($C_{21}H_{32}O_3N_2$·HOOC-COOH·1.5H_2O) C, H, N.

Pharmacology. β_1- and β_2-adrenergic blocking activities were determined in vitro on the isolated guinea pig atria and trachea.

Isolated Guinea Pig Atria. Guinea pigs of either sex, weighing from 250 to 350 g, were stunned by a blow on the head, and their hearts were quickly removed. The left atrium was dissected free and mounted in a muscle chamber containing 25 mL of Krebs solution gassed with O_2 plus CO_2 (95:5). The resting muscle tension applied to all preparations was adjusted to 0.5 g throughout the course of each experimental study. All experiments were performed at 37 °C. Concentration-response curves to isoproterenol-positive inotropic action were determined before and after increasing cumulative doses of drugs. Thus, after control responses to five cumulative concentrations of isoproterenol (10^{-9} to 10^{-7} M; exposure time, 2-3 min) had been obtained, increased concentrations of our new compounds or propranolol (exposure time, 15 min) were added to the bath, and dose-response curves to isoproterenol were determined again. Experiments were performed on groups of at least three preparations. The β-blocking effect of drugs was calculated from the concentration-response curves to isoprenaline (inotropism increase induced) and expressed as IC_{50}, i.e. the drug concentration inhibiting 50% of the response to the isoprenaline concentration giving 90-100% of the maximal effect.

Isolated Guinea Pig Trachea. Trachea spirals cut were equilibrated under an initial tension of 1.50 g in Krebs solution at 37 °C, gassed with O_2 plus CO_2 (95:5). The resting tension was between 0.4 and 1.0 g. The effects of isoproterenol (3×10^{-8} to 3×10^{-6} M) were tested against contraction induced by acetylcholine (ACh 3×10^{-6} M). Antagonists were added to the bath 15 min before ACh, and isoproterenol (3×10^{-8} to 3×10^{-5} M) was added again after the contraction induced by ACh were developed. Experiments were performed on groups of at least three preparations. IC_{50} were assessed as described above.

Acknowledgment. This work was supported by RIKER 3M Society and INSERM (Contract No. 855005).

Registry No. **3** [n = 3, R^1 = CH_3, Z = 2-F(cis-H, OH)], 118017-57-5; **3** (n = 3, R^1 = CH_3, Z = 3-F), 118017-58-6; **3** [n = 3, R^1 = Me, Z = H(cis-H, OH)], 100703-72-8; **3** (n = 4, R^1,R^1 = CH_2CH_2, Z = H), 89874-29-3; **3** (n = 4, R^1 = Me, Z = H), 116822-93-6; **3** (n = 3, R^1,R^1 = CH_2CH_2, Z = H), 89874-28-2; **3** (n = 8, R^1,R^1 = CH_2CH_2, Z = H(trans-H,OH)], 100837-45-4; **4** [n = 3, R^2 = H, Z = H (cis-H,OH)], 116823-15-5; **4** [n = 4, R^2 = H, Z = H (cis-H,OH)], 116823-17-7; **4** [n = 4, R^2 = Me, Z = H (trans-H,OMe)], 116823-07-5; **4** [n = 3, R^2 = Me, Z = H(cis-H,-OMe)], 116823-05-3; **4** [n = 3, R^2 = Me, Z = F(cis-H,OMe)], 118017-59-7; **4** [n = 3, Z = H, R^2 = CH_2CH_2OH(cis-H,-OCH_2CH_2OH)], 116823-09-7; **4** [n = 4, R^2 = CH_2CH_2OH, Z = H(trans-H,OCH_2CH_2OH)], 116823-10-0; **4** [n = 8, R^2 = Me, Z = H (cis-H,OMe)], 116823-21-3; **4** [n = 8, R^2 = H, Z = H (cis-H,OH)], 116908-29-3; **4** [n = 8, R^2 = H, Z = H (trans-H,OH], 116823-20-2; **5** [Z = H, n = 3, R^2 = H (cis-H,OH) syn], 118017-60-0; **5** [Z = H, n = 3, R^2 = H (cis-H,OH) anti], 118017-61-1; **5** (Z = H, n = 4, R^2 = H), 118017-62-2; **5** (Z = H, n = 4, R^2 = Me), 118017-63-3; **5** [Z = H, n = 3, R^2 = Me (cis-H,OMe)], 118017-64-4; **5** [Z = F, n = 3, R^2 = Me(cis-H,OMe)], 118017-65-5; **5** [Z = H, n = 3, R^2 = CH_2CH_2OH (cis-H, OCH_2CH_2OH)], 118017-66-6; **5** [Z = H, n = 4, R^2 = CH_2CH_2OH (trans-H,OCH_2CH_2OH)], 118017-67-7; **5** [Z = H, n = 8, R^2 = Me (cis-H,OMe)], 118017-68-8; **7a**, 118017-69-9; **7b**, 118017-70-2; **7b**·oxalate, 118017-71-3; **7c**, 118017-72-4; **7d**, 118017-73-5; **7d**·oxalate, 118017-74-6; **7e**, 118017-75-7; **7e**·oxalate, 118017-76-8; **7f**, 118017-77-9; **7f**·oxalate, 118017-78-0; **7g**, 118017-79-1; **7g**·oxalate, 118017-80-4; **7h**, 118017-81-5; **7i**, 118017-82-6; **7i**·oxalate, 118070-30-7; **7j**, 118017-83-7; **7j**·oxalate, 118017-84-8; **7k**, 118017-85-9; **7k**·oxalate, 118017-86-0; **7l**, 118017-87-1; **7m**, 118017-88-2; **7m**·HCl, 118017-89-3; **7n**, 118017-90-6; **7o**, 118017-91-7; **7p**, 118017-92-8; **7q**, 118017-93-9; **7r**, 118017-94-0; **7s**, 118017-95-1; *i*-$PrNH_2$, 75-31-0; *t*-$BuNH_2$, 75-64-9; $HUANH_2$, 3213-28-3; 2,2-dimethoxy-1-hydroxycyclohexene, 118017-96-2; *p*-bromofluorobenzene, 460-00-4; epichlorhydrin, 106-89-8.

