Thyroid Hormone Analogues. Synthesis of 3'-Substituted 3,5-Diiodo-L-thyronines and Quantitative Structure–Activity Studies of in Vitro and in Vivo Thyromimetic Activities in Rat Liver and Heart

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Twenty-nine novel 3'-substituted derivatives of the thyroid hormone 3,3',5-triiodo-L-thyronine (T_3) have been synthesized by using established methods and by a new route involving manipulation of a 3'-formyl intermediate. In vitro hormone receptor binding (to intact nuclei) and in vivo thyromimetic activity (induction of mitochondrial 3-phosphoglycerate oxidoreductase, GPDH) were measured in rat liver and heart for these new analogues and for the 18 previously reported 3'-substituted 3,5-diiodo-L-thyronines. Analysis of the binding data using theoretical conformational and quantitative structure–affinity methods implies that the 3'-substituent recognition site on the thyroid hormone receptor is hydrophobic and limited in depth to the length of the natural iodo substituent, but has sufficient width to accommodate a phenyl or cyclohexyl group. Receptor binding is reduced by approximately 10-fold in 3'-acyl derivatives which form strong intramolecular acceptor hydrogen bonds with the adjacent 4'-hydroxyl. The compounds studied showed no differences in their relative affinities for heart and liver nuclei, suggesting that receptors in these tissues are similar. However, the relationships between thyromimetic activity (induction of GPDH) and nuclear binding showed some tissue differences. A high correlation between activity and binding is observed for full agonists in the heart, but an equally significant correlation for the liver data is only seen when 3'-substituent bulk (molar refractivity) is included in the analysis. These results suggest the possibility that differential tissue penetration or access to receptors may occur in vivo.

It is well established that the thyroid hormones (T_3 and T_4, Figure 1) lower plasma cholesterol, probably via increased liver metabolism. However, these hormones cannot be used therapeutically to treat hypercholesterolemia because of the adverse consequences of some of their other actions, notably on the heart. Although several groups of workers have attempted to find analogues that retain hypocholesterolemic activity but lack cardiac effects, all thyromimetics that have been tested in humans to date have shown unacceptable cardiac side effects. Recently, however, we have reported the discovery of a novel class of selective thyromimetics that lower plasma cholesterol without increasing cardiac activity.[1] These novel hypocholesterolemic agents emerged from an analysis of the effect of structure on thyromimetic activity and receptor affinity in both the heart and liver for a wide range of T_3 analogues. In this paper we describe some of our initial SAR studies of 3'-substituted analogues of T_3, the results of which established a clearer molecular basis for the design of selective agents via modification of the 3'-substituent.

The thyroid hormones are believed to exert their physiological effects as a consequence of protein synthesis occurring after hormonal interaction with receptors in cell nuclei.[2] The evidence in support of this hypothesis includes the excellent correlations observed between nuclear binding (in vitro and in vivo) and various thyromimetic activities seen for a range of analogues of T_3 and T_4.[3,4] These studies have suffered from two principal drawbacks: (1) nuclear binding and thyromimetic activity were measured in different tissues; (2) a narrow structural variation between analogues, which is especially evident in 3,3',5,5'-substituted compounds. Although several hundred thyroid hormone analogues have been prepared,[4,5,6] replacement of the 3'-iodo substituent in T_3 has been limited to halogen, small alkyl, phenyl, nitro, and hydroxyl, until our recent studies. Jorgensen and Kollman and their coworkers have used several series of analogues with essentially the same restricted variation in the 3'-substituent to establish structure–activity and structure–affinity relationships.[4] A model was proposed for the interaction of the 3'- and 4'-substituents in which the 3'-substituent binding pocket (located distal to the alanine recognition site (Figure 1)) is lipophilic and strictly size limited to accommodate the natural iodo substituent.[7] The 4'-hydroxyl was suggested to donate a hydrogen bond to the receptor, in a disposition trans to the 3'-substituent[7,8] which is in accord with geometries found from X-ray crystallographic studies.[9] We have shown that the quantitative structure–affinity relationships on which this model is based are derived from a series of compounds in which 3'-substituent lipophilicity and size are covariables.[10] The evidence for both a lipophilic 3'-substituent receptor binding site and the receptor hydrogen bonding of the 4'-hydroxyl was demonstrated to rest solely on the weak affinity of the 3'-nitro derivative. The low affinity of this analogue could be due to reduced lipophilicity,[4] the presence of a strong intramolecular hydrogen bond with the 4'-hydroxyl,[4,8] ionization of the 4'-hydroxyl,[11] or a

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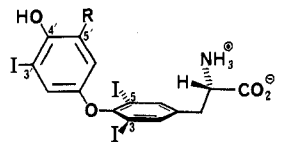
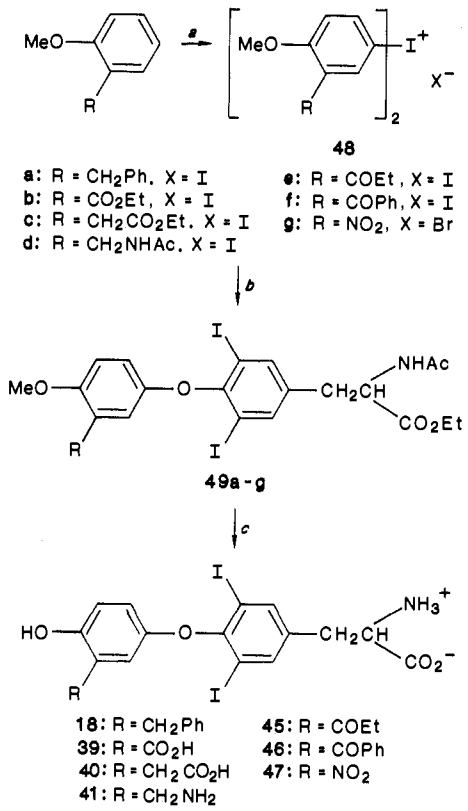


T_3 R = H

T_4 R = I

Figure 1. Structures of the thyroid hormones 3,3′,5-triiodothyronine (T_3) and thyroxine (3,3′,5,5′-tetraiodothyronine, T_4); the diphenyl ether conformation shown, with the 3′-substituent distal to the alanine-bearing ring, is believed to be required for thyromimetic activity (see ref 4 and 5).

Scheme I

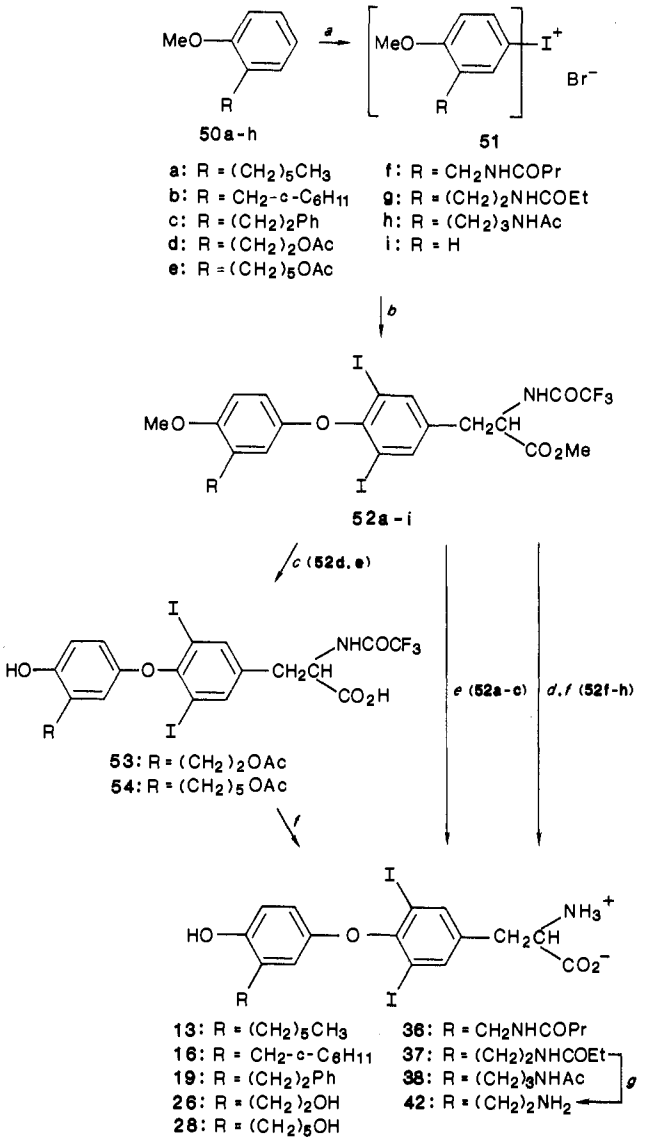


[a] $I(OCOCF_3)_3$ [$(IO)_2SO_4$ for R = NO_2], NaI or NaBr. [b] *N*-Acetyl-3,5-diiodo-L-tyrosine ethyl ester/Cu/Et_3N/MeOH. [c] HBr/HOAc/H_2O.

combination of these factors. There are no alternative hydrophilic 3′-substituted analogues that would permit the relative importance of these properties to be estimated. When the 3′-nitro analogue was omitted, a quantitative structure–affinity study[10] using all the other known 3′-substituted analogues[12] showed that substituent bulk alone can account for the variation in affinity. Consequently, the model proposed by Jorgensen and Kollman[4,7] needs to be tested by novel 3′-substituted analogues with a greater spread of physical properties.

We have synthesized 29 novel 3′-substituted 3,5-diiodo-L-thyronines (Table I) to test and expand the proposed model of receptor binding. The analogues were chosen to explore, as independent variables, the size and shape of the 3′-substituent receptor pocket, its hydrophobic character, and the role of the 4′-hydroxyl in receptor

Scheme II



[a] $I(OCOCF_3)_3$, NaBr. [b] *N*-Trifluoroacetyl-3,5-diiodo-L-tyrosine methyl ester/Cu/Et_3N/MeOH. [c] $AlCl_3$/EtSH. [d] BBr_3. [e] HBr/HOAc/H_2O. [f] NaOH. [g] HCl/HOAc/H_2O.

binding. The affinities of the compounds in Table I for receptors in rat heart and liver nuclei were measured in vitro, and thyromimetic activities were measured in vivo in the same tissues. Quantitative structure–activity relationships derived from these data are used herein to (1) provide a comprehensive model for the 3′-substituent receptor binding site, (2) establish correlations between liver and heart thyromimetic potency and affinity, (3) establish the relationships between potency and affinity in each tissue, and (4) identify differences in tissue responsiveness.

Synthesis

The novel analogues synthesized for this study are shown in Table I, together with all previously reported 3′-substituted 3,5-diiodothyronines, most of which were resynthesized. Many compounds were prepared by variations of established routes[13] to thyroid hormone analogues (Schemes I–III). The key step in these syntheses is diphenyl ether construction, either by arylation of a 3,5-diiodotyrosine derivative with a bis(3-substituted-4-

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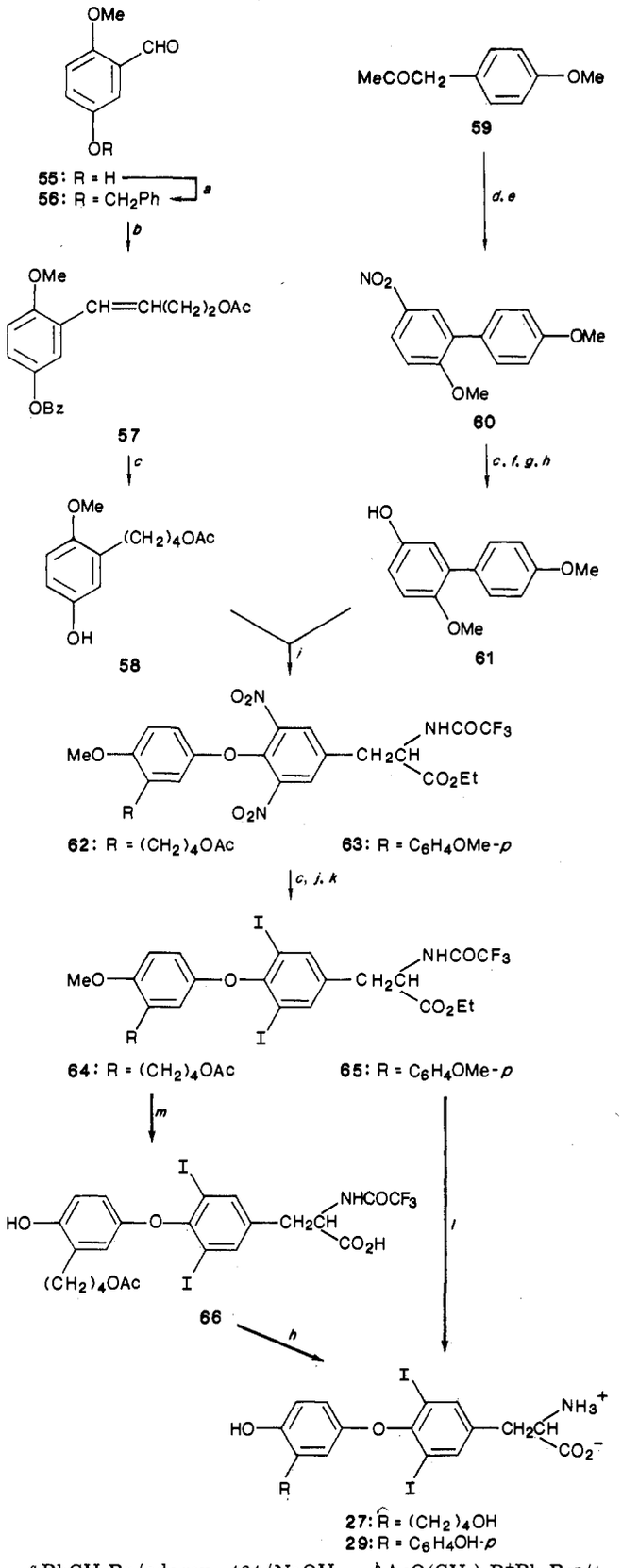
Scheme III



[a] $PhCH_2Br$/adogen 464/NaOH. [b] $AcO(CH_2)_3P^+Ph_3Br^-$/*t*-BuOK/dicyclohexano-18-crown-6. [c] H_2/Pd-C. [d] $NaCNO_2$-$(CHO)_2$/NaOH. [e] Me_2SO_4/adogen 464/NaOH. [f] HBF_4/$C_5H_{11}ONO$. [g] Ac_2O/AcOH. [h] NaOH. [i] *N*-Trifluoroacetyl-3,5-dinitro-L-tyrosine ethyl ester/$MeSO_2Cl$/pyridine. [j] $NaNO_2/H_2SO_4$. [k] KI/I_2. [l] HBr/HOAc. [m] $AlCl_3$/EtSH.

methoxyphenyl)iodonium salt (Schemes I and II) or by condensation of a 3,5-dinitrotyrosine derivative with a 3-substituted 4-methoxyphenol (Scheme III).

The choice of acetyl or trifluoroacetyl as amino protecting group and conditions for the demethylation of the 4′-methoxy group in the protected thyronines **49** (Scheme I), **52** (Scheme II), and **64** and **65** (Scheme III) depended on the nature of the 3′-substituent in the target molecules. Refluxing aqueous hydrobromic acid in acetic acid effectively removed all amino acid protecting groups and the 4′-methoxy group and was used where the target compound was stable to these conditions (**49**, **52a–c**, and **65**). Compounds with 3′-substituents containing mineral acid labile functionality were prepared from the protected thyronines by cleavage of the 4′-methoxy and amino acid ester groups with either boron tribromide[14] (**52f–h**) or aluminum chloride/ethanethiol[15,16] (**52d,e** and **64**), followed by basic hydrolysis of the *N*-trifluoroacetyl group, and the *O*-acetyl groups in **53**, **54**, and **66**.

The selective ester cleavage reactions using aluminum chloride/ethanethiol[15,16] deserve comment. The originators of the method found that aryl methyl ethers were rapidly cleaved by the reagent at 0 °C whereas ester cleavage occurred slowly at room temperature. Methyl esters reacted more rapidly than ethyl or higher esters, and this can be explained by steric inhibition of an S_N2 reaction between ethanethiol and the aluminum chloride–ester complex. We found that the methyl ethers in compounds **52d,e** (Scheme II) and **64** (Scheme III) were, as expected, cleaved by the reagent at 0 °C, with the 3′-acetoxy functionalities being retained. Unexpectedly, the methyl esters of **52d,e** and the ethyl ester of **64** were also quantitatively cleaved under these conditions. Participation of the neighboring trifluoroacetyl amide by complexation with aluminum chloride may facilitate these deesterifications. Use of boron tribromide led to nonselective cleavage reactions, with the acetoxy group in **52d** being converted to the corresponding bromide.

