΔE for the Y-substituted phenol approaches that of unsubstituted phenol once the H_2O and Y substituent are no longer within contact distance.[35] Obviously, the larger Y is the larger the repulsive H_2O–Y interactions are and the larger the ϕ value must be before ΔE returns to the unsubstituted phenol value. The same holds true for phenols and phenoxides acting as proton acceptors (VIa and VIb) replacing ϕ with θ_{COO}.

It was also found for both the CNDO/2 and ab initio calculations that the electronic effects of the X substituent and the electronic and steric effects of the Y substituent on the hydrogen bond energies are essentially additive for V and VI. That is, X and Y act essentially independently in their influence on intermolecular hydrogen-bond formation. That is why we do not explicitly discuss our calculations on compounds in which both X and Y ≠ H.[36]

(d) Out of Plane Movement of H_2O. Variation of ΔE upon movement of the H_2O molecule's oxygen out of the phenol or phenoxide plane was also examined with CNDO/2. Maintaining θ = 110° (V) and the phenolic OH coplanar with the aromatic ring, movement of the H_2O out of the ring plane, and hence loss of O–H- -O colinearity, resulted in a large hydrogen bond energy loss for phenol (V: X = Y = H) for more than about 20° movement of the H_2O oxygen out of the phenol plane. For 2,6-diiodophenol (V: X = Y = I), a similar movement results in loss of H_2O–I repulsion but no significant overall phenol–H_2O attraction. Experimental studies on phenol suggest that the molecule is planar with a rotational barrier for rotation around the CO bond of ~3 kcal/mol. Thus, we did not examine nonplanar Ph-OH structures in this study.[35] Maintaining θ_{COO} = 125° for VIa and the phenolic hydroxyl coplanar with the aromatic ring, movement of the H_2O out of the aromatic plane retains O–H- -O colinearity. Hence, such a movement has almost no effect (loss of only ~0.3 kcal/mol for an out of plane motion of 45°) for phenol (VIa: X = Y = H) and results in a significant (~4.5 kcal/mol) phenol–H_2O attraction for 2,6-diiodophenol (VIa: X = Y = I). A similar result was found for movement of H_2O out of plane for the phenoxide–H_2O model system (VIb) where phenoxide (VIb: X = Y = H) and 2,6-diiodophenoxide (VIb: X = Y = I) were compared. In short, out of plane movement of water results in no significant changes for small Y substituents but is favored in the case of large Y substituents.

(e) 3′-Alkyl Group Conformation. Since the 3′-substituent might influence the cis–trans isomerism of the 4′-OH, we carried out a conformational analysis for *o*-ethyl (VII, $R_6 = R_8 = H$), o-*n*-propyl (VII, $R_6 = H$, $R_8 = CH_3$),



VII

o-isopropyl (VII, $R_6 = CH_3$, $R_8 = H$), and o-*sec*-butyl (VII, $R_6 = R_8 = CH_3$) phenols. The most conspicuous features of these conformational analyses are (a) the low energy conformations have the $C_1C_5C_7$ plane approximately perpendicular to the aromatic plane ($\phi_{2157} \approx 90°$ with ϕ_{1578} fairly unrestricted) and (b) branching on the carbon α to the ring ($R_6 \neq H$) tends to favor conformations with $\phi_{2156} > 90°$ in order to relieve steric repulsion between alkyl side chains and the phenolic oxygen. In general, the alkyl groups tend to extend away from the hydroxyl group. With the OH cis to the alkyl group ($\phi_{1234} = 0°$), conformations with $\phi_{2156} < 60°$ tend to be completely excluded.[36]

On the basis of the above calculations and analysis of various binding affinities, we attempt to answer the questions raised above concerning the probable nature of the interactions between outer-ring substituents of thyroid hormone analogues with groups on the plasma proteins and nuclear receptors.

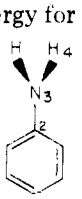
(f) Outer-Ring Substituent–Receptor Interactions. What is the geometry of the hydrogen bond formed between a thyroid hormone and TBG? The binding affinity of L-T_3 to TBG is only 9% that of L-T_4.[13] The 4′-hydroxyl group of T_4 is almost 100% ionized at the pH used. The geometrical orientation of the proton donor on the TBG molecule must be such that the 3′- and 5′-iodines provide little, if any, steric interference to this hydrogen-bond formation. This suggests that the geometrical orientation of the TBG proton donor is such that either θ_{COO} is substantially greater than 125° but less than 180° and/or that the proton donor's approach to the phenoxide ion is substantially out of the phenoxide ring plane (VIb and Figure 5).