Supplementary Material Available: Full IR, UV, ^1H NMR and ^{13}C NMR for all prepared oximes **5** and all prepared (aryloximino)propanolamines (6 pages). Ordering information is given on any current masthead page.

Selective Thyromimetics. Cardiac-Sparing Thyroid Hormone Analogues Containing 3′-Arylmethyl Substituents

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Introduction of specific arylmethyl groups at the 3′-position of the thyroid hormone 3,3′,5-triiodo-L-thyronine (T_3), and its known hormonally active derivatives, gives liver-selective, cardiac-sparing thyromimetics, with potential utility as plasma cholesterol lowering agents. Selectivity-conferring 3′-substituents include substituted benzyl, e.g. *p*-hydroxybenzyl, and heterocyclic methyl, e.g. 2-oxo-1,2-dihydropyrid-5-ylmethyl and 6-oxo-1,6-dihydropyridazin-3-ylmethyl. Correlations between in vivo and in vitro receptor binding affinities show that liver/heart selectivity does not depend on receptor recognition but on penetration or access to receptors in vivo. QSAR studies of the binding data of a series of 20 3′-arylmethyl T_3 analogues show that electronegative groups at the para position increase both receptor binding and selectivity in vivo. However, increasing 3′-arylmethyl hydrophobicity increases receptor binding but reduces selectivity. Substitution at ortho and meta positions reduces both binding and selectivity. Replacement of the 3,5-iodo groups by halogen or methyl maintains selectivity, with 3,5-dibromo analogues in particular having increased potency combined with oral bioavailability. Diphenyl thioether derivatives also have improved potency but are less orally active. At the 1-position, the D enantiomer retains selectivity, but removal of the α-amino group to give a propionic acid results in loss of selective thyromimetic activity.

It is well known that hypothyroidism is accompanied by high levels of circulating cholesterol in low-density lipoprotein (LDL) and increased risk of atherosclerosis, while in hyperthyroidism LDL cholesterol levels are decreased.[1] Lipoprotein abnormalities in hypothyroid subjects are corrected by administration of thyroid hormones.[2] Although the natural hormones T_3 and T_4 (Figure 1) are potent hypocholesterolemics,[3] they cannot be used thera-

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0022-2623/89/1832-0320$01.50/0

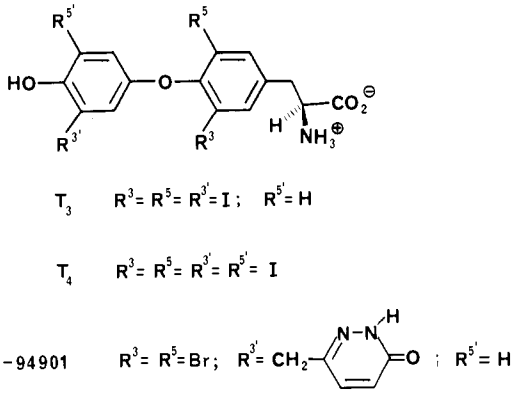

Figure 1. Structures of the natural thyroid hormones 3,3′,5-triiodo-L-thyronine (T_3) and L-thyroxine (T_4) and the selective thyromimetic SK&F L-94901.[10]

peutically in patients with normal thyroid function because of their potential to induce cardiac side effects, which is especially evident in subjects whose cardiac function may already be compromised. These side effects are a consequence of a combination of direct hormone action, resulting in increased cardiac dysrhythmias, and of indirect action as a consequence of the characteristic hormone-induced increase in metabolic rate. A number of postulated metabolites of T_3 and T_4, and a variety of synthetic analogues, have been tested in attempts to dissociate cholesterol-lowering from calorigenic and cardiac effects. These studies however failed to provide a thyromimetic with unequivocally demonstrated selectivity.[4] Despite their low therapeutic ratio, several analogues, including the unnatural enantiomer D-T_4 and more recently etiroxate (α-methyl DL-T_4 ethyl ester),[5] have been reported to lower serum cholesterol in man at doses that do not raise metabolic rate. However, the major clinical trial with D-T_4 was halted after increased mortality was found in the drug-treated group.[6]

Since these early studies with thyromimetics were performed, evidence has accumulated, which strongly suggests that thyromimetic activity results from protein synthesis occurring after hormonal interaction with nuclear receptors in the cells of responsive tissues.[7] Cholesterol-lowering effects are believed to be a consequence of specific enzyme and LDL receptor induction in the liver.[3b,8] We have designed and synthesized novel thyromimetics with the objective of discovering analogues lacking cardiac activity but retaining hepatic activity. Studies with 3′-substituted T_3 analogues[9] possessing high affinity for the nuclear receptor led to a series of 3′-(arylmethyl)-3,5-dihalo-L-thyronines,[10] exemplified by SK&F L-94901 (Figure 1). When administered to rats these compounds were shown to be hypocholesterolemic and have high thyromimetic activity in the liver and little or no direct action on the heart. Although the novel compounds showed no differences in their relative affinities for liver and heart nuclear receptors in vitro, the observed selective thyromimetic activities were consistent with in vivo receptor binding, which showed liver selectivity. Factors influencing *access* of the hormone analogues to receptors in the different tissues appear to be responsible for selective liver activity.