We developed alternative syntheses (Schemes IV–VI) where the required 3′-substituents were introduced into a 3′-unsubstituted thyronine (**52i**). Formylation of **52i** gave the key aldehyde **67**, which was readily transformed to target 3′-alkyl compounds (Scheme IV) by successive Wittig reaction, ionic hydrogenation by triethylsilane and trifluoroacetic acid,[17] and deprotection. To obtain 3′-substituted compounds containing ether and olefin functionality we required precursor thyronines where the 4′-hydroxyl and amino acid moieties were protected by base labile groups. To this end, the aryl methyl ether in **67** was selectively cleaved by boron trichloride[18] to give **70** (Scheme V). Reductive alkoxylation of **70**, followed by basic hydrolysis, gave the 3′-alkoxymethyl compounds **30** and **31**. The labile 3′-hydroxymethyl group in **71a** readily reacted with *n*-butanethiol in trifluoroacetic acid to give the thioether **74**. Base hydrolysis of **70**, **71a**, and **74** gave the corresponding amino acid target compounds (**43**, **25**, and **35**). Protection of the 4′-hydroxyl in **70** with benzenesulfonyl gave **72**, which underwent Wittig reactions

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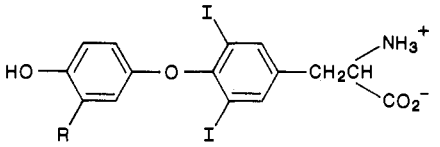
Table I. Physical Properties and Thyromimetic Activities of 3′-Substituted 3,5-Diiodothyronines

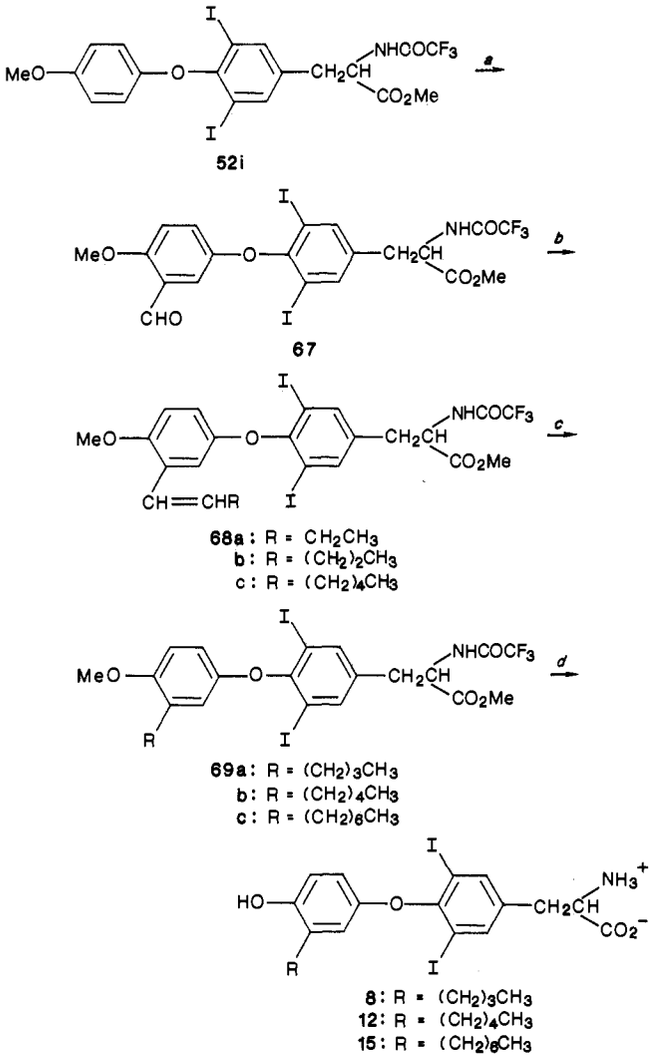


no.[a]	R	mp, °C	$[\alpha]^{25}_D$ (*c*, %)[h]	formula	analyses	liver rel IC_{50}[x]	liver rel ED_{50}[y]	heart rel IC_{50}[x]	heart rel ED_{50}[y]
1[d]	H	258–259 dec	21.7 (1.03)[i]	$C_{15}H_{13}I_2NO_4 \cdot H_2O$	C, H, N; I[l]	0.08	1.1	0.40	0.45
2[b,e]	CH_3			$C_{16}H_{15}I_2NO_4$	C, H, N	0.6	7.5	3.9	3.2
3	CH_2CH_3	252–256 dec	22.8 (0.93)[i]	$C_{17}H_{17}I_2NO_4$	C, H, N, I	56.0	139	44.1	108
4[c,f]	$CH{=}CH_2$	275–278 dec	0.95 (1.05)	$C_{17}H_{15}I_2NO_4$	C, H, N; I[m]	17.0	10.7	15.3	12.5
5	$(CH_2)_2CH_3$	205–207	21.5 (0.95)[i]	$C_{18}H_{19}I_2NO_4 \cdot 0.66H_2O$	C, H, N, I	45.2	88.9	24.5	32.8
6[e]	$CH(CH_3)_2$	207–209 dec	5.1 (0.96)[i]	$C_{18}H_{19}I_2NO_4$	C, H, N, I	87.0	290	119.2	348
7[c,f]	$CH_2CH{=}CH_2$	224–227 dec	1.6 (0.86)	$C_{18}H_{17}I_2NO_4 \cdot 0.5H_2O$	C, H, N; I[n]	32.1	33.7	37.5	15.4
8[c,f]	$(CH_2)_3CH_3$	219–221	4.3 (0.98)	$C_{19}H_{21}I_2NO_4 \cdot 0.75H_2O$	C, H, N, I	43.6	120	88.4	F[z]
9	$CH(CH_3)CH_2CH_3$	210–211	22.3 (1.01)	$C_{19}H_{21}I_2NO_4 \cdot 0.5H_2O$	C, H, N, I	72.1	F	60.4	F
10[e]	$CH_2CH(CH_3)_2$					20.5	F	18.7	F
11[b]	$C(CH_3)_3$	226–227	0.3 (0.95)	$C_{19}H_{21}I_2NO_4 \cdot H_2O$	C, H, N, I	25.0	25.6	30.0	18.1
12[b,f]	$(CH_2)_4CH_3$	199–200 dec	0 (0.98)	$C_{20}H_{23}I_2NO_4 \cdot NaOAc \cdot 0.2NaBr \cdot H_2O$	C, H, N, Br, I	32.2	7.5	24.6	9.7
13[f]	$(CH_2)_5CH_3$	215–220	24.4 (1.04)	$C_{21}H_{23}I_2NO_4$	C, H, N, I	60.0	4.92	40.0	LM (62)[z]
14	c-C_6H_{11}	212–215 dec	20.9 (1.07)	$C_{21}H_{23}I_2NO_4 \cdot H_2O$	C, H, N; I[o]	9.83	F		LM (48)
15[c,f]	$(CH_2)_6CH_3$	223–225 dec	8.4 (1.11)	$C_{22}H_{27}I_2NO_4 \cdot 0.7H_2O$	C, H, N, I	8.74	LM (51)	8.7	LM (16)
16[f]	CH_2-c-C_6H_{11}	238–240	24.0 (0.99)	$C_{22}H_{25}I_2NO_4 \cdot 0.5H_2O$	C, H, N, I	36.4	F	27.5	F
17[b,e]	C_6H_5	195		$C_{21}H_{17}I_2NO_4$	H, N, I; C[p]	7.3	6.6	4.85	4.4
18	$CH_2C_6H_5$	203–205	17.0 (0.76)[i]	$C_{22}H_{19}I_2NO_4 \cdot H_2O$	C, H, N, I	21.7	2.16	13.0	1.43
19[f]	$(CH_2)_2C_6H_5$	240–242	22.8 (1.02)	$C_{23}H_{21}I_2NO_4 \cdot H_2O$	C, H, N; I[q]	1.8	LM (61)	3.61	LM (21)
20	F	226	24.8 (1.03)	$C_{15}H_{12}FI_2NO_4$	C, H, N, I	0.76	F		F
21	Cl	248–249 dec	21.5 (0.87)[i]	$C_{15}H_{12}ClI_2NO_4$	C, H, N, Cl, I	5.1	2.79	12.0	1.92
22	Br	238–239	19.6 (1.02)[i]	$C_{15}H_{12}BrI_2NO_4$	C, H, N, Br, I	13.0	20.2	49.1	10.0
23[g]	I					100	100	100	100
24	OH	242–243 dec	21.3 (0.51)	$C_{15}H_{13}I_2NO_5 \cdot 1.5H_2O \cdot 0.5EtOH$	C, H, N, I	0.04	LM (47)	0.05	LM (55)
25[f]	CH_2OH	>300	24 (1.07)	$C_{16}H_{15}I_2NO_5$	C, H, N, I	0.39	LM (75)	0.59	0.07
26[f]	$(CH_2)_2OH$	256–258 dec	23.2 (1.0)	$C_{17}H_{17}I_2NO_5 \cdot 0.8H_2O$	C, H, N, I	0.45	LM (30)	0.68	LM (36)
27[f]	$(CH_2)_4OH$	218–221 dec	17.9 (0.98)	$C_{19}H_{21}I_2NO_2 \cdot 0.75H_2O$	C, H, N	1.61	LM (27)		LM (40)
28[f]	$(CH_2)_5OH$	239–241	22.6 (0.95)	$C_{20}H_{23}I_2NO_5$	C, H, N, I	5.34	LM (20)	2.33	LM (42)
29[f]	C_6H_4OH-*p*	227 dec	20.0 (1.03)	$C_{21}H_{17}I_2NO_5 \cdot 0.5HBr \cdot 0.05H_2O$	C, H, Br, N; I[r]	0.76	LM (81)	0.50	LM (22)
30[f]	CH_2OCH_3	294–297 dec	21.4 (1.06)	$C_{17}H_{17}I_2NO_5 \cdot 0.5H_2O \cdot 0.5NaOAc$	C, H, N, I	2.55	25.0	1.42	F
31[f]	$CH_2O(CH_2)_3CH_3$	185–188 dec	20.1 (0.99)	$C_{20}H_{23}I_2NO_5 \cdot 0.5H_2O \cdot 0.6NaOAc$	C, H, N; I[s]	5.07	F	2.45	F
32[c,f]	$(CH_2)_3OCH_2CH_3$	198–202	2.2 (0.81)	$C_{20}H_{23}I_2NO_5 \cdot 0.5H_2O$	C, H, N	1.45	F	0.96	LM (55)
33[c,f]	$(CH_2)_4OCH_3$	230–233 dec	5.2 (0.94)	$C_{20}H_{23}I_2NO_5$	C, H, N	10.89	0.78	5.1	F
34[c,f]	*t*-$CH{=}CH(CH_2)_2OCH_3$	188–191	6 (0.49)	$C_{20}H_{21}I_2NO_5 \cdot 0.5H_2O \cdot 0.05NaOAc$	C, H, N	2.24		0.81	
35[f]	$CH_2S(CH_2)_3CH_3$	221–224	20 (1.01)	$C_{20}H_{23}I_2NO_4S \cdot 0.5H_2O \cdot 0.1NaOAc$	C, H, N, I, S	19.0	F	6.3	LM (54)
36[f]	$CH_2NHCO(CH_2)_2CH_3$	225–227	18.1 (0.91)	$C_{20}H_{22}I_2N_2O_5 \cdot 0.25H_2O$	C, H, N, I	0.016	I[z]	0.013	I
37[f]	$(CH_2)_2NHCOCH_2CH_3$	220–221	16.9 (0.98)	$C_{20}H_{22}I_2N_2O_5$	C, H, N, I	0.12	LM (42)	0.061	I
38[f]	$(CH_2)_3NHCOCH_3$	246–248	13.2 (1.02)	$C_{20}H_{22}I_2N_2O_5$	C, H, N, I	0.14	LM (37)	0.22	I
39[f]	CO_2H	292–293	−16.8 (2.7)[j]	$C_{16}H_{13}I_2NO_6$	C, H, N, I	0.004	I	0.01	I
40[f]	CH_2CO_2H	245–246 dec	−5.7 (2.5)[j]	$C_{17}H_{15}I_2NO_6 \cdot H_2O$	C, H, N; I[t]	0.03	LM (25)	0.10	LM (22)
41[f]	CH_2NH_2	247 dec	12.7 (0.92)[i]	$C_{16}H_{16}I_2N_2O_4 \cdot 2HBr$	C, H, N, Br; I[u]	0.01	I	0.03	I
42[f]	$(CH_2)_2NH_2$	281–282	13.3 (1.01)	$C_{17}H_{18}I_2N_2O_4 \cdot 2HCl \cdot 1.5H_2O$	C, H, N, Cl, I	0.005	LM (37)	0.006	I
43[f]	CHO	>305	−14.1 (1.08)	$C_{16}H_{13}I_2NO_5$	C, H, N; I[v]	0.18	LM (50)	0.20	F
44	$COCH_3$	252–253 dec	12.1 (1.01)[k]	$C_{17}H_{15}I_2NO_5$	C, H, N, I	0.37	121	0.54	58
45[f]	$COCH_2CH_3$	202–205 dec	20.8 (0.66)[i]	$C_{18}H_{17}I_2NO_5$	C, H, N, I	0.31	F	1.4	1.6
46[f]	COC_6H_5	212–213 dec	18.7 (1.2)[i]	$C_{22}H_{17}I_2NO_5$	C, H, N, I	0.37	LM (13)	0.41	LM (40)
47	NO_2	243–247	19.2 (0.95)[i]	$C_{15}H_{12}I_2N_2O_6$	H, N; C; I[w]	0.13	0.32	1.0	F

Footnotes to Table I

[a] All compounds are L-alanine derivatives, unless otherwise noted. [b] DL-Alanine derivatives. [c] Partially racemized (see text). [d] Phase Separations Ltd. [e] Supplied by Dr. B. Blank, SK&F Philadelphia. [f] Novel compound, this work. [g] Sigma Chemical Co. Ltd. [h] EtOH/H_2O/10 N HCl (17:2:1) unless otherwise stated. [i] 1 N HCl/EtOH (1:9). [j] 1 N NaOH/EtOH (1:2). [k] HOAc/EtOH/1 N HCl (10:9:1). [l] I: calcd, 46.70; found, 46.22. [m] I: calcd, 46.05; found, 42.60. [n] I: calcd, 44.21; found, 44.87. [o] I: calcd, 40.59; found, 40.11. [p] C: calcd, 41.96; found, 41.17. [q] I: calcd, 39.28; found, 38.55. [r] I: calcd, 38.07; found, 37.56. [s] I: calcd, 37.91; found, 39.02. [t] I: calcd, 42.22; found, 42.66. [u] I: calcd, 35.45; found, 35.91. [v] I: calcd, 45.89; found, 45.05. [w] C: calcd, 31.60; found, 31.12. I: calcd, 44.52; found, 44.11. [x] In vitro nuclear receptor affinity, relative to T_3 = 100% (see text). [y] In vivo potency for stimulation of GPDH activity after 48 h by full agonists in thyroidectomized rats, relative to T_3 = 100% (see text). [z] F = full agonist; I = inactive; LM = low maximum response (% of T_3 maximum response in parentheses) (see text).

Scheme IV



[a] $Cl_2CHOCH_3/SnCl_4$. [b] $RCH_2P^+Ph_3Br^-/t$-BuOK/dicyclohexano-18-crown-6. [c] Et_3SiH/CF_3CO_2H. [d] $HBr/HOAc/H_2O$.

with (alkoxyalkylidene)triphenylphosphoranes to give the olefins **73b,c**. Catalytic hydrogenation followed by hydrolysis gave the desired thyronines **32** and **33**. The trans olefin was isolated from **73c** and converted to **34**.

The basic conditions required for Wittig reactions of the aldehydes **67** and **72** gave rise to partial racemization of the protected amino acid, as evidenced by the relatively low optical rotations of **4**, **8**, **12**, **15**, and **32–34** (Table I) (also see Experimental Section). In the case of **15**, racemization was complete. This racemization is probably of little consequence, since it is well established that the thyromimetic activities of any L- and the corresponding DL-thyronine are essentially the same in a variety of assays.[4,19]

(19) Ellis, D.; Emmett, J. C.; Leeson, P. D.; Underwood, A. H., unpublished results.

The 3′-allyl derivative **7** was prepared by Claisen rearrangement of the ether **78** in refluxing *N,N*-diethylaniline, followed by basic hydrolysis of the product ester mixture of **79** and **80** (Scheme VI).