In hydrogen bonding to the nuclear receptor, is the 4′-phenolic hydroxyl functioning as a proton donor (V), as a proton acceptor (VIa), or both? Also, is the 4′-hydroxy directed "cis" or "trans" to the 3′ position? The possible role of the 4′-hydroxyl as a proton donor or acceptor to receptor is best approached by examining relative in vitro binding potencies of analogues to rat hepatic nuclei.[3-9] If the 4′-hydroxyl were functioning only as a proton acceptor, as it presumably does in TBG, then one would expect the binding affinities of analogues to be directly proportional to electron-withdrawing abilities of the 3′ and 5′ substituents. The in vitro binding studies have shown that the binding of analogues to these nuclear receptors is only slightly effected by the electronic interactions of the 3′ and 5′ substituents with the 4′-hydroxyl. In addition, the binding to nuclear receptors is approximately equal for analogues with 3′-alkyl or 3′-halo substituents of approximately equal lipophilicities. As will be shown below, the relative binding affinities and physical properties of 3′,4′-substituted analogues support the model of the 4′-hydroxyl functioning as a proton donor which is directed "trans" to the 3′ position. The contribution [ΔG^1(4′-OH)] of the 4′-hydroxyl to the free energy of binding to the nuclear receptors can be calculated (eq 3) as the difference in $\Delta G°$ values for binding of a 4′-deoxy analogue and the corresponding 4′-hydroxy compound. The 4′-hydroxyl contributions have been calculated[9,37,38] as −1.23, −1.61, and −1.91 kcal/mol in the presence of $R_{3'}$ = H, CH_3, and *t*-Bu, respectively. The relative magnitude of these values are consistent with what would be expected if the increasing bulk of these 3′-alkyl substituents were orienting the 4′-hydroxyl toward the 5′ position where a proton acceptor resides on the receptor. Similarly, the 4′-hydroxyl contributions in compounds with 3′-monohalo substitution becomes more favorable as the strength of the intramolecular hydrogen bond[19] between the 3′-halo and the 4′-hydroxyl decreases [ΔG(4′-OH) = −1.47, −2.03, −2.48, and −3.60 kcal/mol for 3′-F, 3′-Cl, 3′-Br, and 3′-I, respectively]. This suggests that the easier it is to break the 3′-halo–4′-hydroxyl hydrogen bond and allow the 4′-OH to be oriented toward the 5′ position, the more favorable is the contribution of the 4′-OH to binding. That the 3′-halogen substituents (especially Br and I) enhance the contribution of the 4′-hydroxyl group more than the 3′-alkyl suggests that the 3′-halogens are significantly enhancing the 4′-OH proton donor ability via electron-withdrawing effects. This effect was seen in the ab initio calculations described above.

With this model, it becomes plausible to ascribe at least part of the intolerance of the nuclear receptor to a 5′ substituent on the basis of the interference of this substituent with the 4′-OH--receptor hydrogen bond. Our theoretical studies predict strong repulsive potentials for bulky 5′ substituents if $\phi = 0°$ [V (Figure 1 and 2)]. Although the 5′ bulk is detrimental for in vivo activity and in vitro binding, even a group as large as iodine causes only ~1.27 kcal/mol loss in binding free energy. This suggests that the receptor proton acceptor is probably so oriented that steric interaction with 5′ substituents is reduced, e.g., by orientation either out of the phenolic ring plane and/or with ϕ greater than 0°. Thus, a 5′ substituent sterically interferes with hydrogen-bond formation between the 4′-hydroxyl and receptor, in addition to its probable direct involvement in repulsive steric interactions with the receptor.

In order for the model of the 4′-hydroxyl as a proton donor directed toward the 5′ position to be acceptable, it must also be able to account for the low in vitro binding affinities of various 4′-OCH_3, 4′-H, and 4′-NH_2 analogues.[26] CNDO/2 studies of benzene–H_2O dimers (to model the 4′-H substituted analogues) gave only very small repulsive interactions for H_2O as a proton donor (0.59 kcal/mol) or as a proton acceptor (0.27 kcal/mol). Anisole (to model the 4′-methoxy substituent) was calculated to be as good a proton acceptor as phenol. Using V (X = Y = H), replacing the OH group with a staggered OCH_3 group, the dimer gives rise to substantial repulsive interactions using the minimum energy R from phenol–H_2O. Experimentally, 4′-OCH_3 analogues are generally found to bind less strongly than the corresponding 4′-OH analogues, in agreement with a 4′-OH donor model.

The hydrogen-bond energy for aniline (VIII) (to model



VIII

the 4′-NH_2 substituent) as proton donor to H_2O was calculated by CNDO/2 to be −3.85 kcal/mol with the NH_2 group coplanar with the aromatic ring and with θ_{HNH} = 120°. The microwave spectrum of aniline,[39] however,

predicts that the NH_2 group adopts an out of plane angle of 37.5° with $\theta_{HNH} \approx 113.1°$. With the microwave spectrum geometry for aniline and with H_2O as a proton acceptor (but with the H_2O oxygen coplanar with the ring plane), one finds a hydrogen-bond energy for the dimer of only −2.56 kcal/mol.[40] Assuming the 4′-OH proton donor model, it is clear why the 4′-NH_2 binds less strongly than 4′-OH.