Herein we report the synthesis, receptor affinities in vitro and in vivo, and thyromimetic activities in vivo, of a wider group of analogues related to SK&F L-94901 (Tables I–III). The properties of the 3′-arylmethyl group required for receptor recognition, selectivity, and tissue access are explored with a series of 3,5-diiodo derivatives (**1–20**) (Table II). Subsequent modification to the 3,5-substituents, ether oxygen, and L-alanyl side chain of the selective thyromimetics **15** and **19** were made [compounds **21–33**, Table III], in order to improve potency and gain oral bioavailability.

Synthesis. The syntheses of compounds 1,[8] **14**, **15**, **17–19**,[11] and **32** (SK&F L-94901)[12,13] have been reported. The other analogues (Table I) were prepared as shown in Schemes I–V. The principal routes used to construct the diphenyl ether[8,14] were either condensation of a phenol with a 2,6-dinitrotyrosine derivative (Schemes I and V) or coupling of a diaryliodonium salt with a 2,6-disubstituted phenol (Schemes II–IV). Physical properties and analytical data are given in Table I.

The dinitrophenol route (Scheme I) was generally used for the synthesis of 3′-benzyl derivatives containing electron-releasing substituents, since in these cases attempts to prepare the requisite diaryliodonium salts were often unsuccessful. Precursor phenols (**40**, Scheme I) were synthesized from the aldehyde **34**[15] by treatment with excess of the appropriate aryl Grignard reagent, followed by catalytic hydrogenation of the intermediate carbinols; protection of the free phenol in **34** was not necessary. Phenol **40e** was prepared from reaction of **34** with the Grignard reagent from 4-bromo-2-chloroanisole, which gave

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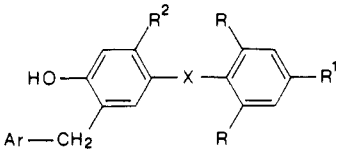
Table I. Physical Properties and Analytical Data of Thyronine Derivatives



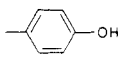
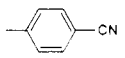
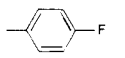
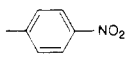
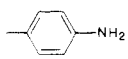
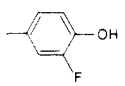
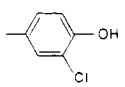
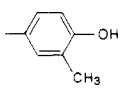
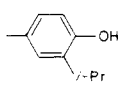
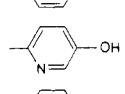
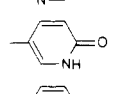
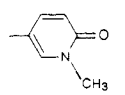
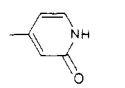
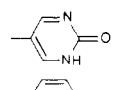
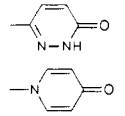
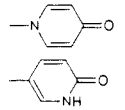
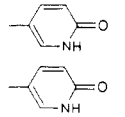
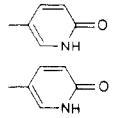
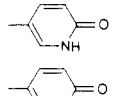
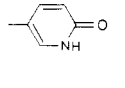
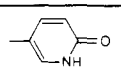
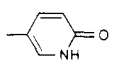
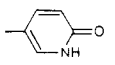
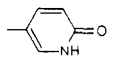
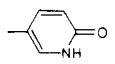
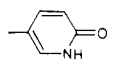
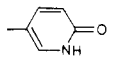
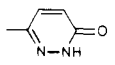
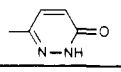
no.[a]	Ar	R	X	R[1]	R[2]	mp,[b] °C	$[\alpha]_D$[c]	formula	analyses
(1)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	203–205	+17.0 (0.76)	$C_{22}H_{19}I_2NO_4 \cdot H_2O$	C, H, N, I
(2)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	251–253	+20.0 (0.96)[d]	$C_{22}H_{19}I_2NO_5$	C, H, N, I
(3)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	269–271	+17.3 (0.51)[d]	$C_{23}H_{18}I_2N_2O_4$	C, H, N, I
(4)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	240–241	+15.4 (0.95)[d]	$C_{22}H_{18}FI_2NO_4$	C, H, N, I
(5)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	244–245	−3.4 (0.88)[e]	$C_{22}H_{18}I_2N_2O_6$	C, H, N, I
(6)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	245–248		$C_{22}H_{20}I_2N_2O_4 \cdot 2HBr \cdot 1.6H_2O$	C, H, N, Br, I
(7)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	235–237	−12.5 (0.28)[e]	$C_{22}H_{19}I_2NO_5$	C, H, N, I
(8)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	255–258	+16.9 (0.97)[d]	$C_{22}H_{18}FI_2NO_5$	C, H, N, I
(9)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	257–259	+19.4 (1.05)[d]	$C_{22}H_{18}ClI_2NO_5$	C, H, N, Cl, I
(10)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	264–266	+17.3 (0.94)[d]	$C_{23}H_{21}I_2NO_5$	C, H, N, I
(11)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	223–224	+16.4 (0.57)[d]	$C_{25}H_{25}I_2NO_5 \cdot 0.5H_2O$	C, H, N, I
(12)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	214–216	+13.5 (1.02)[d]	$C_{21}H_{18}I_2N_2O_4 \cdot 0.5H_2O$	C, H, N; I[h]
(13)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	270–271	−3.7 (0.57)[e]	$C_{21}H_{18}I_2N_2O_4$	C, H, N, I
(14)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	277	+14.0 (1.0)[d]	$C_{21}H_{18}I_2N_2O_5 \cdot 0.35H_2O$	C, H, N, I
(15)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	253–255	−7.2 (1.0)[e]	$C_{21}H_{18}I_2N_2O_5 \cdot H_2O$	C, H, N, I
(16)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	253–255	+16.2 (1.16)[d]	$C_{22}H_{20}I_2N_2O_5 \cdot 0.5H_2O$	C, H, N, I
(17)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	275–276	+19.8 (1.1)[d]	$C_{21}H_{18}I_2N_2O_5$	C, H, N, I
(18)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	>260	+10.6 (0.84)[d]	$C_{20}H_{17}I_2N_3O_5 \cdot H_2O$	C, H, N, I
(19)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	258–262	+10.8 (1.0)[d]	$C_{20}H_{17}I_2N_3O_5 \cdot 0.7H_2O$	C, H, N, I
(20)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	>250	−8.3 (0.49)[f]	$C_{21}H_{18}I_2N_2O_5$	C, H, N
(21)		Br	O	L-$CH_2CH(NH_2)CO_2H$	H	269–271	−10.7 (0.97)[f]	$C_{21}H_{18}Br_2N_2O_5$	C, H, N, Br
(22)		Cl	O	L-$CH_2CH(NH_2)CO_2H$	H	235	−11.2 (0.95)[f]	$C_{21}H_{18}Cl_2N_2O_5 \cdot 0.5H_2O$	C, H, N, Cl
(23)		CH_3	O	DL-$CH_2CH(NH_2)C$-O_2H	H	250–253		$C_{23}H_{24}N_2O_5$	C, H, N
(24)		I	S	L-$CH_2CH(NH_2)CO_2H$	H	270–273	−3.8 (0.92)[e]	$C_{21}H_{18}I_2N_2O_4S$	C, H, N, I, S