Biological Activity

The induction of a specific enzyme, mitochondrial cytochrome C 3-phosphoglycerate oxidoreductase (GPDH),[20] was used to measure thyromimetic activity in the livers and hearts of hypothyroid rats. Details of the methodology have been published.[11] The activity of GPDH was measured 48 h after a single intramuscular injection of test compound in a vehicle of 0.15 M NaCl/0.01 M NaOH (1 mL/kg). Dose–response curves were fitted to a hyperbolic curve by using a nonlinear curve fitting procedure, and ED_{50}'s were calculated. Relative potency (rel ED_{50}) was calculated as the ratio of the ED_{50} for T_3 to that for the test compound, expressed as a percentage. The ED_{50} for T_3 on liver GPDH activity was 1.6×10^{-7} mol/kg; on heart GPDH it was 0.7×10^{-7} mol/kg. Many compounds did not give the same maximal increase in GPDH as did T_3; for these low maximum (LM) compounds, the percentage of the maximum response given by the highest dose used, 50 mg/kg, was calculated. Receptor binding in vitro was determined by using isolated nuclei prepared by sedimentation of homogenates through high density sucrose solutions. Nuclei were incubated with [^{125}I]T_3 and increasing concentrations of test compound by using the conditions of Samuels and Tsai[21] for liver nuclei and Koerner and co-workers[3] for heart nuclei. Bound and free [^{125}I]T_3 were separated by centrifugation, and the concentration of test compound that reduced the binding of [^{125}I]T_3 by 50% (IC_{50}) was determined. Relative binding (rel IC_{50}) was defined as the ratio of the IC_{50} for T_3 to that of the test compound, expressed as a percentage. The IC_{50} for T_3 (which is similar for both nuclei) is 0.2–0.5 nM. The rel ED_{50} and rel IC_{50} values are given in Table I. Logarithms of these activities were used to calculate the quantitative structure–activity relationships described here. Duplicate determinations of rel IC_{50} varied by less than twofold; the variance in log (rel IC_{50}) is therefore approximately ±0.3 and the standard deviation of each equation exceeds this value.

In Vitro Receptor Recognition

Selectivity. The relative in vitro affinities of the compounds in Table I for receptors from liver and heart are very closely correlated:

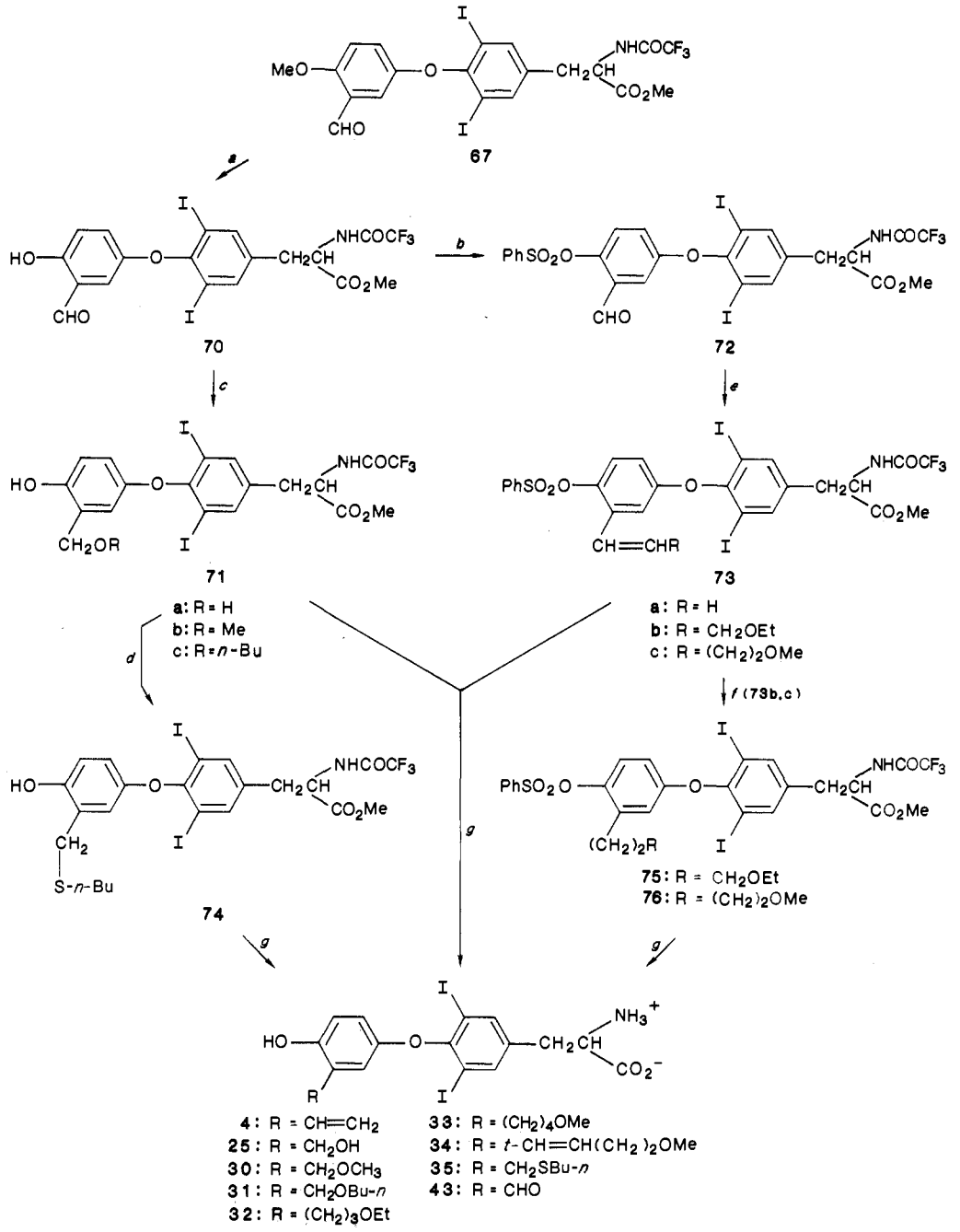
$$\log (\text{rel } IC_{50})_{\text{liver}} = 1.05 \log (\text{rel } IC_{50})_{\text{heart}} - 0.089 \quad (1)$$

$n = 44$, $r = 0.964$, $s = 0.339$, $F = 554$ ($p < 0.001$), $t = 23.64$ ($p < 0.001$)

(20) (a) Hoffman, W. W.; Richert, D. A.; Westerfield, W. W. *Endocrinology (Baltimore)* **1966**, *78*, 1189. (b) Dembri, A.; Michel, R.; Michel, O.; Belkhirin, M.; Jorgensen, E. C. *Mol. Cell. Endocrinol.* **1984**, *37*, 223.

(21) Samuels, H. H.; Tsai, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3488.

Scheme V



[a] BCl_3. [b] $PhSO_2Cl$/pyridine. [c] $NaCNBH_3$/HOAc/ROH. [d] 1-BuSH/HOAc/CF_3CO_2H. [e] $RCH_2PPh_3^+Br^-$/t-BuOK/dicyclohexano-18-crown-6. [f] H_2/Pt-C/HOAc. [g] NaOH.

In eq 1 and in subsequent equations, n is the number of compounds used to derive the equation, r is the correlation coefficient, s is the standard deviation, F is the variance ratio, and t is the ratio of the coefficient to its standard deviation. (The inclusion of each coefficient in this and in the subsequent equations is statistically significant ($p < 0.01$), and t values are given only for the final, key equations.) Compounds that show the largest differences between binding to liver and heart receptors are the methyl (**2**) and nitro (**47**) analogues, which have marginal (6–8-fold) selectivity for heart receptors. However, the high overall correlation between relative receptor binding in heart and liver, together with the slope of unity, implies that the receptors in the two tissues may be structurally similar.

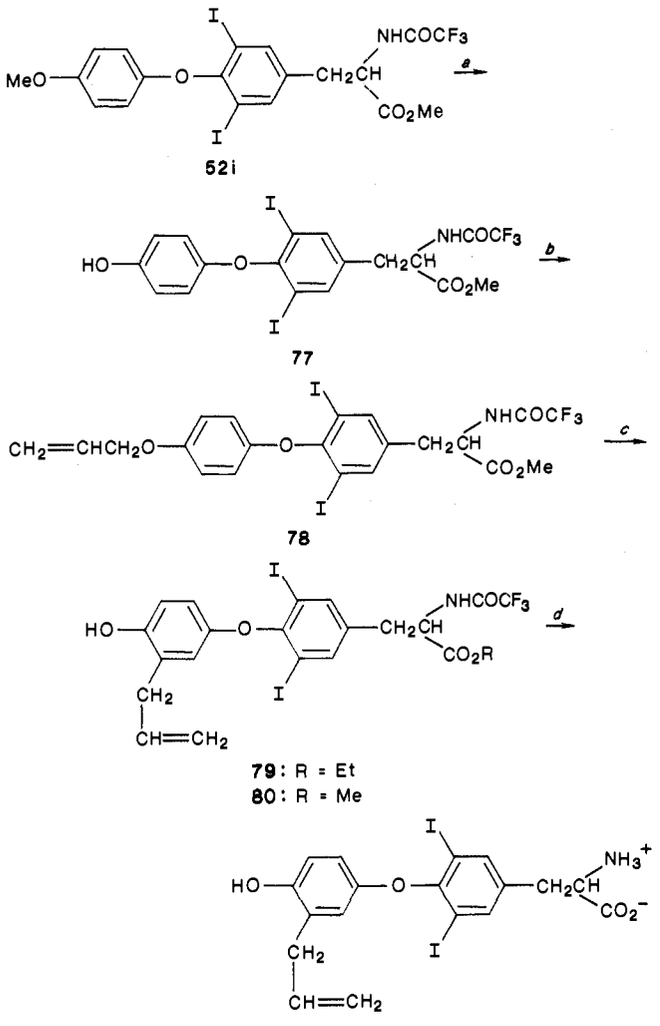
Effects of 3′-Substituent Hydrophobicity and Bulk. Figure 2 shows the relationship between liver rel IC_{50} and the hydrophobicity of the 3′-substituent (derived from octanol–water partition coefficients, Table II). There is a significant correlation:

$$\log (\text{rel } IC_{50})_{\text{liver}} = 0.544\pi - 0.037 \quad (2)$$

$n = 47$, $r = 0.824$, $s = 0.703$, $F = 94.9$ ($p < 0.001$), $t = 9.75$ ($p < 0.001$)

The hydrophobic nature of the 3′-substituent is clearly important for receptor binding, probably as a consequence of a direct, specific effect involved in receptor recognition. Since the relative affinities used to calculate eq 2 were obtained by using in vitro binding to *intact* nuclei, the hydrophobicity requirement may in part reflect nonspecific partitioning or nuclear transport from the aqueous phase to the receptor. However, this seems unlikely to be significant since relative affinities of many T_3 analogues for

Scheme VI



[a] BBr_3. [b] $BrCH_2CH{=}CH_2/K_2CO_3$/dicyclohexano-18-crown-6. [c] $PhNEt_2/\Delta$. [d] NaOH.

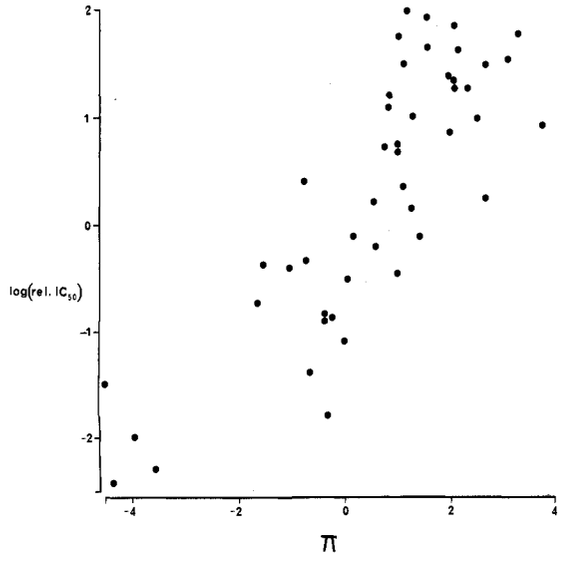


Figure 2. The relationship between in vitro nuclear binding to intact rat liver nuclei (rel IC_{50}, Table I) and 3′-substituent lipophilicity (π, Table II).

a soluble receptor preparation are similar to affinities found for receptors in intact nuclei.[4]

Figure 3 shows the relationship between in vitro liver rel IC_{50} and 3′-substituent volume (V) estimated according

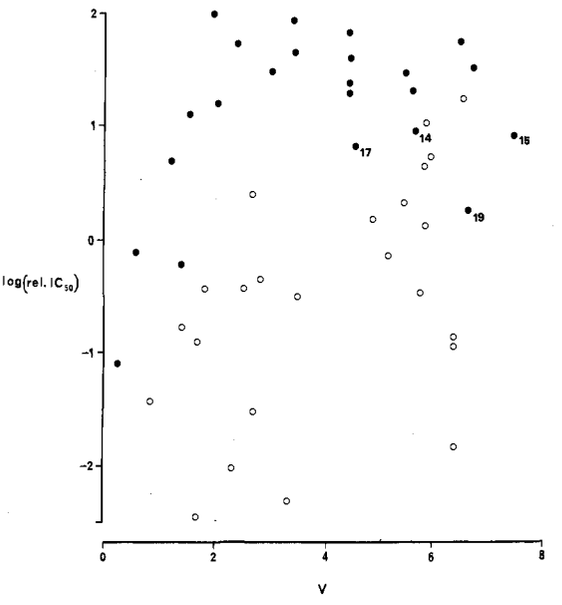


Figure 3. The relationship between in vitro nuclear binding to intact rat liver nuclei (rel IC_{50}, Table I) and 3′-substituent volume (V, Table II). The filled circles (●) represent lipophilic 3′-substituents (compounds 1–23, Table I) and the open circles (○) hydrophilic 3′-substituents (compounds 24–47, Table I). Compounds 14, 15, 17, and 19 (Table I) appear to undergo steric inhibition of binding, and conformations of these analogues are used to help deduce the possible size and shape of the binding pocket (see text).

to Bondi (see Table II). It is evident that there is no overall correlation between binding and volume for the whole data set, but consideration of the nonpolar hydrocarbon and halogen compounds alone (filled circles in Figure 3) reveals a trend. Thus, as volume increases through halogen and small alkyl up to ethyl, there is a sharp increase in receptor binding. Expression increase in volume beyond iodo (**23**) and isopropyl (**6**) apparently has little effect upon binding, with the exception of the phenyl (**17**), cyclohexyl (**14**), phenethyl (**19**), and heptyl (**15**) analogues, all of which show reduced binding. In these cases, steric inhibition at the receptor is presumably occurring.

Interestingly, homologation of the phenyl (**17**) and cyclohexyl (**14**) compounds to benzyl (**18**) and cyclohexylmethyl (**16**), respectively, restores higher binding, which is reduced on further homologation of **18** to phenethyl (**19**) (Table I). In the homologous series from ethyl (**3**) through to *n*-heptyl (**15**), only the last member shows reduced receptor affinity. Inspection of Figure 3 shows that, for any given substituent volume, introduction of hydrophilic substituents decreases binding, as would be expected, given the correlation between binding and hydrophobicity (eq 2).

Overall, the picture of the 3′-substituent thyroid hormone receptor site that emerges from Figures 2 and 3 is of a hydrophobic cavity that has substantially larger volume than an iodine atom. In order to explore the possible dimensions of the binding site we undertook a conformational analysis of the cyclohexyl compounds **14** and **16** and the phenyl compounds **17–19**, where differences in binding appear to be a consequence of steric interference with the receptor. Subsequently, substituent dimensions derived from this study are used together with hydrophobicity in the generation of quantitative structure–affinity relationships (QSARs), which account for the observed receptor affinities. In the use of these methods,