How do large groups at the 3′ position effect binding? In nuclear or TBG binding, steric bulk in the 3′ position is advantageous for hydrophobic groups as large as isopropyl or iodine but detrimental for substituents which extend out from the molecule a distance greater than the size of iodine.[4,9,38,41] The conformational analysis we described above for 3′ substituents suggests that they do not sterically interfere with interactions of the 4′-OH with receptor or TBG. However, the tendency of alkyl chains to orient above the ring and away from the phenolic hydroxyl could be detrimental to binding by exerting unfavorable steric effects in this direction. The fact that any 3′ substituent with more than a two-carbon chain (e.g., 3′-*n*-propyl) is significantly less tightly bound to the nuclear receptor than those of similar total size (3′-*i*-Pr), with only two-carbon extensions from the 3′ position, is consistent with this picture of steric interference.

The relatively low binding affinity of 3′-*t*-Bu analogues and the relative binding order of 4′ substituents OH > OCH_3 > H does indicate the possibility that the 4′-OH is functioning as a proton acceptor from the 3′ side in addition to being a proton donor at the 5′ side. Figure 4 indicates that, with a receptor proton donor approach at $\theta_{COO} \approx 150°$, the presence of steric bulk at the 3′ position (VIa, Y = *i*-Pr) costs only ~0.5 kcal/mol in H-bonding strength. The presence of a receptor proton donor group also helps explain why 4′-OCH_3 analogues are more tightly bound than 4′-H.

Further experiments will be interesting in order to test this "picture" of the receptor. One might test 4′-H or -F, 5′-OH or -CH_2OH compounds in order to ascertain whether 5′ substituents interfere with 4′-H bonding or because there is steric repulsion with the receptor.

Conformation of the Diphenyl Ether Nucleus. In a previous study,[15] we carried out a limited conformation analysis of thyroxine analogues with 3,5 substituents I_2, Br_2, Cl_2, F_2, $(CH_3)_2$, and H_2 and noted the differences in the minimum-energy geometries were qualitatively correlated with biological activity. Those with minimum-energy geometry near ϕ_1,ϕ_2 = 90,0 were the most active. Here, we carry out a more complete investigation of the potential-energy surface as well as analysis of conformer populations. We also broaden our consideration to include variations in the interring linkage, as well as further variations in the 3,5 substituents. We also attempt to quantitatively relate the conformational free energies we obtain to those found in the in vitro binding assays and in vivo biological activities.

X-ray,[42-47] NMR,[48] and theoretical studies[15] suggest that with large 3,5 substituents there are four minimum-energy geometries in which the two aromatic rings are close to being perpendicular and that these conformations ["distal" (III) has the $R_{3'}$ "away from" the inner ring; "proximal" (IV) has the $R_{3'}$ "toward" the inner ring] are of approximately equal energy and are readily interconvertible at room temperature. Adding a 2′-CH_3 substituent leaves only minimum-energy geometries in which the 3′ group is distal to the inner ring.

Our calculations[15] using CNDO/2 indicate that the proximal and the distal conformations differ by not more than 0.1 kcal/mol in all cases studied. Based on this small energy difference, the ratio of proximal and distal conformer populations is 1.2. Cody et al.[42-45] found that in their crystallizing media, all thyroid hormone analogues studied crystallized in the distal conformation. This fact, taken in conjunction with the Camerman's observation of the proximal conformation,[49-51] supports the fact that the two conformations are close in energy so that small modifications of the crystallizing medium favor one conformation or another. Recently, the low-temperature NMR finding of Emmett and Pepper[48] that the population of the distal conformer is 0.5–0.6 (and hence the ratio of proximal/distal = 0.7–1.0) for 1-methyl-3,3′,5-triiodo-4′-hydroxy diphenyl ether again attests to the almost equal energies of the two conformers.

As noted previously in ref 15, at least two effects might play a role in determining the conformation of the diphenyl ether nucleus. These are (a) the mesomeric (*resonance*) *effect* [the lone pair orbitals on the interring are stabilized by conjugation with the p orbitals on the adjacent ring carbon atoms (C_1' and C_4); this effect favors a coplanar conformation of the rings (ϕ_1 and ϕ_2 = 0° and 180°)] and (b) *the steric effect* [the steric interaction of the 3,5 substituents with the outer ring and its substituents favors a perpendicular conformation (ϕ_1 = 90° or 270°, ϕ_2 = 0° or 180°].

In the analysis of the conformations of the various analogues,[52] we sought to test the hypothesis that the biologically active conformation (and the one that binds to the nuclear receptors) is one in which the two rings are perpendicular. We attempted to correlate the free-energy change required to lock the analogue into this conformation (ΔG_{lock}) with biological activity and free energy of binding.