Table I (Continued)

no.[a]	Ar	R	X	R[1]	R[2]	mp,[b] °C	$[\alpha]_D$[c]	formula	analyses
(25)		Br	S	L-$CH_2CH(NH_2)CO_2H$	H	287–289	−7.8 (0.54)[f]	$C_{21}H_{18}Br_2N_2O_4S \cdot H_2O \cdot 0.06EtOH$	C, H, N, Br, S
(26)		I	O	D-$CH_2CH(NH_2)CO_2H$	H	245–248	+3.93 (1.02)[g]	$C_{21}H_{18}I_2N_2O_5 \cdot H_2O$	C, H, N, I
(27)		Br	O	CH_2CO_2H	H	258–259		$C_{20}H_{15}Br_2NO_5$	C, H, N, Br
(28)		Br	O	$(CH_2)_2CO_2H$	H	276–277		$C_{21}H_{17}Br_2NO_5$	C, H, N, Br
(29)		Br	O	$(CH_2)_3CO_2H$	H	280–281		$C_{22}H_{19}Br_2NO_5$	C, H, N, Br
(30)		I	O	CH_3	H	248		$C_{19}H_{15}I_2NO_3$	C, H, N, I
(31)		I	O	L-$CH_2CH(NH_2)CO_2H$	CH_3	>275	−12.4 (0.49)[f]	$C_{22}H_{20}I_2N_2O_5 \cdot 0.4H_2O$	C, H, N, I
(32)		Br	O	L-$CH_2CH(NH_2)CO_2H$	H	283	−11.3 (1.0)[f]	$C_{20}H_{17}Br_2N_3O_5$	C, H, N, Br
(33)		Cl	O	L-$CH_2CH(NH_2)CO_2H$	H	245	−10.0 (0.70)[f]	$C_{20}H_{17}Cl_2N_3O_5 \cdot 1.8H_2O \cdot 0.044NaOAc$	C, H, N, Cl

[a] All compounds had satisfactory ^1H NMR spectra and were of >95% purity as adjudged by HPLC. [b] Usually with decomposition. [c] At 25 °C (percent concentration). [d] 17:2:1 EtOH–H_2O–cHCl. [e] 1:2 1 N NaOH–EtOH. [f] 5:3 5 N NaOH–EtOH. [g] 1:2 0.1 N NaOH–EtOH. [h] I: calcd 40.60, found 41.15.

the 4-metalated derivative exclusively. In this case, in order to prevent hydrogenolysis of the chloro group, the intermediate carbinol was reduced, after acetylation, by ionic hydrogenation with triethylsilane and trifluoroacetic acid. The 2-methylphenol **39** required for the synthesis of the conformationally restricted[16] 2′-methyl analogue **31** was obtained from the parent phenol **36**[11] (Scheme I). Formylation gave **37**, which was reduced and acetylated to the benzyl acetate **38**. Reduction of **38** with sodium borohydride in dimethoxyethane[17] gave **39**.