Table II. Substituent Parameters Used for Quantitative Structure–Activity Relationships and Observed and Calculated Activities

									log (rel IC_{50})$_{liver}$		log (rel ED_{50})$_{liver}$	
no.	π[a]	π'[a]	MR[a]	A[b]	V[b]	L<I[c]	D[d]	H[e]	obsd[f]	calcd[g]	obsd[h]	calcd[i]
1	0.00	0.00	0.103	0.68	0.252	2.17	0.0	0	−1.0969	−1.1766	0.0414	−0.2406
2	0.56	0.56	0.565	2.12	1.367	1.23	0.0	0	−0.2218	0.0287	0.8751	0.4397
3	1.02	1.02	1.030	3.47	2.390	0.00	0.0	0	1.7482	1.5668	2.1430	1.9652
4	0.82	0.82	1.099	2.94	2.041	0.00	0.0	0	1.2304	1.5753	1.0294	1.5493
5	1.55	1.55	1.496	4.82	3.413	0.00	0.0	0	1.6551	1.5589	1.9489	1.8126
6	1.53	1.53	1.496	4.81	3.412	0.00	0.0	0	1.9395	1.5482	2.4624	2.0370
7	1.10	1.10	1.449	4.29	3.064	0.00	0.0	0	1.5065	1.3997	1.5276	1.7072
8	2.13	2.13	1.959	6.17	4.436	0.00	0.0	0	1.6395	1.5846	2.0792	1.6058
9	2.04	2.04	1.961	6.16	4.435	0.00	0.0	0	1.8579	1.5269		1.7769
10	2.04	2.04	1.959	6.16	4.435	0.00	0.0	0	1.3118	1.5269		1.3473
11	1.98	1.98	1.962	6.36	4.434	0.00	0.0	0	1.3979	1.4328	1.4082	1.4134
12	2.67	2.67	2.424	7.52	5.459	0.00	0.0	0	1.5079	1.5835	0.8751	1.1039
13	3.21	3.21	2.890	8.87	6.482	0.00	0.0	0	1.7782	1.5823	0.6920	0.6893
14	2.51	2.51	2.669	6.75	5.679	0.00	2.2	0	0.9926	0.9108		0.3928
15	3.75	3.75	3.355	10.22	7.505	0.00	1.1	0	0.9415	1.1949		−0.7556
16	3.05	3.05	3.134	8.10	6.702	0.00	0.0	0	1.5611	1.6823		0.1201
17	1.96	1.96	2.536	5.33	4.584	0.00	2.2	0	0.8633	0.9241	0.8195	0.4639
18	2.01	2.01	3.001	6.68	5.607	0.00	0.4	0	1.3365	1.2263	0.3345	0.1643
19	2.66	2.66	3.465	8.03	6.630	0.00	3.2	0	0.2553	0.3157		−1.4962
20	0.14	0.14	0.092	1.10	0.580	1.58	0.0	0	−0.1192	−0.4317		0.5309
21	0.71	0.71	0.603	1.81	1.200	0.71	0.0	0	0.7076	0.8863	0.4456	1.1716
22	0.86	0.86	0.888	2.13	1.512	0.40	0.0	0	1.1139	1.3022	1.3054	1.4769
23	1.12	1.12	1.394	2.51	1.964	0.00	0.0	0	2.000	1.8924	2.0000	2.1091
24	−0.67	−0.67	0.285	1.46	0.804	1.49	0.0	0	−1.3979	−0.9556		−0.4808
25	−1.03	−1.03	0.719	2.81	1.827	0.26	0.0	0	−0.4089	0.0323		0.2857
26	−0.77	−0.77	1.184	4.16	2.850	0.00	0.0	0	−0.3468	0.1800		0.2942
27	0.55	0.55	2.105	6.86	4.896	0.00	0.0	0	0.2068	0.3387		0.3758
28	1.00	1.00	2.578	8.21	5.919	0.00	0.0	0	0.7275	0.2772		0.3041
29	1.44	1.44	2.718	6.11	5.136	0.0	2.8	0	−0.1192	0.1545		−0.5523
30	−0.78	−0.78	1.206	4.07	2.760	0.00	0.0	0	0.4065	0.1975	1.3979	0.8854
31	0.99	0.99	2.600	8.12	5.829	0.00	0.0	0	0.7050	0.2947		0.2579
32	1.28	1.28	2.600	8.12	5.829	0.00	0.0	0	0.1614	0.4893		−0.1710
33	1.28	1.28	2.600	8.12	5.829	0.00	0.0	0	1.0370	0.4893	−0.1079	0.5199
34	1.08	1.08	2.394	7.58	5.477	0.00	2.1	0	0.3502	−0.2370		0.2235
35	2.31	2.31	3.191	9.02	6.539	0.00	0.0	0	1.2788	0.9381		−0.2005
36	−0.30	−0.30	2.885	8.76	6.360	0.00	0.0	0	−1.7959	−0.7431		−2.1226
37	−0.38	−0.38	2.885	8.76	6.360	0.00	0.0	0	−0.9208	−0.7968		−1.4322
38	−0.39	−0.39	2.885	8.76	6.360	0.00	0.0	0	−0.8539	−0.8035		−1.3794
39	−4.36	−4.36	0.605	2.38	1.678	0.82	0.0	0	−2.4437	−2.8114		−1.3146
40	−4.53	−4.53	0.977	3.73	2.701	0.00	0.0	0	−1.5229	−2.2270		−0.6105
41	−3.92	−3.92	1.002	3.84	2.323	0.00	0.0	0	−2.0000	−1.8473		−0.9892
42	−3.52	−3.52	1.464	5.19	3.346	0.00	0.0	0	−2.3010	−1.9424		−1.3003
43	−0.65	0.22	0.688	2.28	1.422	0.70	0.0	1	−0.7447	−0.9547		0.0222
44	−0.55	0.44	1.118	3.72	2.537	0.17	0.0	1	−0.4318	−0.5084	2.0828[j]	0.2618[j]
45	0.06	1.05	1.583	5.05	3.560	0.00	0.0	1	−0.5086	−0.2370		0.0805
46	1.05	2.04	3.033	6.93	5.754	0.00	0.4	1	−0.4318	−0.2194		−1.2831
47	−0.28	0.31	0.736	2.55	1.680	0.79	0.0	1	−0.8861	−1.0835	−0.4949	−0.0916

[a] π and π' are the substituent lipophilicity constants from the benzene and 2-substituted phenol systems, respectively, and MR is molar refractivity × 10^{-1}; π, π', and MR were taken from: Hansch, C.; Leo, A *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley-Interscience: New York, 1979. π values for **39–42** are for the charged species. [b] V is substituent volume (cm^3 mol^{-1} × 10^1) and A is substituent surface area (cm^2 mol^{-1} × 10^9) calculated from: Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441. [c] Substituent length less than that of iodo, in angstroms, along the axis of the C-3′–substituent bond (see text). [d] Substituent extension in angstroms beyond the optimal AA′ envelope (Figure 4c; see text). [e] Indicator variable, equal to 1 for strong intramolecular hydrogen bond with 4′-hydroxyl (see Table IV). [f] From Table I. [g] From eq 7. [h] From Table I, potencies of full agonists. [i] From eq 18. [j] Not used in the derivation of eq 18; see text.

assumptions are made that the shape of the receptor will be complementary to those ligands for which it has greatest affinity and that the diphenyl ether moiety of each compound has the same mode of binding to the receptor.

Conformational Analysis. The compounds used for this study were model ortho-substituted phenols, where the substituent has one (as in **14** and **17**), two (as in **16** and **18**), and three (as in **19**) rotatable bonds. Restricting the numbers of rotatable bonds to three permits conformational space to be examined comprehensively by construction of one-, two-, and three-dimensional energy maps. The results are summarized in Figure 4. Computation of a two-dimensional energy map for 2-(cyclohexylmethyl)-phenol, corresponding to the most tightly bound analogue (**16**), revealed three low-energy conformations, labeled A, B, and C (Figure 4a). The cyclohexyl ring conformation was assumed to be in the low-energy chair form, with the benzyl substituent equatorial. A similar analysis of 2-benzylphenol, corresponding to the next most tightly bound analogue (**18**), produced the expected symmetrical map (Figure 4b). The conformers labeled A′, B′, and C′ are those corresponding to conformers A, B, and C, respectively, where the planes of the phenyl and cyclohexyl rings are parallel. Superimpositions of each pair of conformers AA′, BB′, and CC′ are shown in Figures 4c–e; in each case orthogonal views of stick representations are shown, together with van der Waals volumes of the overlaid pair of molecules. Comparison of the structures in Figures 4c–e shows that the van der Waals surfaces of conformers AA′ extend out perpendicularly 4.6 Å from the 2-position of the phenyl ring (C-3′ in the analogues), whereas conformers BB′ and CC′ extend out perpendicularly 6.7 Å. (This definition of substituent length is similar to that defined by Verloop and co-workers.)[22] 2-

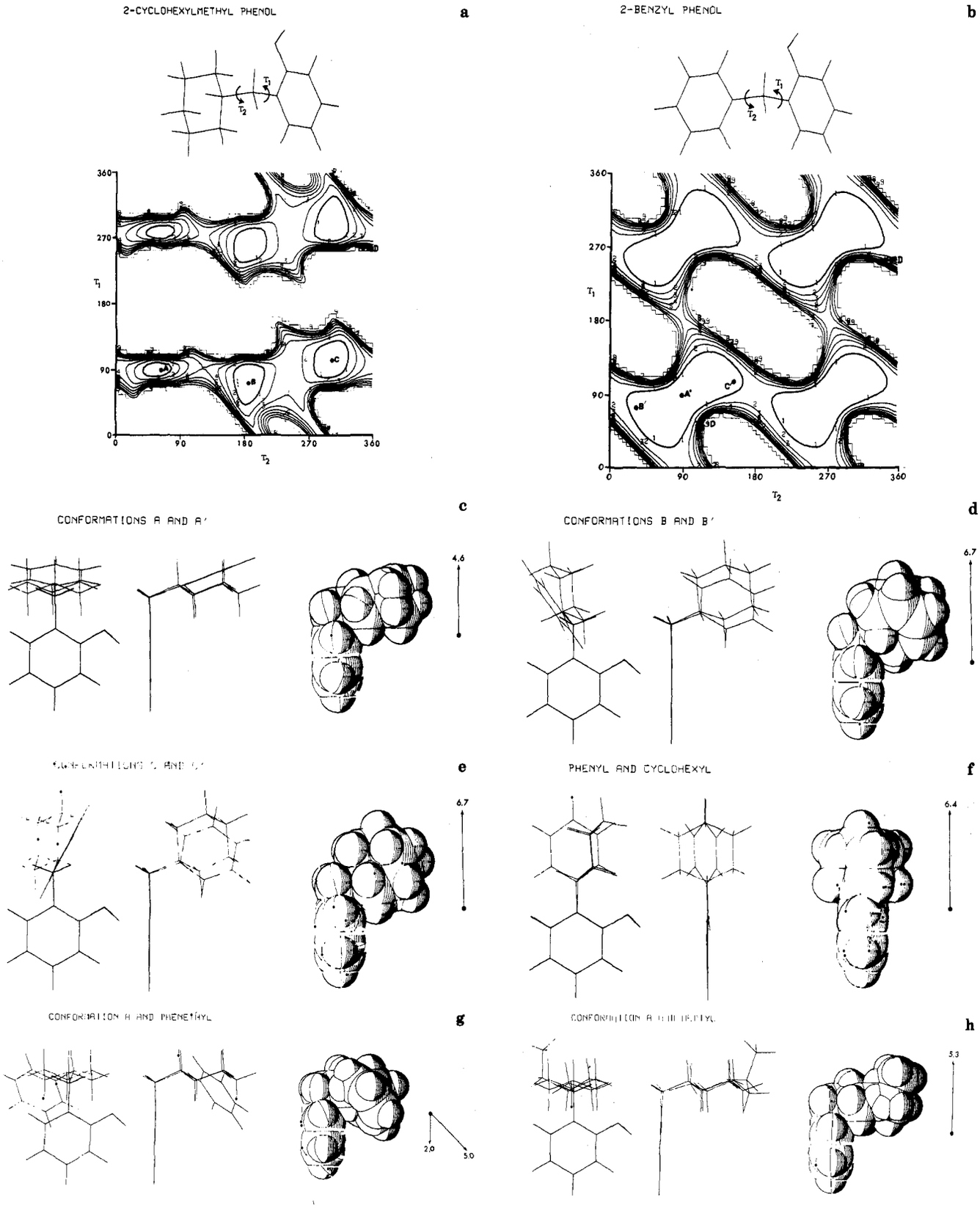


Figure 4. (See text.) Conformational studies of model 2-substituted phenols corresponding to compounds 14–19 (Table I). (a) Ramachandran energy plot (hard sphere approximation) for 2-(cyclohexylmethyl)phenol (corresponding to 16) showing low-energy conformers A, B, and C. (b) Ramachandran energy plot for 2-benzylphenol showing low-energy conformers A′, B′, and C′, where the phenyl ring of the benzyl substituent lies parallel to the plane of the cyclohexyl ring in conformers A, B, and C respectively. (c,d,e) Superimpositions of conformers A and A′, B and B′, C and C′ respectively, derived from the torsion angles in a and b. In c–h, orthogonal views of the overlaid structures are shown from three views: (i) with the phenolic ring in the plane of the paper, (ii) with the phenolic ring perpendicular to the paper, and (iii) a space-filling (van der Waals radii) representation of ii. The dimensions given are the distances in angstroms, along orthogonal *xyz* axes, of the surfaces of the van der Waals envelope which extend from the 3′-carbon, the vertical *y* axis corresponding to the 3′-carbon–substituent bond. (f) Superimposition of 2-phenylphenol and 2-cyclohexylphenol, corresponding to analogues 14 and 17, respectively. (g,h) Superimpositions of conformation A of 2-(cyclohexylmethyl)phenol with low-energy conformers of 2-phenethylphenol (analogue 19) and 2-heptylphenol (analogue 15) respectively.

Table III. Cross-Correlation Matrix (r) for QSAR Parameters

	π'	π	MR	V	A	L<I	D	H
π	0.989							
MR	0.559	0.572						
V	0.512	0.529	0.983					
A	0.466	0.485	0.952	0.989				
L<I	−0.234	−0.239	−0.662	−0.662	−0.663			
D	0.301	0.313	0.423	0.347	0.258	0.174		
H	0.022	−0.127	−0.140	−0.165	−0.175	0.079	−0.099	
λ^a	−0.857	−0.833	−0.077	−0.002	0.057	−0.122	−0.189	−0.127

[a] Derived from π' and A according to eq 9.

Phenylphenol and 2-cyclohexylphenol have relatively well-defined minimum energy conformations, in which both substituents, corresponding to analogues **17** and **14**, extend out a similar distance, 6.4 Å, to BB′ and CC′ (Figure 4f). Since **14** and **17** have reduced affinity in comparison with their homologues (**16** and **18**, Table I), it appears that conformers BB′ and CC′ do not represent likely receptor-bound structures of the 3′-substituent in **16** and **18**. This analysis, which assumes that steric inhibition of binding occurs as substituent perpendicular length increases, suggests that conformations AA′ more realistically represent the receptor-bound conformations of **16** and **18** than do the energetically equivalent, but lengthier, alternative conformers of BB′ and CC′. These findings support the suggestion of Jorgensen and co-workers[4] that 3′-substituents that extend out from the 3′-carbon further than does the natural iodo substituent (4.2 Å) will sterically interfere with binding. However, it is evident that this apparent iodo-limiting steric inhibition of binding is directional and is not a function of an average, nondirectional 3′-substituent length, as used by Jorgensen and co-workers.[4] Thus, extension of 3′-substituent bulk into the volume occupied by the phenyl and cyclohexyl rings of **16** and **18** as represented by the union of conformations A and A′ (Figure 4c) results in high affinity for the receptor. Compounds in which the 3′-substituent extends beyond the AA′ volume have reduced affinities, for example, the phenethyl (**19**) and *n*-heptyl (**15**) analogues. A three-dimensional energy map was constructed for 2-phenethylphenol, and conformers that were within 1 kcal mol^{-1} of the global minimum were identified. Those conformers that extended out perpendicularly from the 2-position carbon by ≤4.6 Å (see Figure 4a) were selected and compared with the AA′ union. In all instances, the phenyl ring of 2-phenethylphenol extended beyond the envelope provided by AA′. Figure 4g shows an overlay of conformation A and a typical low-energy 2-phenethylphenol conformer, together with the van der Waals representation showing the additional volume occupied by the phenethyl substituent. Similarly, allowable conformations of 2-*n*-heptylphenol cannot fit within the volume of the cyclohexylmethyl moiety of conformation A (Figure 4h). In contrast, the 3′-substituents in the homologous series from ethyl to *n*-hexyl, which have equivalent and high affinities for the receptor (compounds **3**, **5**, **8**, **12**, and **13**, Table I), each possess allowable conformations within the "high affinity" AA′ envelope (data not shown). The precise nature of the steric inhibition of binding seen with 2-phenethyl and *n*-heptyl substitution cannot be deduced because of the very many conformations open to these analogues. Consequently, the representations in Figures 4g and 4h are not proposed receptor-bound conformers of **15** and **19**, but are those minimum-energy conformers that most closely resemble conformation A of the tightly bound cyclohexylmethyl analogue **18**. The substituent dimensions given for **15** and **19** in Figures 4g and 4h can, however, be used to generate substituent size parameters for quantitative structure–affinity relationships.

Quantitative Structure–Affinity Relationships (QSARs). The dependence of receptor binding upon 3′-substituent partition coefficient (Figure 2, eq 2) and bulk (Figure 3), together with the conformational analysis (Figure 4), serves to provide a new model for 3′-substituent recognition by the thyroid hormone receptor. This model proposes that the binding pocket is essentially lipophilic and is limited in depth to approximately the length of an iodine substituent, but has sufficient width to accommodate a cyclohexyl or phenyl ring. To test this model, we have constructed multiple regression equations describing receptor affinity in terms of the 3′-substituent properties of hydrophobicity, bulk, length, and hydrogen-bonding interaction with the 4′-hydroxyl. As will be demonstrated, the influence on affinity of intramolecular hydrogen bonds between 3′-substituent and 4′-hydroxyl becomes clearly apparent only when the effects of the other 3′-substituent properties are quantified. The parameters used are given in Table II and the cross-correlation matrix for these properties in Table III.