Because the ϕ_1,ϕ_2 energy surface will depend on the bond lengths (r_1 and r_2) and internal angle (θ) of the diphenyl ether linkage, we first determined the minimum-energy values of these geometrical parameters for Z = O, CH_2, and S. Except for X = S, the calculated and experimental values are in reasonable agreement (Table III).[55]

The potential-energy maps obtained for some of the analogues are shown in Figures 6 and 7. At the temperature of the experiment (310 K), 95% of the population were within the 2 kcal/mol contours. The maps show the following several interesting features:[55] (a) Comparison of the maps shows that in all cases [except for R_3 = I, R_5 = H (Figure 7A)] the two regions ϕ_1 = 0–180° and ϕ_1 = 180–360° are identical. This is to be expected due to the equivalence of the two surfaces of the inner ring. (b) Comparing the map of Z = O and $R_3 = R_5$ = I, Br, Cl, F, and H (Figures 6A–F and 7A) shows that as the size of the 3,5 substituent decreases the allowed areas of the map (2 kcal/mol in energy) increase. The most dramatic jump is seen in going from 3,5-dichloro to 3,5-difluoro and -dihydro; here large areas that were completely forbidden in the former become populated in the latter two compounds. (c) Comparing the maps for Z = O, CH_2, and S (Figure 6C,B,A) shows that for the latter two cases the area within the 1 kcal/mol contour is broader (larger range in ϕ_2) than for the oxygen-bridged case. This may be interpreted on the basis that the lone-pair orbitals on the interring oxygen atom can be conjugated with the p orbitals of the adjacent outer-ring carbon atoms, C_1, to a greater extent than the hyperconjugation of the C–H bonds or the conjugation of the sulfur orbitals [r_2(S) > r_2(O)], with the result of a greater rotational freedom around the Z–C_1 bond for Z = CH_2 and S than for Z = O. It is also interesting to note here that the allowed ranges of ϕ_1 are

Table III. Calculated and Observed Values of r_1, r_2, and θ for Various Interring Linkages (II: $R_1 = R_{5'} = H$, $R_3 = R_5 = I$, $R_{4'} = OH$)

	calcd			obsd			
Z	r_1[d]	r_2[d]	θ[d]	r_1[e]	r_2[e]	θ[e]	ref
O[a]	1.38	1.38	109	1.32-1.43	1.35-1.43	117-126	42, 43, 45, 46
O[b]	1.40	1.40	122				
CH_2[a]	1.48	1.48	114	1.51	1.52	114	56
S[c]	1.55	1.55	104	1.79	1.76	103	57

[a] $\phi_1 = 90°$, $\phi_2 = 0°$. The values of the geometrical parameters used in determining the potential energy maps were $r_1 = 1.38$, $r_2 = 1.40$, and $\theta = 117$. [b] $\phi_1 = \phi_2 = 90°$. [c] $\phi_1 = 90°$, $\phi_2 = 0°$. The experimental r_1, r_2, and θ were used in constructing the ϕ_1,ϕ_2 maps. [d] r_1 and r_2 in angstroms, θ in degrees. [e] r_1 and r_2 in angstroms, θ in degrees; these analogues have different ring-substitution patterns.

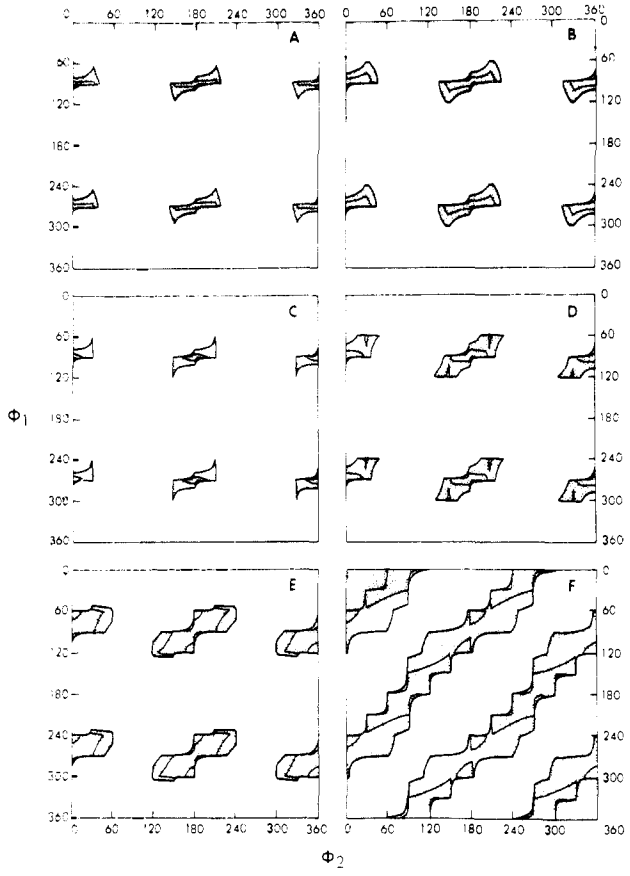


Figure 6. ϕ_1,ϕ_2 energy maps for diphenyl-Z analogues (see II); $R_{3'} = I$, $R_{5'} = H$, and $R_1 = CH_2CH(NH_2)COOH$ energy contours are 1 and 2 kcal/mol and the shaded areas represent energies <2 kcal/mol above the minimum energy. The structures are: (A) $R_3 = R_5 = I$, Z = S; (B) $R_3 = R_5 = I$, Z = CH_2; (C) $R_3 = R_5 = I$, Z = O; (D) $R_3 = R_5 = Br$, Z = O; (E) $R_3 = R_5 = Cl$, Z = O; (F) $R_3 = R_5 = F$, Z = O.