2-Substituted anisoles (**45**, Scheme II) were prepared from Grignard reagent **43** and the appropriate benzaldehyde, followed by reduction of the product carbinols. Ionic hydrogenation was again useful for carbinol reduction where groups susceptible to catalytic hydrogenolysis were present, for example cyano (**45b**) and nitro (**45c**). Synthesis of 1,3,5-substituted analogues of **15** was accomplished with the iodonium salt **50** (Scheme III). The D enantiomer **26** was prepared from a D-tyrosine precursor by the same method as described for **15**,[11] and the 3,5-dichloro compound **33** was synthesized by the route developed for SK&F L-94901 (**32**).[13]

Preparation of the 3,5-dimethyl analogue **23** required arylation of 2,6-dimethyl-4-formylphenol (**52**, Scheme IV). This was achieved either by Ullmann coupling with the iodide **53** [obtained as the byproduct in reactions of **50** (Scheme III)] or by coupling of the potassium salt of **52** with iodonium salt **50** in the presence of 18-crown-6. The resulting aldehyde **54** was converted to the DL-amino acid **23** via the azlactone **55**.

For the synthesis of diphenyl thioethers **24** and **25**, the thiophenol **58** was required. This was obtained from anisole **49** by thiocyanation followed by basic hydrolysis of the thiocyanate **57** (Scheme V). The hydrolysis procedure gave substantial quantities of the disulfide of **58**, which was converted to **58** by reduction with triphenylphosphine and hydrogen chloride. Conversion of the dinitrothyronine **59** to the dibromo precursor **60b** was effected by treatment of the bis(diazonium) salt from **59** with cuprous bromide and hydrobromic acid.

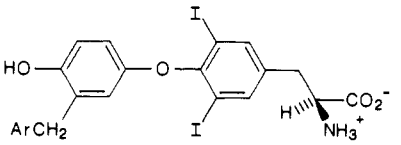
Biological Activity. Thyromimetic activities in vivo and in vitro were measured in rats by using the methods described previously.[10,18] Potency in vivo [ED_{50} for induction of mitochondrial cytochrome C 3-phosphoglycerate oxidoreductase (GPDH)] and receptor affinities in vitro (IC_{50} for intact nuclei) and in vivo (ID_{50} 1 h after intravenous administration) were determined in both liver and heart. Activities relative to T_3 = 100% are given in Tables II and III. Tissue access is defined as the ratio of in vivo to in vitro relative affinities (rel ID_{50}/rel IC_{50}) and in vivo liver/heart selectivity as the ratio (rel $ID_{50})_{liver}$/(rel $ID_{50})_{heart}$ (*S*, Table II). In the in vivo binding studies, the relationship between administered dose and free plasma concentration may be different for each analogue. Using the liver/heart in vivo binding *ratio* (*S*) for structure–selectivity studies has the advantage of eliminating tissue nonspecific pharmacokinetic differences between compounds. Overall thyromimetic activity was determined in euthyroid rats by measurement of the percent increase in metabolic rate (whole body oxygen consumption) after seven daily doses of test compound. The observed increases in oxygen consumption were submaximal relative to T_3, consistent with thyromimetic activity being confined to fewer tissues.[10] Comparisons of increases in oxygen consumption after oral or intramuscular administration are used to assess oral bioavailability of selective thyromimetics.

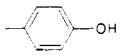
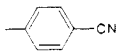
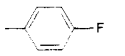
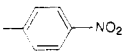
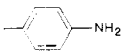
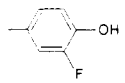
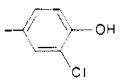
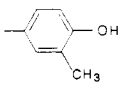
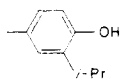
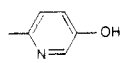
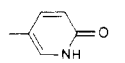
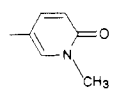
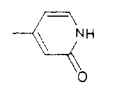
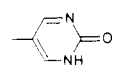
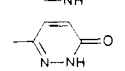
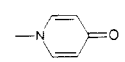
3′-(Arylmethyl)-3,5-diiodo-L-thyronines

Selective Thyromimetics. The analogues **1**–**20** (Table II) were prepared to explore the structural properties of the 3′-substituent, which are essential for the selectivity and thyromimetic activity of the two key lead compounds, the 4-hydroxybenzyl analogue **2** and its 3-aza derivative, the pyridone **15**.[10] Selective thyromimetics are defined as those analogues possessing full agonist activity (rel ED_{50})

(16) Jorgensen, E. C. In *Hormonal Peptides and Proteins*; Li, C. H., Ed.; Academic: New York, 1978; Vol. VI, Chapter 3.
(17) Crelling, J. K.; Quick, J. *J. Org. Chem.* **1978**, *43*, 155.
(18) Benson, M. G.; Ellis, D.; Emmett, J. C.; Leeson, P. D.; Pearce, N. J.; Shah, V. P.; Underwood, A. H. *Biochem. Pharmacol.* **1984**, *33*, 3143.