The influence of hydrophobicity and bulk, expressed in Figures 2 and 3, can be described quantitatively by eq 3, in which addition of a bulk descriptor (MR, Table II) significantly ($p < 0.001$) improves eq 2. In eq 3, π is the

$$\log (\text{rel } IC_{50})_{\text{liver}} = 0.663\pi + 2.42\text{MR} - 3.56 \log (0.1 \times 10^{\text{MR}} + 1) - 0.910 \quad (3)$$

$$n = 47,\ r = 0.920,\ s = 0.498,\ F = 78.7$$

substituent hydrophobicity parameter as used in eq 2 and MR is substituent molar refractivity; MR in eq 3 can be replaced by either substituent volume V or surface area A (see Table II) but gives slightly better correlations than either of these alternative bulk descriptors. The bilinear function in MR best describes the overall effect of substituent size, receptor affinity increasing rapidly (slope 2.42) with increasing MR up to the optimal value of 1.36 (cf. iodine 1.394) and then declining more slowly with increasing MR beyond the optimum (slope −1.14). Equation 3 is very similar to a previous analysis[10] using only 27 of the compounds in Table I and confirms the findings of this earlier work on the effects of 3′-substituent lipophilicity and bulk on receptor affinity. Examination of the residuals from eq 3 strikingly showed that the affinities of compounds **43–47** are overestimated by up to an order of magnitude. Each of these 3′-acyl and -nitro compounds has the capacity to form a strong intramolecular hydrogen bond with the 4′-hydroxyl, with energies in the region of 6–8 kcal mol^{-1} (Table IV). Addition of an indicator variable H, equal to 1 for compounds **43–47** and 0 for all remaining compounds, to eq 3 results in a

(22) Verloop, A.; Hoogenstraaten, W.; Tipker, J. In *Drug Design*; Ariens, E. J., Ed.; Academic: New York, 1976; Vol. 7, p 165.

Table IV. Intramolecular Hydrogen Bond Energies (E, kcal mol^{-1}) and pK_a Values of 2-Substituted Phenols

corresponding analogue	R	E	pK_a[l]
1	H	0.00	9.99
2	CH_3	-0.40[a]	10.29
6	$CH(CH_3)_2$	-0.60[b]	10.47[m]
7	$CH_2CH{=}CH_2$	0.00[c]	10.28
11	$C(CH_3)_3$	-1.38[d]	10.62
17	Ph	1.0[e]	10.01
18	CH_2Ph	-0.20[c]	10.12[m]
20	F	1.44[f]	8.73
21	Cl	1.62[f]	8.56
22	Br	1.57[f]	8.45
23	I	1.45[f]	8.51
24	OH	2.29[g]	9.34
25	CH_2OH	3.00[h]	9.92
30	CH_2OCH_3	3.90[h]	9.85[m]
43	CHO	7.20[i]	8.37
44	$COCH_3$	8.10[j]	10.06
47	NO_2	6.70[k]	7.23

[a] Schaefer, T.; Chum, K. *Can. J. Chem.* **1978**, *56*, 1788. [b] Schaefer, T.; Addison, B. M.; Sebastian, R.; Wildman, T. A. *Can. J. Chem.* **1981**, *59*, 1656. [c] Schaefer, T.; Sebastian, R.; Wildman, T. A. *Can. J. Chem.* **1979**, *57*, 3005. [d] Carlson, G. L.; Fately, W. G. *J. Phys. Chem.* **1973**, *77*, 1157. [e] Kinugasa, T.; Nakamura, M.; Ueji, S. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1663. [f] Carlson, G. L.; Fateley, W. G.; Manocha, A. S.; Bentley, F. F. *J. Phys. Chem.* **1972**, *76*, 1553. [g] Reference 8. [h] Takasuka, M.; Matsui, Y. *J. Chem. Soc., Perkin Trans. 2* **1979**, 1743. [i] Schaefer, T.; Sebastian, R.; Laatikainen, R.; Salman, S. R. *Can. J. Chem.* **1984**, *62*, 326. [j] Calculated by the method of Schaefer: Schaefer, T. *J. Phys. Chem.* **1975**, *79*, 1888. [k] Schaefer, T. *J. Phys. Chem.* **1975**, *79*, 1888. [l] Taken from the following: Albert, A.; Serjeant, E. P. *The Determination of Ionization Constants*, 3rd ed.; Chapman and Hall: 1984. Kortum, G.; Vogel, W.; Andrussow, K. *Dissociation Constants of Organic Acids in Aqueous Solution*; Butterworths: 1961. [m] Calculated according to: Barlin, G. B.; Perrin, D. D. *Q Rev., Chem. Soc.* **1966**, *20*, 75.

statistically significant improvement; $F_{1,42}$ (for addition of H) = 13.1 ($p < 0.001$).

$$\log (\text{rel } IC_{50})_{\text{liver}} = 0.655\pi + 2.48\text{MR} - 3.67 \log (0.1 \times 10^{\text{MR}} + 1) - 0.752H - 0.823 \quad (4)$$

$$n = 47,\ r = 0.939,\ s = 0.442,\ F = 77.8$$

$$t_\pi = 15.19,\ t_{\text{MR}} = 6.13,\ t_{\log(0.1\times10^{\text{MR}}+1)} = 7.04,\ t_H = 3.54\ (p < 0.001)$$

Although 2-nitrophenol, as well as, by analogy, analogue **47**, possesses a strong intramolecular hydrogen bond in nonpolar media, the nitro group increases the acidity of the 4′-hydroxyl substantially over a normal phenol, the pK_a for **47** being 6.85 at 37 °C. Thus, at the pH (7.8) of the liver binding assay, compound **47** exists as approximately 90% 4′-phenoxide. If the receptor will only recognize an un-ionized 4′-hydroxyl, then the relative loss of binding seen with **47** could be due to this ionization. However, from the present data, it is not possible to ascertain if a 4′-phenoxide will bind to the receptor; consequently, although the use of the H parameter in eq 4 for **47** permits quantitative treatment of this compound, it may have no physical significance. For the acyl derivatives **43–46**, substantial 4′-hydroxyl ionization is not expected to occur under the assay conditions (on the basis of the pK_a values for the parent phenols, Table IV) and the use of the H parameter for these compounds is justifiable. Removal of the nitro analogue **47** from the analysis results in an equation of quality identical with that of eq 4, with the inclusion of H equally statistically significant. Use of 4′-hydroxyl pK_a, with or without the addition of 3′-substituent σ values,[4] instead of H did not lead to any improvement in eq 4. Addition of a wide range of substituent electronic parameters including F, R, σ_I, and σ_R similarly did not improve eq 4.

The π values used in deriving eq 2–4 are taken from the benzene system; the presence of strong intramolecular hydrogen bonds in 2-acylphenols increases the π values of the acyl group by around 1 unit (Table II). Using π values calculated from the log P determinations of 2-substituted phenols (π', Table II) gives eq 5. In eq 5, the coefficient

$$\log (\text{rel } IC_{50})_{\text{liver}} = 0.657\pi' + 2.46\text{MR} - 3.65 \log (0.1 \times 10^{\text{MR}} + 1) - 1.34H - 0.804 \quad (5)$$

$$n = 47,\ r = 0.939,\ s = 0.441,\ F = 78.7$$

$$t_{\pi'} = 15.26,\ t_{\text{MR}} = 6.10,\ t_{\log(0.1\times10^{\text{MR}}+1)} = 7.04,\ t_H = 6.27\ (p < 0.001)$$

of H has decreased to −1.34 from −0.752 to compensate for the increased π' values of compounds **43–47**. The coefficients of H in eq 4 and 5 suggest that the interaction of a free 4′-hydroxyl with the receptor contributes 1.1 and 1.9 kcal mol^{-1}, respectively, to the apparent free energy of binding. These values, which are in excellent agreement with those obtained from comparisons of the binding affinities of 3′-substituted T_3 analogues with the corresponding 4′-deoxy compounds,[12,23] strongly support the model proposed by Kollman and co-workers, in which the 4′-hydroxyl donates a hydrogen bond to the receptor.[7] Interestingly, several of the other 3′-substituents examined have the potential for forming acceptor intramolecular hydrogen bonds with the 4′-hydroxyl, but of lesser strength (Table IV). In these cases, it is probable that the intramolecular hydrogen bonds do not persist to any great extent in the aqueous assay medium, and consequently free 4′-hydroxyl groups are available for receptor binding. The intramolecular hydrogen bond in 2-nitrophenol is similarly broken by weakly basic solvents.[24] In contrast, the stronger hydrogen bonding seen with 3′-acyl analogues **43–46** probably persists appreciably in water, as evidenced by the higher π values for the substituents in the 2-phenol system relative to the benzene system. Assuming that these hydrogen bonds in **43–46** are not substantially broken either by water or by the receptor, then the π' values used in eq 5 are more appropriate for QSAR purposes than the π values of eq 4. Equation 5 provides a reasonable quantitative description of the data preented in Figures 2 and 3 and additionally highlights how a specific structural property, intramolecular 3′-substituent–4′-hydroxyl hydrogen bonding, influences receptor binding. However, the use of the bulk properties MR and π' gives little indication of the effects of substituent shape and specific hydrophilic character. In addition, although the correlation between MR and π' is not high for all 47 analogues (r = 0.559, Table III), for the apolar 3′-substituents in compounds **1–23**, there is a very significant correlation (r = 0.944). Thus, it is not possible, by using eq 5, to assess confidently the relative contributions of substituent size and lipophilic character to receptor binding.

The conformational analysis presented above provides a means of assessing the effects of substituent size which can be checked quantitatively. Two parameters were developed for this purpose: L<I and D (Table II). The term L<I applies to those 3′-substituents whose length L along the axis of the 3′-carbon–substituent bond is less than that of the natural iodo substituent; L<I is then calculated by using eq 6. The value of L_I (4.2 Å) was taken from the

(23) Somack, R.; Andrea, T. A.; Jorgensen, E. C. *Biochemistry* **1982**, *21*, 163.
(24) Kamlet, M. J.; Taft, R. W. *J. Org. Chem.* **1982**, *47*, 1734.

$$L<I = L_I - L_R \quad (6)$$

compilation of Verloop and co-workers.[22] The values of L_R either were taken from the same source or were calculated from energy-minimized structures of the appropriate 2′-substituted phenols. Many of the analogues with flexible 3′-substituents possess low-energy conformations where L_R can be equal to L_I; for these compounds, L<I = 0. The term D applies to bulky substituents that extend beyond the optimal envelope expressed by the union AA′ (Figure 4c); D is not directional and is defined as the distance (in angstroms) that the substituent protrudes from the AA′ cyclohexyl/phenyl surface. For **17** and **29**, D is the distance these substituents extend out, along the 3′-carbon–substituent bond, further than iodo.

It was found that the parameters L<I and D could replace log $(0.1 \times 10^{MR} + 1)$ in eq 5, resulting in a significantly improved equation ($r = 0.955$); a further improvement was obtained if substituent area A (Table II) was used instead of MR:

$$\log (\text{rel } IC_{50})_{liver} = 0.671\pi' - 0.269A - 1.30L<I - 0.351D - 1.40H + 1.82 \quad (7)$$

$$n = 47,\ r = 0.963,\ s = 0.351,\ F = 104.4$$

$$t_{\pi'} = 20.37,\ t_A = 8.84,\ t_{L<I} = 9.18,\ t_D = 5.10,\ t_H = 8.19\ (p < 0.001)$$

Calculated log (rel IC_{50}) values from eq 7 are given in Table II. Use of substituent volume V, instead of A, gave a similar equation ($r = 0.960$). The bulk parameters MR, V, and A are closely correlated (Table III) and are therefore interchangeable. For the apolar analogues **1–23**, π' and A are highly correlated:

$$\pi' = 0.371A - 0.175 \quad (8)$$

$$n = 23,\ r = 0.984,\ s = 0.180,\ F = 659$$

Thus, for compounds **1–23**, the negative coefficient of A and the positive coefficient of π' in eq 7 essentially cancel each other, i.e., $(0.671\pi' - 0.269A)$ is close to 0 for these nonpolar 3′-substituents. The affinities of compounds **1–23** are effectively accounted for by the size parameters L<I and D alone. For the 3′-substituents with polar functionality (**24–47**), π' and A are not linearly related, and $(0.671\pi' - 0.269A)$ is not equal to 0. Using eq 8, we developed the parameter λ, calculated for each compound according to eq 9. The term λ has values close to 0 for

$$\lambda = 0.371A - \pi' - 0.175 \quad (9)$$

nonpolar substituents and increases for increasingly hydrophilic substituents. Similar parameters, which can be considered as estimates of hydrophilicity, have been developed by Moriguchi and co-workers[25] using log P and by Testa and Seiler,[26] who used the hydrophobic fragmental constant f. Replacing π' and A by λ in eq 7 gives eq 10.

$$\log (\text{rel } IC_{50})_{liver} = -0.671\lambda - 1.23L<I - 0.360D - 1.38H + 1.58 \quad (10)$$

$$n = 47,\ r = 0.962,\ s = 0.349,\ F = 131.4$$

$$t_\lambda = 20.48,\ t_{L<I} = 11.46,\ t_D = 5.34,\ t_H = 8.22\ (p < 0.001)$$

Because relative affinities for heart and liver receptors are well correlated (eq 1), the same 3′-substituent properties also effectively account for the heart data, eq 11 and 12 corresponding to the liver eq 4 and 10, respectively. Thus,

$$\log (\text{rel } IC_{50})_{heart} = 0.633\pi' + 2.18MR - 3.48 \log (0.1 \times 10^{MR} + 1) - 1.06H - 0.411 \quad (11)$$

$$n = 44,\ r = 0.928,\ s = 0.456,\ F = 60.4$$

$$t_{\pi'} = 14.08,\ t_{MR} = 4.54,\ t_{\log(0.1\times10^{MR}+1)} = 5.78,\ t_H = 4.79$$

$$\log (\text{rel } IC_{50})_{heart} = -0.635\lambda - 0.890L<I - 0.378D - 1.06H + 1.50 \quad (12)$$

$$n = 44,\ r = 0.938,\ s = 0.422,\ F = 71.4$$

$$t_\lambda = 15.95,\ t_{L<I} = 6.27,\ t_D = 4.37,\ t_H = 5.21\ (p < 0.001)$$

eq 10 and 12 effectively account for the observed variation in nuclear binding affinity; the parameters used to calculate these equations are not significantly interrelated (Table III).

Summary. The conformational and QSAR studies described here provide a detailed picture of the nature of the 3′-substituent's binding pocket in the thyroid hormone receptor. The main features are summarized as follows. (1) The site appears to be limited in depth to approximately the length of the natural iodo substituent (4.2 Å). Substituents that do not extend fully into the pocket cause loss of analogue binding affinity in direct proportion to the decrease in substituent length from iodo, probably reflecting loss of favorable substituent–receptor van der Waals binding. (2) Substituents of greater bulk than iodo can be tolerated, especially if conformations are accessible where substituent length, along the C-3′–substituent bond, is not substantially greater than that of iodo. (3) These studies have convincingly demonstrated for the first time that the substituent binding pocket is essentially hydrophobic in character; affinity for the receptor decreases as substituent hydrophilic character increases. However, introduction of polar groups by methylene substitution of the high binding hexyl analogue **13** gave isomeric ethers **31–33** and amides **36–38** with different relative affinities, suggesting that the binding site does not possess uniform hydrophobic character. The different affinities of the 3′-methoxybutyl compound **33** (10.9% of T_3) and the isomeric 3′-ethoxypropyl analogue **32** (1.5% of T_3) imply that there is an element of the binding pocket that can tolerate a polar group. (4) 3′-Acyl derivatives, which form strong acceptor intramolecular hydrogen bonds with the 4′-hydroxyl, have apparent binding energies 1.1–1.9 kcal mol^{-1} less than expected on the basis of their size and lipophilicity, suggesting that a free 4′-hydroxyl is required for optimal affinity, possibly by hydrogen bonding to the receptor.

In Vivo Thyromimetic Activity

Selectivity. Significant relationships were found to exist between both maximal GPDH responses (% max) and potencies for full agonists (rel ED_{50}) in heart and liver:

$$(\%\ max)_{liver} = 0.738(\%\ max)_{heart} + 24.4 \quad (13)$$

$$n = 44,\ r = 0.823,\ s = 20.3,\ F = 88.2\ (p < 0.001)$$

$$\log (\text{rel } ED_{50})_{liver} = 0.911 \log (\text{rel } ED_{50})_{heart} + 0.277 \quad (14)$$

$$n = 15,\ r = 0.974,\ s = 0.173,\ F = 241\ (p < 0.001),\ t = 15.51\ (p < 0.001)$$

Expression of thyromimetic activity will depend upon intrinsic nuclear receptor affinity, as well as those transport factors that influence penetration to receptors. Since these compounds are not able to distinguish between receptors in the two tissues (eq 1), the high overall correlations between activities are to be expected. Examination of Table

(25) Moriguchi, I.; Kanada, Y.; Komatsu, K. *Chem. Pharm. Bull.* **1976**, *24*, 1799.
(26) Testa, B.; Seiler, P. *Arzneim.-Forsch.* **1981**, *31*, 1053.

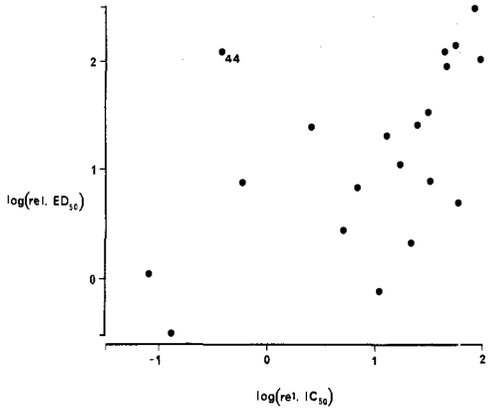


Figure 5. The relationship between in vivo thyromimetic activity (rel ED_{50}) and in vitro nuclear binding (rel IC_{50}) for liver full agonists (Table I). The 3′-acetyl analogue **44** shows the greatest difference between activity and binding (see text).