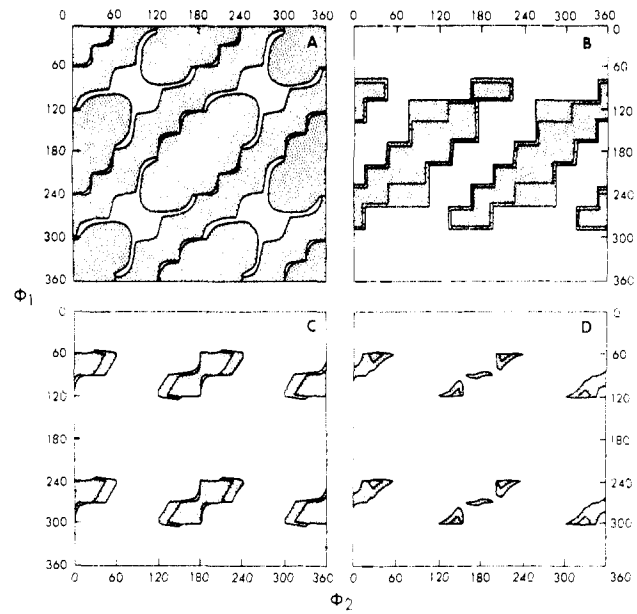


Figure 7. ϕ_1,ϕ_2 energy maps for diphenyl-Z analogues (see II); $R_{3'} = I$, $R_{5'} = H$, and $R_1 = CH_2CH(NH_2)COOH$ energy contours are 1 and 2 kcal/mol and the shaded areas represent energies <2 kcal/mol above the minimum energy. The structures are: (A) $R_3 = R_5 = H$, Z = O; (B) $R_3 = I$, $R_5 = H$, Z = O, $\phi_1 = 180$ places the outer ring nearer $R_5 = H$ than $R_3 = I$; (C) $R_3 = R_5 = CH_3$, Z = O; (D) $R_3 = R_5 = i$-Pr, Z = O.

similar for the three cases. This is in agreement with the idea that steric interaction between the outer ring and the 3,5-iodine atoms is stronger than the resonance of the bridging atom with the inner ring. (d) For large 3,5 substituents (I_2, Br_2, Cl_2; Figures 6C–E) there are only two global minima at positions in which the phenyl rings are perpendicular, whereas for smaller groups (F_2 and H_2; Figures 6F and 7A) there are four global minima positioned on both sides of the perpendicular ring conformation. (e) Finally, for every global minimum, there is a local minimum (which differs by not more than 0.1 kcal/mol in energy from the global minimum) obtained by changing ϕ_2 by 180°. The global and local minima would correspond to the case of 3′-iodine held proximally or distally.

To further examine the relative roles of the resonance and steric effects, the minimum-energy geometries were determined for analogues of 2,6,3′-triiodo-4′-hydroxydiphenyl ether with Z = CO, SO, and NH. These three compounds have minimum-energy geometries of ϕ_1,ϕ_2 = 90,10, 90,10, and 90,0, respectively (Table V), indicating that the steric effect of the bulky inner-ring iodine atoms is the main determinant of conformation. Finally, replacing the 3,5-iodine atoms by 3,5-nitro groups (Z = O) causes the minimum-energy geometry to change from ϕ_1,ϕ_2 = 90,0 to 50,60. This change can be accounted for by either of two possible explanations: (1) the smaller steric effects of the nitro groups in a direction perpendicular to the inner ring (this is due to the coplanarity of the nitro groups with the inner ring and the small Van der Waals radii of nitrogen and oxygen compared to iodine) and (2) the delocalization of the π electron system onto the nitro groups enhances the conjugation of the lone-pair orbital of the interring oxygen with the inner ring π system and tends to orient the two rings in a coplanar conformation.

A large number of crystal structures of diphenyl ethers and related compounds has been determined by X-ray crystallography. The compounds with Z = O and $R_3 = R_5$ = I have ϕ_1 and ϕ_2 in the ranges of 64–90 and 4–34.[42-46,54] The causes for such broad variations are not obvious. Our calculated minimum-energy geometry is ϕ_1,ϕ_2 = 90,0 for this substitution pattern. It must be mentioned, however, that crystal forces seem to have an effect on the values of ϕ_1 and ϕ_2. This crystal effect is demonstrated by com-