Table II. Thyromimetic Activities of 3′-(Arylmethyl)-3,5-diiodo-L-thyronines

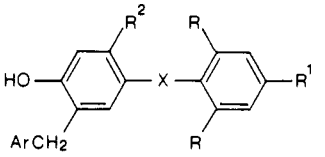


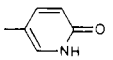
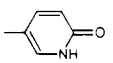
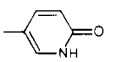
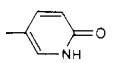
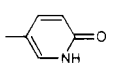
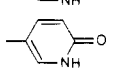
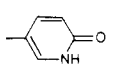
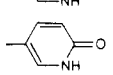
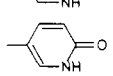
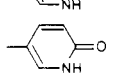
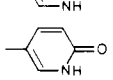
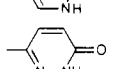
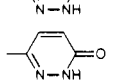
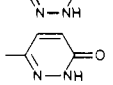
		liver[a]			heart[a]			
no.	Ar	rel IC_{50}[b]	rel ID_{50}[c]	rel ED_{50}[d]	rel IC_{50}[b]	rel ID_{50}[c]	rel ED_{50}[d]	S[e]
(1)		15	5.1	2.2	13	0.30	0.50	17.0
(2)		17	19	2.2	56	0.70	<0.10	27.1
(3)		13	6.1	LM	6.5	0.22	I	27.7
(4)		60	98	5.7	43	8.6	3.7	11.4
(5)		20	9.8	LM	64	0.71	0.90	13.8
(6)		0.63	0.21	I	1.1	0.023	I	9.13
(7)		0.81	0.024	LM	2.2	0.024	LM	1.00
(8)		31	0.60	LM	31	0.37	I	1.62
(9)		5.9	0.07	LM	15	0.07	I	1.00
(10)		7.4	0.35	LM	9.7	0.10	I	3.50
(11)		0.67	0.11	LM	4.4	0.11	LM	1.00
(12)		0.96	2.4	F	3.5	0.54	LM	4.44
(13)		8.5	7.0	LM	15	1.6	LM	4.38
(14)		20	31	1.7	15	2.5	LM	12.4
(15)		2.3	10	1.7	2.7	0.20	I	50.0
(16)		0.76	4.3	LM	0.58	0.058	I	74.1
(17)		0.042	0.070	LM	0.045	0.0035	I	20.0
(18)		0.15	0.28	I	0.58	0.0062	I	45.2
(19)		2.0	38	2.4	4.0	0.40	<0.10	95.0
(20)		0.25	2.7	1.4	0.40	0.013	I	208

[a] Determined in rats by using the methods described in ref 10 and 18. All activities are quoted relative to T_3 = 100%. [b] In vitro binding to isolated nuclei: IC_{50} values for T_3: liver, 0.2–0.5 nM; heart, 0.2–0.5 nM. [c] In vivo binding to nuclei determined 1 h after intravenous administration of test compounds: ID_{50} values for T_3: liver, 2.4 nM/kg; heart, 2.4 nM/kg. [d] Potency for induction of GPDH (mitochondrial cytochrome C 3-phosphoglycerate oxidoreductase) 48 h after single intramuscular injection; ED_{50} values for T_3: liver, 160 nM/kg; heart, 70 nM/kg; LM = low maximum response after two daily doses of 50 mg/kg; F = full agonist, potency not determined; I = inactive after two daily doses of 50 mg/kg. [e] Selectivity, defined as the ratio $(\text{rel } ID_{50})_{liver}/(\text{rel } ID_{50})_{heart}$.

in the liver and little or no activity in the heart. It is evident that replacement of the hydroxyl group in the 3′-substituent of **2** by alternative polar groups, for example cyano (**3**), fluoro (**4**), nitro (**5**), and amino (**6**), results in loss of liver-selective activity. Similarly, substitution ortho to the hydroxyl in **2** by halogen or alkyl (compounds **8**–**11**)

Table III. Thyromimetic Activities of 1,3,5-Substituted and Ether Link Analogues of the Selective Thyromimetics **15** and **19**



						liver[a]			heart[a]			% increase O_2 consump (dose, mg/kg)[b]	
no.	Ar	R	X	R^1	R^2	rel IC_{50}	rel ID_{50}	rel ED_{50}	rel IC_{50}	rel ID_{50}	rel ED_{50}	im	po
(15)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	2.3	10	1.7	2.7	0.20	I	19 (3.3)	I (50)
(21)		Br	O	L-$CH_2CH(NH_2)CO_2H$	H	2.0	23	6.9	1.8	0.30	I	32 (3.3)	31 (5)
(22)		Cl	O	L-$CH_2CH(NH_2)CO_2H$	H	0.49	13	1.2	0.53	0.5	LM	26 (3.3)	31 (5)
(23)		CH_3	O	DL-$CH_2CH(NH_2)CO_2H$	H	0.083		1.1	0.14		I	28 (10)	18 (5)
(24)		I	S	L-$CH_2CH(NH_2)CO_2H$	H	14.8		16.6	19.4		I	26 (3.3)	I (50)
(25)		Br	S	L-$CH_2CH(NH_2)CO_2H$	H	9.8		14.5	10.2		LM		16 (5.3)
(26)		I	O	D-$CH_2CH(NH_2)COH$	H	1.1		1.0	1.9		I		I (50)
(27)		Br	O	CH_2CO_2H	H	3.0	4.9	2.0	7.2	0.05	I	21 (20)	I (50)
(28)		Br	O	$(CH_2)_2CO_2H$	H	31.6	13.8	2.0	33.2	4.2	0.04		
(29)		Br	O	$(CH_2)_3CO_2H$	H	21.7	3.3	0.69	21.7	0.26	I		19 (5)
(31)		I	O	L-$CH_2CH(NH_2)CO_2H$	CH_3	0.028	0.080		0.039	0.002			
(19)		I	O	L-$CH_2CH(NH_2)CO_2H$	H	2.0	38	2.4	4.0	0.40	<0.1	19 (3.3)	20 (10)
(32)		Br	O	L-$CH_2CH(NH_2)CO_2H$	H	0.9	50	18	2.0	1.3	<0.1	20 (0.1)	20 (0.1)
(33)		Cl	O	L-$CH_2CH(NH_2)CO_2H$	H	0.48		25	0.8		LM		15 (0.04)