I shows that several analogues display tissue differences in thyromimetic activity; none of these compounds is a full agonist in both tissues, and consequently they were not used in the generation of eq 14. The differences seen relate to differential maximal GPDH responses (% max) used to calculate eq 13. For example, 3′-hexyl (**13**), 3′-cyclohexyl (**14**), 3′-ethoxypropyl (**32**), and 3′-(butylthio)methyl (**35**) analogues possess full agonist activity in the liver, but are submaximal agonists in the heart. Liver selectivity is also seen with the amides **37** and **38** and the amine **42**, which are submaximal agonists in the liver and inactive in the heart. Two compounds, the 3′-hydroxymethyl (**25**) and formyl (**43**) analogues, show some heart selectivity, being full agonists in this tissue and submaximal agonists in the liver. As will be demonstrated below, some differences between heart and liver also emerge when the relationships between in vitro affinity and activity are compared in the two tissues.

Relationships with in Vitro Nuclear Binding. Correlations between in vitro nuclear binding and thyromimetic activity serve as the basis for the derivation of quantitative relationships between structure and overall activity. Such correlations also provide a test for the nuclear receptor hypothesis of thyroid hormone action; for example, high correlations between rat liver nuclear binding and antigoiter activity[3,4] have been used in support of the hypothesis. The data in Table I provide an opportunity to examine relationships between binding and activity in the same tissues.

For those full agonists where potencies were obtained, the relationships between potency (rel ED_{50}) and binding (rel IC_{50}) are shown in Figures 5 and 6 for liver and heart, respectively. In both tissues, the 3′-acetyl analogue **44** shows the widest discrepancy between binding and activity, with activity exceeding in vitro binding by 2 orders of magnitude. However, we have shown that this analogue does not challenge the nuclear receptor hypothesis, since its affinity for receptors when measured in vivo is fully consistent with the observed thyromimetic activity.[11] When this compound was removed from the analysis, the following equations linking potency and in vitro binding were established:

$$\log (\text{rel ED}_{50})_{\text{liver}} = 0.637 \log (\text{rel IC}_{50})_{\text{liver}} + 0.434 \quad (15)$$

$$n = 19,\ r = 0.693,\ s = 0.613,\ F = 15.8\ (p < 0.001)$$

$$\log (\text{rel ED}_{50})_{\text{heart}} = 1.14 \log (\text{rel IC}_{50})_{\text{heart}} - 0.379 \quad (16)$$

$$n = 16,\ r = 0.894,\ s = 0.437,\ F = 55.7\ (p < 0.001)$$

$$t = 7.47\ (p < 0.001)$$

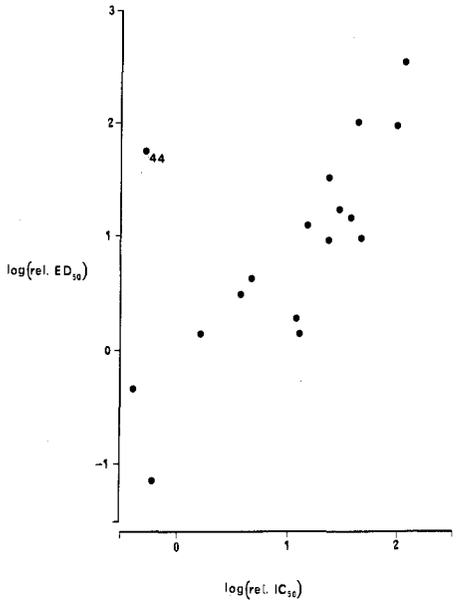


Figure 6. The relationship between in vivo thyromimetic activity (rel ED_{50}) and in vitro nuclear binding (rel IC_{50}) for heart full agonists (Table I).

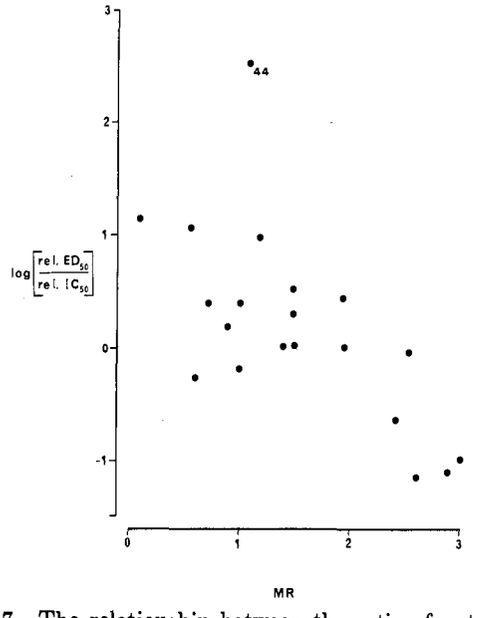


Figure 7. The relationship between the ratio of potency to nuclear binding (rel ED_{50}/rel IC_{50}) and 3′-substituent molar refractivity (MR, Table II) for the liver full agonists in Table I.

Activity in the heart is well explained by relative affinity (eq 16), but liver activity shows greater variance (eq 15); also compare Figures 5 and 6. We explored the effects of 3′-substituent properties by sequentially adding, to eq 15, the parameters in Table II. Addition of molar refractivity (MR) resulted in a highly significant improvement:

$$\log (\text{rel ED}_{50})_{\text{liver}} = 0.948 \log (\text{rel IC}_{50})_{\text{liver}} - 0.591\text{MR} + 1.03 \quad (17)$$

$$n = 19,\ r = 0.854,\ s = 0.457,\ F = 21.5\ (p < 0.001)$$

Addition of alternative bulk parameters V ($r = 0.832$) and A ($r = 0.817$) also significantly improved eq 15, as did, to a lesser extent, π' ($r = 0.785$). The effect of MR upon the potency–affinity correlation is graphically expressed in Figure 7. It is evident that, at low MR, activity is greater than affinity, whereas at high MR, affinity is greater than activity. The apparent curvature of the plot in Figure 7

suggested that a nonlinear function in MR would improve eq 17:

$$\log (\text{rel ED}_{50})_{\text{liver}} = 0.789 \log (\text{rel IC}_{50})_{\text{liver}} - 1.95 \log (0.005 \times 10^{MR} + 1) + 0.630 \quad (18)$$

$$n = 19,\ r = 0.887,\ s = 0.405,\ F = 29.6\ (p < 0.001)$$

$$t_{\log(\text{rel IC}_{50})_{\text{liver}}} = 7.14,\ t_{\log(0.005\times10^{MR}+1)} = 4.80\ (p < 0.001)$$

Use of L<I or D parameters (Table II) instead of MR did not improve eq 18. Values of log (rel ED_{50}) calculated using eq 18 are given in Table II.

Summary. The correlations between activity and nuclear receptor binding (eq 15–18) in rat heart and liver provide excellent support for the nuclear receptor hypothesis of thyroid hormone action.[1,2] Molar refractivity (MR) in eq 17 and 18 probably reflects the combined effect of many pharmacokinetic phenomena that influence in vivo transport to liver receptors. There is accumulating evidence that in vivo nuclear concentrations of thyromimetics may be controlled by energy-dependent transport processes in both plasma membrane and nucleus.[27,28] The relative efficiency of these processes in liver may be affected by alteration of analogue 3′-substituent MR. In any event, rat liver and heart appear to express some differences in their abilities to respond to these thyromimetics as evidenced by the requirement of a 3′-substituent property to describe liver activity (eq 17 and 18) and the lack of such a requirement in the heart (eq 16). The large difference between in vitro nuclear binding and activity of the acetyl analogue **44**[11] is not readily explained by the physical properties of this compound. Related 3′-acyl analogues **43**, **45**, and **46** do not show such large discrepancies between binding and activity.

Generally, in vivo nuclear binding provides a better correlation with activity than does in vitro binding.[1,10] Thus, increased in vivo binding accounts for the activity of **44**[11] and reduced in vivo binding is seen with **13**, **15**,[10] and **18**.[19] The reasons for this lack of correlation between in vitro and in vivo binding are not known, but would be expected if a structurally selective nuclear pump controlled access to the receptor in vivo.[1,27,28]

Conclusions

These studies have shown that the 3′-substituent binding pocket on the thyroid hormone receptor is lipophilic and is substantially larger than the natural iodo substituent. Furthermore, a free 4′-hydroxyl is necessary for high receptor binding. The quantitative structure–affinity relationships expressed by eq 10 and 12 provide a basis for the design of new, tightly bound thyromimetics. For example, these studies suggest that the size and shape and specific polar character of the binding site should be probed further by the testing of substituted analogues of the high binding benzyl (**18**) or cyclohexylmethyl (**16**) compounds. The restricted conformational mobility of such semirigid analogues should allow more precise mapping of the receptor boundaries than is presently possible.

The correlations between thyromimetic activities and in vitro nuclear binding strongly support the nuclear receptor hypothesis of thyroid hormone action. Analysis of the correlations observed in liver and heart for full agonists however revealed some tissue differences. Activity in the heart was readily explained by nuclear binding alone (eq 16) whereas addition of a 3′-substituent property, molar refractivity, was required to obtain a similarly significant correlation in the liver (eq 18). Since these compounds do not differentiate between heart and liver receptors in vitro (eq 1), it is probable that tissue differences in vivo result from pharmacokinetic effects controlling analogue penetration to receptors, supporting our recent findings that manipulation of the 3′-substituent in T_3 analogues can give selective thyromimetics with high cholesterol lowering and GPDH activity in the liver but very weak cardiac GPDH activity.[1]

Experimental Section

Melting points were determined with a Büchi capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 580B or 577 spectrophotometer. Proton magnetic resonance spectra and ^{13}C NMR spectra were recorded on either a JEOL JNM PFT 100 or a JEOL JNM FX60Q spectrometer using tetramethylsilane or DSS as the internal standard. Mass spectra were recorded on a VG Micromass 7070F mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. All IR and NMR spectra of isolated intermediates and target compounds were consistent with the assigned structures.

Merck Kieselgel 60 (Art. 7734) was used for gravity column chromatography, and Merck Kieselgel 60 (Art. 15111) was used for medium-pressure column chromatography.

The purity of isolated intermediates and target compounds was determined by thin-layer chromatography and/or analytical high-pressure liquid chromatography (HPLC), which was performed by using a Perkin-Elmer Series 3B/LC 75 or a Constametric/LC 75 system. C_{18}/μBondapak column packing was used and detection measured at UV 240 nm at ambient temperature. The mobile phase used was CH_3CN/1% AcOH (containing 1 g of camphorsulfonic acid per liter) for target diiodothyronines and CH_3CN/H_2O or CH_3CN/1% AcOH for intermediate diiodothyronines. All target diiodothyronines (Table I) were of ≥95% purity by HPLC.

1-*n*-Hexyl-2-methoxybenzene (50a). To a stirred and cooled solution of 2-methoxybenzaldehyde (76.01 g, 0.558 mol), *n*-pentyltriphenylphosphonium bromide (300 g, 0.726 mol), and dicyclohexano-18-crown-6 in dry CH_2Cl_2[29] (1.6 L) was added KOBu-*t* (63 g). The mixture was stirred at room temperature overnight, and then additional *n*-pentyltriphenylphosphonium bromide (69 g) and KOBu-*t* (18.7 g) were added in three portions over 1 h. The mixture was filtered, the filtrate evaporated, and the residue extracted with petroleum ether/Et_2O (1:1). The extracts were evaporated, and the residue was chromatographed on silica gel to give the olefin (80 g), which was hydrogenated in EtOH (600 mL) with 10% Pd/C (5 g). When uptake of hydrogen was complete, the mixture was filtered and distilled to give **50a** (72.82 g, 68%), bp 68–73 °C (0.15 mm). Anal. ($C_{13}H_{20}O$) C, H.

1-(Cyclohexylmethyl)-2-methoxybenzene (50b). The alcohol (75.45 g, 0.366 mol) obtained from Grignard reaction of 2-methoxybenzaldehyde with bromocyclohexane was hydrogenated at 40 °C (50 psi) in EtOH (300 mL) and water (30 mL) in the presence of 10% Pd/C (16 g). After 20 h, the uptake of hydrogen was complete. The mixture was filtered, the filtrate evaporated to dryness, and the residue combined with a smaller batch (from 11.0 g of precursor alcohol) and purified by column chromatography on silica gel to give **50b** as a colorless oil (30.82 g, 44%): ^1H NMR ($CDCl_3$) δ 0.7–1.8 (11 H, m, cyclohexyl *H*), 2.50 (2 H, d, Ar CH_2), 3.79 (3 H, s, OCH_3), and 6.87–7.10 (4 H, m, Ar*H*). Anal. ($C_{14}H_{20}O$) C, H.

(4-Acetoxybutyl)triphenylphosphonium bromide was prepared in 80% yield from (4-bromobutyl)triphenylphosphonium bromide by the method of Creasy and Schweizer:[30] mp 120–123 °C; ^1H NMR ($CDCl_3$) δ 1.80 (4 H, m, $(CH_2)_2$), 1.94 (3 H, s, $OCOCH_3$), 3.90 (2 H, m, $Ph_3P^+CH_2$), 4.11 (2 H, m, CH_2OCOCH_3), and ~7.75 (m, Ar*H*). Anal. ($C_{24}H_{26}BrO_2P$) H, Br; C: calcd, 63.03; found, 62.28.

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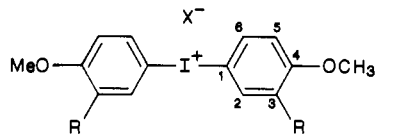
Table V. Bis(3-substituted-4-methoxyphenyl)iodonium Salts



R	X	mp, °C	δ_{C-1}[a]	formula	analyses
(48a) CH_2Ph	I	162–164	107.3[b]	$[C_{28}H_{26}IO_2]^+I^-$	C, H, I
(48b) CO_2Et	I	155–157	107.8	$[C_{20}H_{22}IO_6]^+I^-$	C, H, I
(48c) CH_2CO_2Et	I	122–123	106.4	$[C_{22}H_{26}IO_6]^+I^-$	C, H; I[c]
(48d) CH_2NHAc	I	142–145	106.5	$[C_{20}H_{24}IN_2O_4]^+I^-\cdot 0.5Me_2NCHO$	C, H, N
(48e) COEt	I	155–156	108.7	$[C_{20}H_{22}IO_4]^+I^-$	C, H, I
(48f) COPh	I	165–167	108.5	$[C_{28}H_{22}IO_4]^+I^-$	C, H, I
(48g) NO_2	Br	203–205	*d*	$[C_{14}H_{12}IN_2O_6]^+Br^-\cdot 0.1NaBr$	C, H, N
(51a) $(CH_2)_5CH_3$	Br	174–175	108.4	$[C_{26}H_{38}IO_2]^+Br^-$	C, H, Br, I
(51b) CH_2-c-C_6H_{11}	Br	175–176	107.8	$[C_{28}H_{38}IO_2]^+Br^-$	C, H
(51c) $(CH_2)_2Ph$	Br	197–198	107.9	$[C_{30}H_{30}IO_2]^+Br^-\cdot 0.25H_2O$	C, H, Br, I
(51d) $(CH_2)_2OAc$	I	154–155	107.5	$[C_{22}H_{26}IO_6]^+I^-$	C, H, I
(51e) $(CH_2)_5OAc$	Br	133–135	108.2	$[C_{28}H_{38}IO_6]^+Br^-$	C, H, Br, I
(51f) $CH_2NHCOPr$	Br	203–204	108.0	$[C_{24}H_{32}IN_2O_4]^+Br^-\cdot 0.5H_2O$	C, H, N, Br, I
(51g) $(CH_2)_2NHCOEt$	Br	170–171	107.6	$[C_{24}H_{32}IN_2O_4]^+Br^-\cdot 0.5H_2O$	C, H, N, Br, I
(51h) $(CH_2)_3NHAc$	Br	171–172	108.6	$[C_{24}H_{32}IN_2O_4]^+Br^-\cdot 0.45KBr$	C, H, N, I
(51i) H	Br		109.1[e]		

[a] From ^{13}C NMR spectra in DMSO-d_6. [b] δ_{C-2}, 136.2; δ_{C-3}, 132.7; δ_{C-4}, 159.2; δ_{C-5}, 113.9; δ_{C-6}, 134.9. [c] I: calcd, 39.64; found, 40.07. [d] Decomposition occurred. [e] $\delta_{C-2,6}$, 136.2; $\delta_{C-3,5}$, 166.9; δ_{C-4}, 160.3.