Table IV. A Comparison of Calculated and Observed Structures and Rotational Barriers for Thyroid Hormone Analogues (II: R_1 = H, R_3' = I, R_5' = H)

					minimum energy geometry				rotational barrier	
					calcd[a]		obsd[b]			
compd	R_3	R_5	R_4'	Z	ϕ_1	ϕ_2	ϕ_1	ϕ_2	calcd ($\Delta E^{\ddagger}$)[c]	obsd ($\Delta G^{\ddagger}$)[d]
1	I	I	OH	CH_2	90	0			4.74	
2	I	I	OH	CO	90	10			2.73	
3	I	I	OH	S	90	0			2.04	
4	I	I	OH	SO	90	10			0.89	
5	I	I	OH	NH	90	0	89	27[e]	13.41	
6	I	I	OH	O	90	0	64-90	13-33[f]	10.64	7.9[i]
7	Br	Br	OH	O	90	0			5.78	
8	Cl	Cl	OH	O	90	0			2.47	
9	F	F	OH	O	30	30			2.29	
10	NO_2	NO_2	OH	O	50	60			18.43	
11	*i*-Pr	*i*-Pr	OH	O	60	30	77.2 79.5	5.7[g] 2.1	6.68	<10[j]
12	Me	Me	OH	O	60	30			6.06	
13	H	H	OH	O	30	30	37	67[h]	1.19	
14	I	H	OH	O	180	90			2.94	
15	I	I	COOEt						11.15	9.3[i]

[a] Minimum energy calculated here with CNDO/2; see footnote 16, 24, and 25 for the geometrical parameters employed. [b] As determined by X-ray crystallography. [c] Calculated energy barrier. [d] Observed free energy barrier via NMR. [e] Reference 58. [f] References 42, 43, and 59. [g] Reference 60. [h] Reference 61. [i] Reference 48. [j] Reference 62.

paring the values of ϕ_1 and ϕ_2 for each of three pairs of compounds that are chemically identical and exist in the same crystal, yet their values of ϕ_1 and ϕ_2 differ by 2.3, 8.5, and 13.7°, respectively, for ϕ_1 and 3.6, 3.4, and 4.2°, respectively, for ϕ_2.[59,63,64] Table IV shows a comparison of calculated and the range of observed values for ϕ_1 and ϕ_2 for different 3,5-substituents and interring linkages.

Our conformational maps (Figures 6 and 7) make clear why the ϕ_1,ϕ_2 deviations from 90,0 are correlated, as has been noted by Cody.[43] For example, for the 3,5-substituted diphenyl ethers, Figures 6 and 7 show clearly that the 60,30 conformation is significantly lower in energy than the 60,−30 conformation. As one decreases ϕ_1 from 90°, the outer ring $C_{2'}$ begins to approach C_3; twisting ϕ_2 to +30° relieves this steric repulsion, twisting ϕ_2 to −30° shortens the $C_{2'}$–C_3 distance and increases the steric repulsion.

Another parameter that can be determined from the potential-energy surface is the barrier to internal rotation.[65] Table IV lists the calculated barriers and shows that they are in good agreement with the two precise experimental values available in the literature. The data in Table IV show other interesting features: (a) The highest rotational barrier is (18.34 kcal/mol) for 2,6-dinitro-3′-iodo-4′-hydroxydiphenyl ether (compound **10**). This can be understood on the basis that the lone-pair orbital of the interring oxygen is delocalized into the inner ring to a greater extent in this compound than in any other compound in this series. Thus, there is significant π bond character in the O–C_4 bond. In addition, the O–C_1' bond has some π bond character. The highest energy geometry along the path of internal rotation is ϕ_1,ϕ_2 = 90,90. In this geometry, the conjugation between the interring oxygen and the two rings is weakened, and hence a higher barrier to internal rotation is found. (b) Replacing the 3,5-diiodo atoms (Z = O) by dibromo, dichloro, difluoro, and dihydro (compounds **6–9** and **13**) causes a progressive decrease in the calculated rotational barrier. This can be explained on the basis of the progressive decrease in the size of the 3,5 substituents, which leads to less steric constraint on rotation. (c) Replacing Z = O by CH_2, CO, S, and SO (compounds **1–4** and **6**) lowers the rotational barrier. There are two factors which might contribute to this: (1) r_1 and r_2 (II) are larger for Z = CH_2, CO, S, and SO than for Z = O (thus, the steric constraint to rotation arising from the 3,5 substituents is less in the first four cases than in the last one), and (2) the overlap of the lone-pair orbitals on the oxygen atom (Z = O) with the π electrons of the outer ring is larger in magnitude than in the case of Z = CH_2 (no lone-pair orbitals available), CO, S, and SO (larger r_1 and r_2), and hence the bonds connecting the Z atom to the outer rings have more double-bond character in the first case than in the last four. (d) Finally, the rotational barrier in 3,5-*i*-Pr_2 (compound **11**) is almost the same as that of the 3,5-Me_2 (compound **12**), in spite of the much larger bulk of the former. This can be explained on the basis that each isopropyl group can rotate about the bond connecting it to the inner ring in a concerted manner, with the rotation of the outer ring (an effect which we investigated) keeping its methine hydrogen atom rather than the bulky groups pointed "toward" the outer ring. Thus, the outer ring "feels" more or less the same repulsion from the 3,5-*i*-Pr_2 groups as from the 3,5-Me_2 groups.