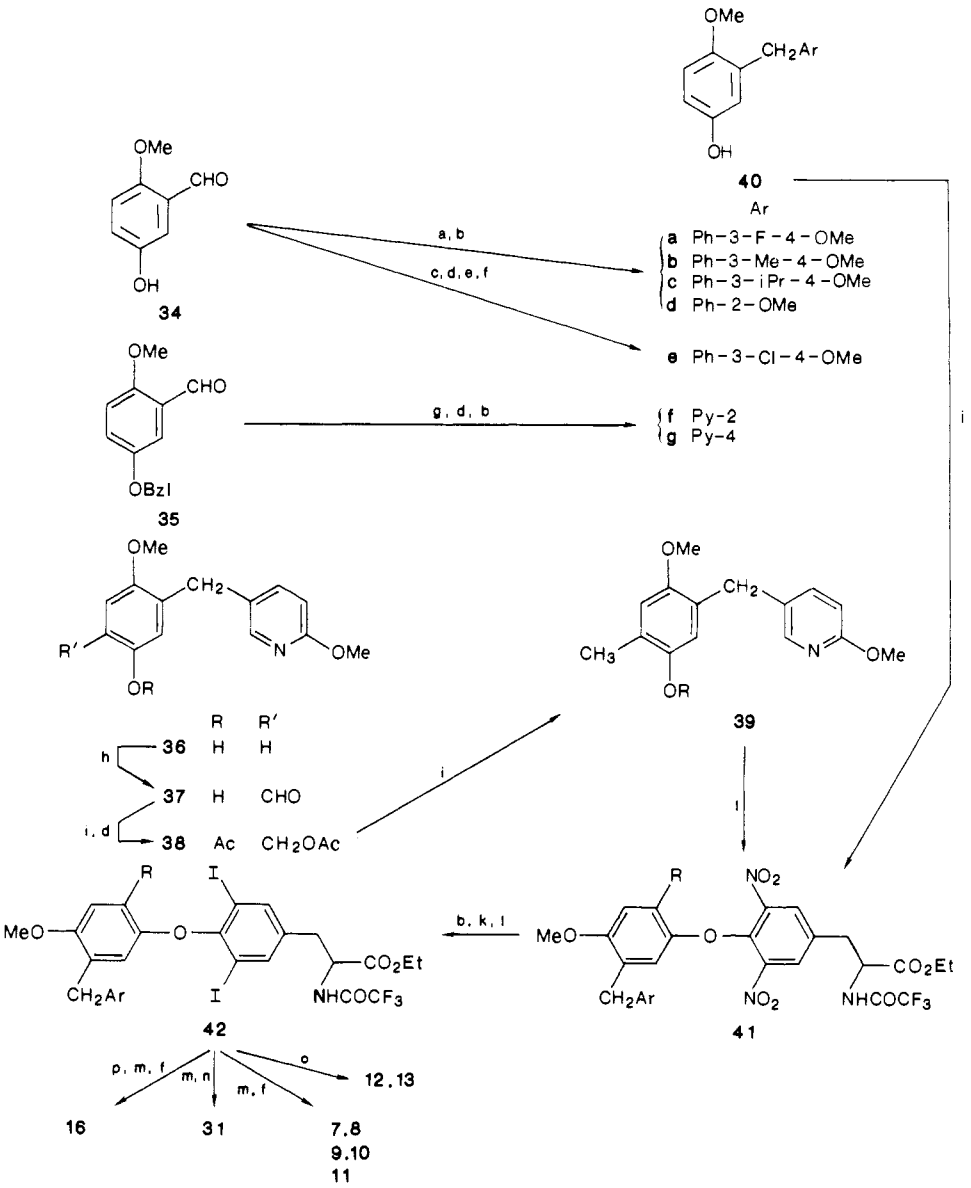
[a] See footnotes, Table II. [b] Percent increase in oxygen consumption after seven daily doses of test compound, given intramuscularly (im) or orally (po) to euthyroid rats (see ref 10 for details of the method used). I = inactive. These compounds produce characteristic submaximal increases in oxygen consumption relative to T_3 (see ref 10).

greatly reduces liver agonist activity and tissue selectivity. The loss of potency and selectivity in the 2-hydroxybenzyl analogue **7** shows that the location of the hydroxyl group in **2** is critical. Among the heterocyclic analogues, 4-oxy substitution is also critical for selectivity and activity,[11] with the isomeric pyridone **15**, 3-hydroxypyridine **14**, and 4-pyridone **20** all being selective thyromimetics. Although the tautomer of **15** that is required for activity is not known,[11] it is evident from the activities of **14** and **20** that both 4-oxo and 4-hydroxy substitution in 3′-heteroarylmethyl analogues induce selective activity. Aza analogues of **15**, the pyrimidone **18** and the pyridazinone **19**, have different activities, selective thyromimetic activity being observed only with **19**.