5-(2-Methoxyphenyl)pentyl acetate (50e) was prepared from 2-methoxybenzaldehyde and (4-acetoxybutyl)triphenylphosphonium bromide, as described for **50a**: bp 131–138 °C (1.5 mm); ^1H NMR ($CDCl_3$) δ 1.50 (6 H, m, $(CH_2)_3$), 2.02 (3 H, m, $OCOCH_3$), 2.61 (2 H, t, Ar CH_2), 3.81 (3 H, s, OCH_3), 4.04 (2 H, t, CH_2OCOCH_3), and ~7.0 (4 H, m, Ar *H*). Anal. ($C_{14}H_{20}O_3$) C, H.

1-[(Acylamino)alkyl]-2-methoxybenzenes 50f and 50g. To a cold stirred mixture of the 1-(aminoalkyl)-2-methoxybenzene (0.35 mol) and the acid anhydride (0.38 mol) was slowly added 2 N aqueous NaOH to pH 14. The mixture was stirred for 2 h and then extracted with Et_2O. The combined dried Et_2O extracts were distilled to give the pure amides. **50f**: 89%; bp 145–148 °C (0.3 mm). Anal. ($C_{12}H_{17}NO_2$) C, H, N. **50g**: 85%; mp 46–47 °C. Anal. ($C_{12}H_{17}NO_2$) C, H, N.

1-(3-Acetamidopropyl)-2-methoxybenzene (50h). A solution of diethyl (cyanomethyl)phosphonate (200 g, 1.12 mol) in CH_2Cl_2 (500 mL) was added dropwise to a colled and stirred mixture of 2-methoxybenzaldehyde (146 g, 1.07 mol), tetrabutylammonium iodide (7.0 g, 0.018 mol), CH_2Cl_2 (1 L), and 2 N aqueous NaOH (1.1 L), such that the temperature did not exceed 35 °C. After 15 min, the organic layer was removed, washed with saturated NaCl solution, dried with anhydrous $MgSO_4$, and evaporated to dryness. The residue was purified by column chromatography to give a mixture of the *cis* and *trans*-2-methoxycinnamonitriles (158 g, 93%). This mixture was dissolved in ethanolic HCl (1.4 L) and hydrogenated at 48 °C (40 psi) with 10% Pd/C (30 g). When uptake of hydrogen was complete, the mixture was filtered, the filtrate evaporated, and the residue triturated with Et_2O to give 1-(3-aminopropyl)-2-methoxybenzene hydrochloride (113 g, 64%). This hydrochloride was treated with 1 N aqueous NaOH (600 mL) to give the oily free base, which was suspended in water (200 mL), and acetic anhydride (100 mL) was added. After 1 h, the mixture was extracted with Et_2O, and the combined extracts were dried over anhydrous $MgSO_4$ and evaporated to give **50h** as a colorless oil (105 g, 92%): ^1H NMR ($CDCl_3$) δ 1.80 (2 H, m, CH_2), 1.97 (3 H, s, $NHCOCH_3$), 2.69 (2 H, Ar CH_2), 3.25 (2 H, m, $CH_2NHCOCH_3$), 3.83 (3 H, s, OCH_3), ~6.0 (1 H, br resonance, N*H*), and ~7.0 (4 H, m, Ar *H*). Anal. ($C_{12}H_{17}NO_2\cdot 0.15CH_3CO_2H$) C, H, N.

Preparation of Bis(3-substituted-4-methoxyphenyl)iodonium Salts. 2-Substituted methoxybenzenes were treated with iodine tris(trifluoroacetate) or with iodyl sulfate according to the general methods of Beringer and co-workers.[31] The salts were isolated as bromides or iodides and were characterized by elemental analyses and by the appearance in their ^{13}C NMR spectra of the characteristic C–I^+ signal at ~108 ppm (Table V).

Coupling Reactions of Iodonium Salts with 3,5-Diiodotyrosine Derivatives. The method of Blank and co-workers[32] employing Cu catalysis in Et_3N/MeOH was used. The product 3,5-diiodothyronine derivatives were isolated by column chromatography (Table VI).

3′-(5-Acetoxypentyl)-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine (54). 3′-(5-Acetoxypentyl)-3,5-diiodo-*N*-(trifluoroacetyl)-*O*-methyl-L-thyronine methyl ester (**52e**) (2.49 g, 3.2 mmol) in CH_2Cl_2 (10 mL) was added to a colled (0 °C) stirred solution of $AlCl_3$ (2.40 g) in ethanethiol (8 mL).[16] After 1 h at 0 °C, the mixture was evaporated to dryness, the residue partitioned between water and EtOAc, and the organic layer washed with saturated NaCl solution, dried with anhydrous sodium sulfate, and evaporated to dryness. Purification by column chromatography and then recrystallization from aqueous methanol gave **54** (1.20 g, 86%): mp 140–142 °C; ^1H NMR (DMSO-d_6) δ 1.1–1.7 (6 H, m, $(CH_2)_3$), 1.97 (3 H, s, $OCOCH_3$), 2.5 (2 H, m, Ar CH_2), 2.7–3.4 (2 H, m, Ar CH_2CH), 3.96 (2 H, t, CH_2OCOCH_3), 4.54 (1 H, m, Ar CH_2CH), 6.3 (2 H, m, 2′,6′-*H*), 6.67 (1 H, m, 5′-*H*), 7.80 (2 H, s, 2,6-*H*), and 9.62 (1 H, d, N*H*). Anal. ($C_{24}H_{24}F_3I_2NO_7$) C, H, N, I. Under the same conditions, **52a** gave **53** (74%): mp 186–187 °C. Anal. ($C_{21}H_{18}F_3I_2NO_7$) C, H, N, I. Similarly prepared from the ethyl ester **64** was the acid **66** (86%): mp 110–112 °C. Anal. ($C_{23}H_{22}F_3I_2NO_7$) C, H, N.

3′-(5-Hydroxypentyl)-3,5-diiodo-L-thyronine (28). To a stirred suspension of thyronine **54** (1.09 g, 1.5 mmol) in EtOH (4 mL) was added 10% aqueous NaOH (3 mL). The yellow solution was warmed on a steam bath for 10 min and then treated with glacial HOAc to pH 6. Addition of a few drops of water promoted precipitation. The product was recrystallized from aqueous ethanolic NaOH on addition of glacial HOAc to give **28** (0.72 g, 79%): ^1H NMR (1 N NaOD) δ ~1.4 (6 H, m, $(CH_2)_3$), 2.42 (2 H, t, Ar CH_2), 2.70 (2 H, m, Ar CH_2CH), 3.50 (3 H, m, Ar CH_2CH and CH_2OH), 6.45 (3 H, m, 2′,5′,6′-*H*), and 7.76 (2 H, s, 2,6-*H*).

3′-[3-(Acetylamino)propyl]-3,5-diiodo-L-thyronine (38). To a stirred solution of 3′-[(acetylamino)propyl]-3,5-diiodo-*N*-(trifluoroacetyl)-*O*-methylthyronine methyl ester (**52h**) (3.74 g, 0.005 mol) in dry CH_2Cl_2 (110 mL) at −50 °C was added a solution of boron tribromide (10.0 g, 0.04 mol) in dry CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 20 h and then poured into ice/water. The precipitate was collected and dissolved in excess 2 N aqueous NaOH. After 0.5 h, the solution

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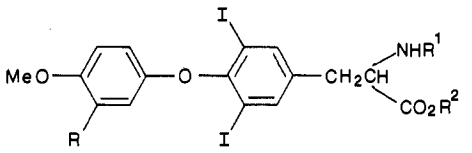
Table VI. Protected 3,5-Diiodothyronines from Arylations of 3,5-Diiodotyrosines with Bis(3-substituted-4-methoxyphenyl)iodonium Salts



R	R^1	R^2	yield, %	mp, °C	formula	analyses
(**49a**) CH_2Ph	Ac	Et	26	82–84	$C_{27}H_{27}I_2NO_5$	C, H, N, I
(**49b**) CO_2Et	Ac	Et	40	143–144	$C_{23}H_{25}I_2NO_7$	C, H, N; I[a]
(**49c**) CH_2CO_2Et	Ac	Et	36	155–116	$C_{24}H_{27}I_2NO_7$	C, H, N, I
(**49d**) CH_2NHAc	Ac	Et	55	136–138	$C_{23}H_{26}I_2N_2O_6$	C, H, N, I
(**49e**) COEt	Ac	Et	47	*b*	$C_{23}H_{25}I_2NO_6$	*c*
(**49f**) COPh	Ac	Et	47	*b*	$C_{27}H_{25}I_2NO_6$[d]	C, H, N, Cl, I
(**49g**) NO_2	Ac	Et	18	147–148	$C_{20}H_{20}I_2N_2O_7$	C, H, N, I
(**52a**) $(CH_2)_5CH_3$	$COCF_3$	Me	43	97–100	$C_{25}H_{28}F_3I_2NO_5$	C, H, N, I
(**52b**) CH_2-c-C_6H_{11}	$COCF_3$	Me	37	108–109	$C_{26}H_{28}F_3I_2NO_5$	C, H, N, I
(**52c**) $(CH_2)_2Ph$	$COCF_3$	Me	38	97–98	$C_{27}H_{24}F_3I_2NO_5$	C, H, N, I
(**52d**) $(CH_2)_2OAc$	$COCF_3$	Me	15	60–64	$C_{23}H_{22}F_3I_2NO_7$	C, H, N, I
(**52e**) $(CH_2)_5OAc$	$COCF_3$	Me	41	*b*	$C_{26}H_{28}F_3I_2NO_7$	C, H, N
(**52f**) $CH_2NHCOPr$	$COCF_3$	Me	39	165–166	$C_{24}H_{25}F_3I_2N_2O_6$	C, H, N, I
(**52g**) $(CH_2)_2NHCOEt$	$COCF_3$	Me	27	150–151	$C_{24}H_{25}F_3I_2N_2O_6$	C, H, N, I
(**52h**) $(CH_2)_3NHAc$	$COCF_3$	Me	35	153–154	$C_{24}H_{25}F_3I_2N_2O_6$	C, H, N, I
(**52l**) H	$COCF_3$	Me	46	168–169	$C_{19}H_{16}F_3I_2NO_5$	C, H, N, I

[a] I: calcd, 37.26; found, 38.40. [b] Glass. [c] Not analyzed; deprotected compound (**45**) analyzed (Table I). [d] Contains 0.035$CHCl_3$.

was acidified to pH 6 with glacial HOAc and the precipitate was washed successively with water, EtOH, and then Et_2O to give **38** (2.40 g, 77%).

3′-(Cyclohexylmethyl)-3,5-diiodo-L-thyronine (16). Protected thyronine **52b** (0.745 g, 1.0 mmol) in HOAc (80 mL) and 49% aqueous HBr (40 mL) was heated under reflux for 5 h. The solution was concentrated and treated with excess water to precipitate the hydrobromide of **16**, which was dissolved in aqueous 4 N NaOH/EtOH (1:2). The solution was filtered and acidified to pH 4 with HOAc to give **16** (0.52 g, 84%).

3′-(2-Aminoethyl)-3,5-diiodo-L-thyronine (42). Thyronine **37** (0.625 g, 1.0 mmol) in HOAc (100 mL) and 10 N aqueous HCl (50 mL) was heated under reflux for 48 h. The solution was evaporated to dryness and the residue suspended in Et_2O and filtered to give **42** as the dihydrochloride (0.64 g, 100%).

5-(Benzyloxy)-2-methoxybenzaldehyde (56). To a stirred suspension of 5-hydroxy-2-methoxybenzaldehyde[33] (91.28 g, 0.60 mol), benzyl bromide (123.17 g, 0.72 mol), and adogen 464 (23.2 g) in CH_2Cl_2 (450 mL) was added NaOH (36.0 g, 0.90 mol) in H_2O (450 mL). After 2 h, the organic layer was removed, washed with water and then saturated NaCl, dried with anhydrous $MgSO_4$, and evaporated to give **56** (141.37 g, 97%): mp 99–100 °C. Anal. ($C_{15}H_{14}O_3$) C, H.

3-(4-Acetoxybutyl)-4-methoxyphenol (58). Wittig reaction of **56** (20.0 g, 0.0825 mol) and (3-acetoxypropyl)triphenylphosphonium bromide (151 g, 0.331 mol) as described for **50a** gave 2-(4-acetoxybut-1-enyl)-4-(benzyloxy)-1-methoxybenzene (**57**) (15.1 g, 56%) as a yellow oil. Anal. ($C_{20}H_{22}O_4$) H; C: calcd, 73.60; found, 74.15. This olefin (15.0 g) was hydrogenated from 10 °C (40 psi) in EtOH (150 mL) in the presence of 10% Pd/C (1.5 g). The mixture was filtered, the filtrate evaporated, and the residue crystallized from aqueous EtOH to give **58** (6.28 g, 63%): mp 100–102 °C; ^1H NMR ($CDCl_3$) δ 1.65 (4 H, m, $(CH_2)_2$), 2.06 (3 H, s, $OCOCH_3$), 2.60 (2 H, m, Ar CH_2), 3.78 (3 H, s, OCH_3), ~4.10 (2 H, m, CH_2OCOCH_3), 5.05 (1 H, br s, Ar OH), and 6.65 (3 H, m, Ar H); MS, *m/e* 238 (M^+). Anal. ($C_{13}H_{18}O_4$) H; C: calcd, 65.53; found, 63.39.

4-Methoxy-3-(4-methoxyphenyl)nitrobenzene (60). Reaction of **59** with sodium nitromalonaldehyde by the method of Blank and co-workers[32] gave 2-hydroxy-3-(4-methoxyphenyl)-nitrobenzene, mp 134–135 °C. Anal. ($C_{13}H_{11}NO_4$) C, H, N. This phenol (43 g, 0.175 mol) was stirred for 2.5 h with dimethyl sulfate (53.3 g, 0.422 mol), NaOH (10.32 g, 0.248 mol), and adogen 464 (5.16 g) in CH_2Cl_2 (500 mL) and H_2O (500 mL). The organic layer was removed, washed with water, dried with anhydrous $MgSO_4$, and evaporated to give an oil, which crystallized from Et_2O/petroleum spirit to give **60**, mp 131–132 °C. Anal. ($C_{14}H_{13}NO_4$) C, H, N.

4-Methoxy-3-(4-methoxyphenyl)phenol (61). The nitro compound **60** (15.0 g, 0.058 mol) was hydrogenated in EtOH (200 mL) with 10% Pd/C (1.5 g). When uptake of hydrogen was complete, the mixture was filtered and added to tetrafluoroboric acid (25.2 g). The mixture was cooled (0 °C) and stirred while *tert*-amyl nitrite (10.2 g) was added. After 2 h, the mixture was diluted with Et_2O (800 mL) and left at 5 °C for 72 h and the diazonium tetrafluoroborate (21 g) collected by filtration. This salt was added, at room temperature, to a solution of Ac_2O (200 mL) in AcOH (1400 mL) that had previously been heated at reflux for 2 h. The mixture was heated gradually to reflux over 1.5 h and after a further 1 h evaporated to dryness. The residue was dissolved in EtOH (300 mL) and 2 N NaOH (200 mL), heated (steam bath) for 1.5 h, then concentrated to 100 mL, and neutralized with 2 N HCl. The product was extracted with $CHCl_3$ and purified by chromatography on silica gel, eluting with $CHCl_3$, to give **61** (10.0 g, 75%) as an oil: ^1H NMR (DMSO-d_6) δ 3.63 (3 H, s, OCH_3), 3.78 (3 H, s, OCH_3), ~6.7 (2 H, m, 2,6-H), 6.89 (1 H, d, 5-H), 6.95 (2 H, m, 3′,5′-H), 7.35 (2 H, m, 2′,6′-H), and 8.98 (1 H, s, OH); MS, *m/e* 230 (M^+). Anal. ($C_{14}H_{14}O_3$·0.12$CHCl_3$) C, H.

3′-(4-Acetoxybutyl)-3,5-dinitro-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine Ethyl Ester (62). To 3,5-dinitro-*N*-(trifluoroacetyl)-*L*-tyrosine ethyl ester (11.31 g, 0.0286 mol) in dry pyridine (30 mL) was added methanesulfonyl chloride (3.28 g, 0.0286 mol). The dark solution was stirred and refluxed for 10 min, and then to it was added 3-(4-acetoxybutyl)-4-methoxyphenol (**58**) (6.20 g, 0.026 mol) in dry pyridine (20 mL). The mixture was refluxed for 1 h, evaporated to dryness, taken up in $CHCl_3$, washed successively with water, 2 N HCl, saturated $NaHCO_3$, and water, then dried with anhydrous magnesium sulfate, and evaporated. The residue was purified by exhaustive chromatography on silica gel (63–200 μm), eluting with ethyl acetate/petroleum ether to give **62** (5.30 g, 34%): mp 80–81 °C; ^1H NMR ($CDCl_3$) δ 1.36 (3 H, t, CH_2CH_3), ~1.60 (4 H, m, $(CH_2)_2$), 2.04 (3 H, s, $OCOCH_3$), 2.58 (2 H, t, Ar CH_2), 3.35 (2 H, m, Ar CH_2CH), 3.78 (3 H, s, OCH_3), 4.05 (2 H, t, CH_2OCOCH_3), 4.32 (2 H, q, CH_2CH_3), 4.85 (1 H, m, Ar CH_2CH), ~6.65 (3 H, m, 2′,5′,6′-H), 7.25 (1 H, br d, NH), and 7.91 (2 H, s, 2,6-H). Anal. ($C_{26}H_{28}F_3N_3O_{11}$) C, H, N.