We analyzed the free energy required to lock (ΔG_{lock}) the analogues into the mutually perpendicular conformation. The values of ΔG(X) (nuclear binding), Δ ln BA(X) (in vivo activity), and ΔG_{lock}(X) are given in Table V. Comparing the relative binding affinities to nuclear receptor and "locking free energies" (relative to T3), we find:

$$\Delta G(X) = 2.79\Delta G_{lock}(X) - 0.62 \quad (7)$$

with a correlation coefficient r = 0.89. We interpret the equation as follows: if the 3,5-iodine atoms or the interring oxygen atom are replaced by other atoms or groups such that it becomes more difficult to "lock" the compound into the perpendicular conformation, there is a parallel decrease in the ability of the compound to bind to nuclear receptors. This statement provides support for Jorgensen's hypothesis.[2] Moreover, the slope of 2.79 (±1) shows that if a test group(s) causes an increase in the binding energy (loss of binding) relative to the reference group the observed increase is greater than the calculated free energy required to "lock" the compound. This is in agreement with the concept that the 3 and 5 substituents could be involved in *direct* interactions, in addition to their effects on determining a biologically favorable geometry.[37] Similar conclusions come for a comparison of in vivo activities and ΔG_{lock} (Figure 8).

For analogues with R_3 = R_5 = I, Br, Cl, CH_3, F, and H, the relative biological [Δ ln BA(X)] activities follow the

Table V. Observed Binding Free Energies (Relative to T3) and Calculated ΔG_{lock} Values for Various Thyroid Hormone Analogues (II: R_4' = OH, R_1 = R_5' = H, R_3' = I)[a,b]

R_3	R_5	Z	ΔG_{lock}	$\Delta(\Delta G_{lock}(X))$[c]	$\Delta G(X)$[d]	Δ ln $BA(X)$[e]
I	I	CH_2	+5.20	+0.48	-0.56	(-1.63)-(-4.61)
I	I	S	+5.13	+0.41	-0.90	(-0.91)-(-1.21)
I	I	O	+4.72	0.00	0.00	0.00
Br	Br	O	-5.03	+0.31	+0.65	(-1.15)-(-2.64)
Cl	Cl	O	+5.33	+0.61		(-4.61)-(-1.90)
F	F	O	+7.00	+2.28		
i-Pr	*i*-Pr	O	+5.91	+1.19	+3.83	
Me	Me	O	+5.93	+1.21	+3.08	(-3.22)-(-5.30)
H	H	O	+6.39	+1.67	+3.26	-11.2
I	H	O	+6.33	+1.61	+2.97	-11.2

[a] The experimental studies had the amino acid side chain [R_1 = $CH_2CH(NH_2)COOH$] and different R_3' and R_5' substituents. [b] Energies in kcal/mol. [c] $\Delta(\Delta G_{lock})$ is the relative binding free energy (with T3 = 0.00). [d] Relative binding affinities to solubilized nuclear receptor; see ref 26. [e] Relative in vivo biological activity; see ref 2 and 8.

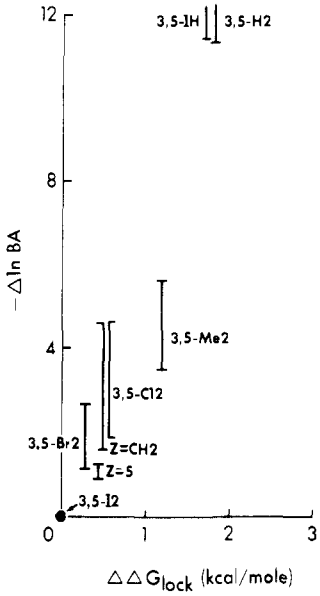


Figure 8. Plot of Δ ln (biological activity) vs. ΔG_{lock} for compounds in Table V.

order one would expect on the basis of hydrophobicity. However, the fact that the 3,5-diisopropyl has no activity seems to rule out the exclusive role of hydrophobic effects in contributing to $\Delta G(X)$ for 3,5 substituents, since the isopropyl partition coefficient is similar to that of I. From the fact that this compound is much less active than eq 7 indicates one might conclude that the 3,5-*i*-Pr_2 groups are involved in a detrimental steric interaction with the receptor. Another possibility is that our calculated ΔG_{lock} for 3,5-diisopropyl is too low because we did not consider rotation around the C–C bond of the *i*-Pr groups, considering the R_3 = R_5 = *i*-Pr compound to be just like R_3 = R_5 = Me. Conformations where one Me group points at the outer ring would cause the outer ring to rotate from the 90,0 conformation and thus increase ΔG_{lock}.[66] In addition, the fact that the R_3 = I, R_5 = H compound has a biological activity similar to that for R_3 = R_5 = H is consistent with the calculated ΔG_{lock} but not with models based exclusively on size or hydrophobicity.

The relative binding affinities of analogues with 0–4 iodines in the 3,5,3′ and 5′ positions to prealbumin do not correlate well with ΔG_{lock}, emphasizing an essential difference between the conformational requirements of nuclear receptor and prealbumin.[14]

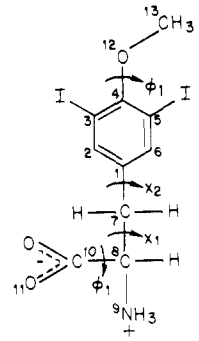
Conformation of the Alanine Side Chain. The length, absolute configuration, and associated charges of the L-alanine side chain of the thyroid hormones and analogues are extremely important in determining in vivo (see ref 2 and 8) and in vitro activities. Hence, a CNDO/2 conformational analysis study of the naturally occurring alanine side chain was undertaken.

Table VI. CNDO/2 Conformational Energy Local Minima of Alanine Side Chain (IX)

X_2, degrees	X_1, degrees	ΔE, kcal mol
105	60	3.12
285	60	2.87
90	180	~0[a]
270	180	~0[a]
75	300	0.00
255	300	0.19

[a] See text.

For analysis of the amino acid side-chain conformation, 4-methoxy-3,5-diiodo-L-phenylalanine (IX) was used as a



IX

model system. The CH_3 and NH_3^+ groups were assumed to be staggered.[67] Conformation studies were performed utilizing variations in X_2 = $PhC_6C_1C_7C_8$, X_1 = $PhC_1C_7C_8N_9$, and ψ_1 = $N_9C_8C_{10}O_{11}$. Taking X_2 = 270° and X_1 = 180° (the fully extended "transoid" conformer with the least expected steric repulsions of the NH_3^+ and COO^- with the aromatic ring), 15° variations in ψ_1 led to a minimum energy at 345°. ψ_1 was taken as 345° in all further calculations. Variations of 15° in X_2 and X_1 led to the local minima in the energy listed in Table VI. Besides these minima, there are two very steep minima at X_2 = 60°/240° and X_1 = 210°, which are about 20 kcal/mol more stable than the local minimum X_2 = 75°, X_1 = 300°. Bonds orders show that this stabilization is due to an unexplainable attractive interaction between the carboxyl group and C_2-H and C_6-H, and its magnitude is apparently an artifact of the approximations of the CNDO/2 method. From the calculations it can be concluded that for the model system (IX) studied (1) there is no great preference for either a cisoid ($X_2 \approx 90°$) or a transoid ($X_2 \approx 270°$) conformation; (2) X_1 may assume any of the expected staggered values of approximately 60°, 180°, or 300°; (3) $\psi_1 \approx 345°$; and (4) the various X_2,X_1 conformers are readily interconvertible with barriers

between them ($\lesssim$7.5 kcal/mol). These theoretical results are in agreement with a large number of X-ray crystallographic studies[42-46,54,68,69] of aromatic amino acids and thyroid hormone analogues and calculations based on empirical potential functions (see ref 54), with the small exception that the transoid conformation is usually observed. The theoretical and X-ray studies emphasize the fact that the amino acid side chain can probably assume whatever conformation is required to maximize binding interactions.

Summary and Conclusions. We have developed a formalism for the analysis of the binding of small molecules to macromolecules in terms of individual group contributions and have presented a number of theoretical studies of thyroid hormones and their analogues using quantum mechanical methods. We have used the formalism and the calculations to enable us to better understand available structure–activity relationships of in vivo activity as well as in vitro test systems (nuclear receptor and plasma protein binding).

Our results suggest the following three concepts: (1) The 4′-OH of thyroid hormone analogues is functioning as a proton donor to the nuclear receptor in vitro and in vivo with the O–H pointed in the "proximal" direction. This H bond is influenced by (a) the electron-withdrawing properties of the 3′ and 5′ substituent, (b) the effect of the 3′ and 5′ group on the 4′-OH cis–trans isomerism, and the (c) direct steric interactions of the 3′ and 5′ substituents with the receptor. The structure–activity relationship for the outer ring appears different for the plasma proteins than the nuclear receptor and suggest that it is the 4′-phenoxide that is present at the protein receptor site. We thus suggest a specific geometric model to represent the 4′-O$^-$···receptor interaction. (2) The nuclear receptor has a preference for a conformation of the aromatic rings where they are approximately perpendicular; there is also a specific, size-limited interaction of the 3,5 groups with groups at the receptor site. The plasma proteins have much less structural specificity for the diphenyl ether linkage and appear to bind strongly those analogues whose minimum-energy conformations are different from 90,0. (3) The structure–activity relationships of side-chain binding to nuclear receptor and plasma protein suggest specific ionic interactions of the side chain with receptor. Our conformation analysis of the side chain finds that a large part of conformation space is allowed. Thus, if the side chain is not too "long" or "short", it can assume the appropriate conformation for receptor interaction.

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