Similarly prepared from **61** was **63** (78%). Anal. ($C_{27}H_{24}F_3N_3O_{10}$) H; C calcd 54.82, found 52.77; N calcd 7.10, found 6.45.

3′-(4-Acetoxybutyl)-3,5-diiodo-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine Ethyl Ester (64). The dinitro compound

(33) Ulrich, H.; Rao, D. V.; Tucker, B.; Sayigh, A. A. R. *J. Org. Chem.* 1974, *39*, 2437.

62 (5.28 g, 0.00858 mol) was hydrogenated in acetic acid (50 mL) with 10% Pd/C (1.0 g). When uptake of hydrogen was complete (0.5 h), the mixture was filtered and the filtrate added to a cooled (−5 °C) stirred solution of sulfuric acid (3.36 g, 0.0343 mol) in water (50 mL). The resulting solution was stirred and cooled to −10 °C while a solution of sodium nitrite (1.48 g, 0.0214 mol) in water (10 mL) was added dropwise, the temperature being maintained at below −7 °C during the addition. The mixture was poured into a cooled (3 °C) vigorously stirred mixture of iodine (3.2 g), potassium iodide (15 g), and urea (1.1 g) in water (140 mL) and $CHCl_3$ (140 mL). After 1.5 h, sodium metabisulfite (20 g) was added and the organic layer separated, washed with water and then with saturated $NaHCO_3$ solution, dried with anhydrous $MgSO_4$, and evaporated to leave a red gum (5.37 g). Purification by exhaustive medium-pressure chromatography on Kieselgel 60 (15–40 μm), eluting with 5% Et_2O/petroleum ether, gave **64** (0.91 g, 14%): mp 78–80 °C; ^1H NMR ($CDCl_3$) δ 1.32 (3 H, t, CH_2CH_3), ~1.65 (4 H, m, $(CH_2)_2$), 2.02 (3 H, s, $OCOCH_3$), 2.60 (2 H, m, Ar CH_2), 3.15 (2 H, m, Ar CH_2CH), 3.77 (3 H, s, OCH_3), 4.05 (2 H, m, CH_2OCOCH_3), 4.18 (2 H, q, CH_2CH_3), 4.80 (1 H, m, Ar CH_2CH), 6.47 (1 H, d of d, 6′-*H*), 6.69 (1 H, d, 2′-*H*), 6.72 (1 H, d, 5′-*H*), 7.05 (1 H, br d, N*H*), and 7.69 (2 H, s, 2,6-*H*). Anal. ($C_{26}H_{28}F_3I_2NO_7$) C, H, N, I. Similarly prepared from **63** was **65** as a glass (11% after exhaustive column chromatography). Anal. ($C_{27}H_{24}F_3I_2NO_6$) C, H, N, I.

3,5-Diiodo-3′-formyl-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (67). To a cooled (−72 °C) stirred solution of 3,5-diiodo-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine methyl ester (**52i**) (20.02 g, 0.0312 mol) in dry CH_2Cl_2 (200 mL) were added stannic chloride (48.76 g, 0.187 mol) and 1,1-dichlorodimethyl ether (7.17 g, 0.0624 mol) in dry CH_2Cl_2 (40 mL). The mixture was stirred to 0 °C, and after 4 h, 2 N aqueous HCl (80 mL) was added slowly with vigorous stirring. The organic layer was removed, washed with saturated $NaHCO_3$ and then water, then dried, and evaporated to dryness. The residue was chromatographed on silica gel, eluting with $CHCl_3$ to give a pale yellow foam, which was recrystallized from EtOH/water to give **67** (16.61 g, 79%): mp 127–128 °C. Anal. ($C_{20}H_{16}F_3I_2NO_6$) C, H, N, I.

3′-*n*-But-1-enyl-3,5-diiodo-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (68a). To a stirred mixture of **67** (6.80 g, 0.010 mol), *n*-propyltriphenylphosphonium bromide (5.0 g, 0.013 mol), and dicyclohexano-18-crown-6 (80 mg) in dry CH_2Cl_2 (60 mL) was added KOBu-*t* (1.57 g, 0.014 mol) in portions. After 3 h, additional phosphonium bromide (5.0 g) and KOBu-*t* (1.57 g) were added, and after 2 h, 2 N HCl (40 mL) and then H_2O (40 mL) were added. The organic layer was separated, washed with water, dried, and evaporated. The residue was purified by column chromatography on silica gel, eluting with EtOAc/petroleum ether to give **68a** (5.80 g, 82%): mp 121–124 °C. Anal. ($C_{23}H_{22}F_3I_2NO_5$) C, H, N, I. Similarly prepared were **68b** and **68c**. **68b**: 65%; mp 105–107 °C. Anal. ($C_{24}H_{24}F_3I_2NO_5$) C, H, N, I. **68c**: 60%. Compounds **68a–c** were mixtures of cis and trans isomers, separable by analytical HPLC.

Racemization of 67. Compound **67** (0.20 g, 0.00044 mol), KOBu-*t* (0.10 g, 0.00088 mol), and dicyclohexano-18-crown-6 (10 mg) were stirred in dry CH_2Cl_2 (6 mL) at room temperature for 5 and 20 h, and then **67** was isolated and its optical rotation measured at 25 °C in EtOH/H_2O/concentrated HCl (17:2:1): $[\alpha]_D$ (**67**) −5.8° (*c* 1.1), (5-h reaction time) −3.6° (*c* 1.1), (20-h reaction time) 0° (*c* 1.0).

3′-*n*-Butyl-3,5-diiodo-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (69a). To a solution of **68a** (5.30 g, 0.00754 mol) in triethylsilane (20 mL) and dry CH_2Cl_2 (3 mL) was added, dropwise, trifluoroacetic acid (8.5 mL, 0.110 mol). The solution was stirred and heated at 50 °C for 18 h, cooled and then poured into water (100 mL). The mixture was extracted with $CHCl_3$, and the combined organic extracts were washed with water, 10% Na_2CO_3 solution, water, and then saturated NaCl solution, dried, and evaporated to give a pale yellow gum. Purification by chromatography on silica gel, eluting with toluene, gave **69a** (1.44 g, 27%): mp 85–86 °C. Anal. ($C_{23}H_{24}F_3I_2NO_5$) C, H, N, I. Similarly prepared were **69b** and **69c**. **69b**: 49%; mp 103–104 °C. Anal. ($C_{24}H_{26}F_3I_2NO_5$) C, H, N, I. **69c**: 61%; mp 95–97 °C. Anal. ($C_{26}H_{30}F_3I_2NO_5$) C, H, N, I.

3′-Formyl-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (70). To **67** (20.0 g, 0.0295 mol) in dry CH_2Cl_2 (250 mL) at −70 °C was added, dropwise, boron trichloride (200 mL of a 1 M solution in CH_2Cl_2). The mixture was stirred to room temperature and, after 20 h, cooled to 0 °C and treated cautiously with excess ice/water. The organic layer was removed, washed successively with water, dilute $NaHCO_3$, and water, then dried, and evaporated to dryness. The residue was purified by chromatography on silica gel by elution with $CHCl_3$ and then crystallization from aqueous EtOH to give **70** (11.20 g, 57%): mp 182–183 °C. Anal. ($C_{19}H_{14}F_3I_2NO_6$) C, H, N, I.

3′-(Hydroxymethyl)-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (71a). To a stirred solution of **70** (2.80 g, 0.0042 mol) in glacial HOAc (40 mL) was added sodium cyanoborohydride (0.39 g, 0.0062 mol). After 1.5 h, the mixture was diluted with water and extracted with EtOAc and the organic extracts were washed with water and saturated $NaHCO_3$, then dried, and evaporated. The residue crystallized from aqueous MeOH to give **71a** (2.40 g, 85%): mp 169–170 °C. Anal. ($C_{19}H_{16}F_3I_2NO_6$) C, H, N, I.

3′-(Methoxymethyl)-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (71b). To a stirred solution of **70** (2.02 g, 0.0030 mol) in glacial HOAc (5 mL) and MeOH (20 mL) was added sodium cyanoborohydride (0.25 g, 0.0040 mol). Workup as for **71a**, followed by chromatography on silica gel, eluting with EtOAc/petroleum ether, and recrystallization from aqueous methanol, gave **71b** (1.15 g, 56%): mp 147–148 °C; ^1H NMR ($CDCl_3$) δ 3.15 (2 H, m, Ar CH_2CH), 3.43 (3 H, s, CH_3OCH_2), 3.84 (3 H, s, CH_3O), 4.61 (2 H, s, CH_3OCH_2), 4.85 (1 H, m, Ar CH_2CH), 6.48 (1 H, d, 2′-*H*), 6.59 (1 H, d of d, 6′-*H*), 6.84 (1 H, d, 5′-*H*), 6.96 (1 H, br d, N*H*), 7.23 (1 H, s, O*H*), and 7.59 (2 H, s, 2,6-*H*). Anal. ($C_{20}H_{18}F_3I_2NO_6$) C, H, N, I.

3′-(*n*-Butoxymethyl)-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (71c). To **70** (2.80 g, 0.0042 mol) in glacial HOAc (15 mL) and 1-butanol (30 mL) was added sodium cyanoborohydride (0.39 g, 0.0062 mol). After 1.5 h, trifluoroacetic acid (1 mL) was added, the mixture refluxed for 4 h, then cooled, and worked up, and the product isolated as described for **71b** to give **71c** (0.70 g, 26%): mp 97–99 °C. Anal. ($C_{23}H_{24}F_3I_2NO_6$) C, H, N, I.

3′-[(*n*-Butylthio)methyl]-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (74). A solution of **71a** (1.60 g, 0.0024 mol) in glacial HOAc (5 mL), 1-butanethiol (5 mL), and trifluoroacetic acid (1 mL) was refluxed for 4 h. The solvents were evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/petroleum ether, and then crystallization from aqueous MeOH to give **74** (0.94 g, 53%): mp 129–130 °C. Anal. ($C_{23}H_{24}F_3I_2NO_5S$) C, H, N, I, S.

***O*-Benzenesulfonyl-3′-formyl-3,5-diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (72).** To a solution of **70** (8.0 g, 0.0121 mol) in dry pyridine (8 mL) was added benzenesulfonyl chloride (3.18 g, 0.018 mol). After 3 h, the mixture was diluted with water and extracted with $CHCl_3$. The organic extracts were washed with 2 N HCl, saturated $NaHCO_3$, and water, then dried, evaporated to give a brown gum, and purified by column chromatography using silica gel to give **72** (7.24 g, 74%): mp 147–148 °C. Anal. ($C_{25}H_{18}F_3I_2NO_8S$) C, H, N, I, S.

(3-Methoxypropyl)triphenylphosphonium Bromide. To a solution of triphenylphosphine (32.48 g, 0.123 mol) in toluene (80 mL) was added 3-methoxypropyl bromide (21.06 g, 0.138 mol), and the solution was refluxed for 18 h. The product was collected by filtration and then recrystallized from CH_2Cl_2/Et_2O to give the product (33.25 g, 65%): mp 200–203 °C. Anal. ($C_{22}H_{24}BrOP$) H, Br; C: calcd, 63.62; found, 60.95. Similarly prepared from 2-ethoxyethyl bromide was **(2-ethoxyethyl)triphenylphosphonium bromide** (84%): mp 162–167 °C. Anal. ($C_{22}H_{24}BrOP$) H, Br; C: calcd, 63.62; found, 62.80.

***O*-Benzenesulfonyl-3,5-diiodo-3′-(4-methoxybutyl)-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (76).** Aldehyde **72** (7.61 g, 0.0089 mol) was treated with (3-methoxypropyl)triphenylphosphonium bromide as described for **68a** to give the olefin mixture **73c** (3.38 g, 44%): mp 86–89 °C. Anal. ($C_{29}H_{26}F_3I_2NO_8S$) C, H, N, I, S. Mixture **73c** (3.25 g, 0.0038 mol) was hydrogenated in glacial HOAc (80 mL) with 5% Pt/C (2.5 g) at 38 °C (40 psi). The product mixture was purified by medium-pressure chromatography on silica gel (15–40 μm) using 15%

Et_2O/petroleum ether as eluent to give **76** (0.79 g, 24%): mp 110–111 °C. Anal. ($C_{29}H_{28}F_3I_2NO_8S$) C, H, N. Also isolated was the trans olefin **73c** (0.25 g): mp 117–118 °C. ^1H NMR ($CDCl_3$) 5.99 δ (1 H, m, CH=C*H*CH_2) and 6.28 (1 H, d, C*H*=$CHCH_2$). Anal. ($C_{29}H_{26}F_3I_2NO_8S$) H, N; C: calcd, 40.56; found, 41.26.

The following were also prepared. Compound **72** and methyltriphenylphosphonium bromide gave **73a** (16%): mp 157–158 °C. Anal. ($C_{26}H_{20}F_3I_2NO_7S$) C, H, N, I, S. Catalytic hydrogenation of **73b** as described for **73c** gave **75** (31%): mp 82–85 °C; MS, *m/e* 861 (M^+). Anal. ($C_{29}H_{28}I_2F_3NO_8S$) H, N; C: calcd, 40.44; found, 41.78.

3,5-Diiodo-3′-(4-methoxybutyl)-L-thyronine (33). To a stirred suspension of **76** (0.72 g, 0.84 mmol) in EtOH (3 mL) was added 10% aqueous NaOH (3 mL), and the resulting solution was heated at 60 °C for 2.5 h. The cooled solution was treated with HOAc to pH 6 and the precipitate collected and recrystallized from aqueous EtOH/NaOH by addition of HOAc to give **33** (0.40 g, 78%).

3,5-Diiodo-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (77). To a stirred solution of 3,5-diiodo-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine methyl ester (**52i**) (6.50 g, 0.010 mol) in dry CH_2Cl_2 at −74 °C was added boron tribromide (1.91 mL, 0.020 mol). The mixture was maintained between 0 and −5 °C for 2 h, decomposed with water, and then extracted with EtOAc. The combined organic extracts were washed with water, saturated $NaHCO_3$, and water, then dried, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/petroleum ether, followed by crystallization from aqueous MeOH to give **77** (3.29 g, 52%): mp 163–165 °C. Anal. ($C_{18}H_{14}F_3I_2NO_5$) C, H, N, I.

3,5-Diiodo-*O*-prop-2-enyl-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (78). A suspension of **77** (2.35 g, 0.0037 mol), anhydrous K_2CO_3 (0.51 g, 0.0037 mol), dicyclohexano-18-crown-6 (10 mg), and 3-bromopropene (0.45 g, 0.0037 mol) in dry CH_2Cl_2 (25 mL) was stirred at room temperature for 17 h. Water (25 mL) and $CHCl_3$ (20 mL) were added, and the organic layer was separated, washed with 10% NaOH solution and then water, dried, and evaporated to give a white solid. Recrystallization from aqueous MeOH gave **78** (0.74 g, 30%): mp 125–126 °C. Anal. ($C_{21}H_{18}F_3I_2NO_5$) C, H, N, I.

Claisen Rearrangement of 78. A solution of **78** (1.10 g, 0.0016 mol) was heated for 5 h in diethylaniline (6 mL) at 200–210 °C under a nitrogen atmosphere. The cooled solution was diluted with $CHCl_3$, washed with 2 N HCl and then water, dried, and evaporated to give a gum, which was subjected to column chromatography using silica gel with EtOAc/petroleum ether as eluent to give the ethyl ester **79** and the methyl ester **80**. **79**: 0.32 g, 29%; mp 107–109 °C. Anal. ($C_{22}H_{20}F_3I_2NO_5$) C, H, N. **80**: 0.23 g, 21%; mp 135–137 °C; ^1H NMR ($CDCl_3$) δ 3.15 (2 H, m, Ar C*H*$_2$CH), 3.35 (2 H, m, Ar C*H*$_2$CH=CH_2), 3.84 (3 H, s, OC*H*$_3$), 4.78 (1 H, s, Ar O*H*), 4.85 (1 H, m, Ar CH_2C*H*), 5.15 (2 h, m, CH=C*H*$_2$), 6.00 (1 H, m, C*H*=CH_2), 6.47 (1 H, d of d, 6′-*H*), 6.59 (1 H, d, 2′-*H*), 6.74 (1 H, d, 5′-*H*), 6.94 (1 H, br s, N*H*), and 7.59 (2 H, s, 2,6-*H*)]. Anal. ($C_{21}H_{18}F_3I_2NO_5$) C, H, N.

Computational Details. Multiple regression analyses were performed by using the statistics program MINITAB.[34] Molecular modelling and conformational studies were performed with COSMIC, SK&F's in-house package for computational chemistry;[35] structures were built and minimized by conventional molecular mechanics techniques. Three bond conformational maps were computed by using the program CUBE.[36]

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