Since most of the compounds in Table II are not full agonists, quantitative correlations between potency and receptor binding[9] cannot be established for this series. However, for the liver full agonists, there is a reasonable qualitative relationship between potency (rel ED_{50}) and in vivo receptor binding (rel ID_{50}). Inspection of Table II shows that several compounds that are not full agonists or are inactive have differing in vivo receptor affinities. In vivo activity will depend not only on receptor binding and access to receptors but also on pharmacokinetic and metabolic phenomena, including mobilization, distribution, plasma protein binding, solubility, and rate of elimination. These factors could contribute to the relatively poor overall relationship between activity and binding. Differences in efficacy also cannot be ruled out. Relative receptor affinity is however clearly relevant to tissue-selective activity, since each of the selective thyromimetics shows liver selectivity in in vivo receptor binding (*S*, Table II) and access. We have used the in vivo and in vitro binding data to establish those 3′-arylmethyl substituent properties necessary for intrinsic receptor recognition (rel IC_{50}), tissue selectivity (*S*), and access to receptors in vivo [(rel ID_{50})/(rel IC_{50})].

In Vitro Receptor Recognition. Previously, we have shown that the 3′-substitutent binding pocket on the T_3 receptor is hydrophobic and limited in depth to approximately the length of the natural iodo substituent but is wide enough to accommodate a phenyl or cyclohexyl ring.[9] This model was suggested by conformational studies of

Scheme I[a]



[a] Reagents: (a) ArMgX; (b) H_2–Pd/C; (c) 4-bromo-2-chloroanisole–Mg; (d) Ac_2O–pyridine; (e) Et_3SiH–CF_3CO_2H; (f) NaOH; (g) PyBr/*n*-BuLi/−100 °C; (h) boric acid–hexamine; (i) $NaBH_4$; (j) *N*-trifluoroacetyl-3,5-dinitro-L-tyrosine ethyl ester–$MeSO_2Cl$–pyridine; (k) $NaNO_2$–H_2SO_4–H_2O; (l) KI–I_2; (m) BBr_3; (n) HCl–HOAc–H_2O; (o) HBr–HOAc–H_2O; (p) CH_3I–EtOH, reflux (see ref 11 for preparation of starting material).

several semirigid 3′-substituted T_3 analogues, and supported by a quantitative structure-affinity relationship, eq 1, by using a structurally diverse series of analogues. In

$$\log (\text{rel } IC_{50})_{\text{liver}} = -0.671\lambda - 1.23(L < I) - 0.360D - 1.38H + 1.58 \quad (1)$$

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

this and in subsequent equations, n is the number of compounds included, r is the correlation coefficient, s is the standard deviation, and F is the variance ratio. In eq 1, λ is a measure of substituent hydrophilicity, derived from hydrophobicity (π) and substituent surface area (A) according to eq 2.[9] Use of λ permits the steric effects of

$$\lambda = 0.371A - \pi - 0.175 \quad (2)$$

substituents to be more clearly dissociated from the gross effects of bulk, which contribute to hydrophobic interactions;[9] $L < I$ is substituent perpendicular length in angstroms less than iodo, along the axis of the C-3′R bond; D is the nondirectional length, in angstroms, that substituents extend beyond an optimum van der Waals envelope defined by the union of the high affinity 3′-benzyl and 3′-cyclohexylmethyl analogues; the H is an indicator variable, equal to unity for 3′-acyl analogues, which can form strong acceptor intramolecular hydrogen bonds with the 4′-hydroxyl.

Multiple regression analyses using the in vitro binding data (rel IC_{50}) of compounds **1–20** were performed, in order to establish whether or not the affinities of these 3′-arylmethyl analogues were in accord with the above model. The parameters $L < I$ and H in eq 1 are not applicable to this series, and D was replaced by substituent length, greater than hydrogen, of ortho and meta (D-o,m) and para (D-p) substituents on the aryl rings of each analogue. These length parameters correspond to the extension of each 3′-substituent beyond the apparent optimum size defined in the earlier study.[9] The appearance of high affinity when polar substituents are present at the para position of benzyl analogues (compounds **2–4**) suggests the possibility that a dipolar attraction with the receptor may enhance the affinity of these compounds. The effects of electrical contributions to affinity were assessed by using the partial charges on each substituent and ring atom obtained from Gaussian 80 ab initio quantum mechanical

Table IV. 3′-Substituent Parameters Used for Quantitative Structure–Affinity/Selectivity Studies and Observed and Calculated in Vitro Receptor Affinities and in Vivo Selectivity

no.	π^a	A^b	MR[a]	λ^c	D-o,m[d]	Q-p[e]	p-OH[f]
1	2.01	6.68	3.00	0.293	0.00	0.06	0
2	1.34	7.46	3.18	1.253	0.00	−0.08	1
3	1.42	8.19	3.53	1.443	0.00	−0.12	0
4	2.13	7.10	2.99	0.329	0.00	−0.14	0
5	1.71	8.55	3.63	1.287	0.00	−0.26	0
6	0.76	7.74	3.44	1.937	0.00	−0.05	0
7	1.34	7.46	3.18	1.253	0.68	0.06	0
8	1.57	7.88	3.17	1.178	0.59	−0.08	1
9	2.01	8.59	3.68	1.002	1.46	−0.06	1
10	1.90	8.12	3.65	0.938	0.94	−0.09	1
11	2.74	11.59	4.58	1.385	2.05	−0.09	1
12	0.51	6.23	2.77	1.626	0.00	0.07	0
13	0.51	6.23	2.77	1.626	0.00	−0.25	0
14	−0.16	7.01	3.04	2.586	0.00	−0.08	1
15	−0.72	7.01	3.14	3.146	0.00	−0.32	0
16	−0.37	8.36	3.59	3.297	0.94	−0.31	0
17	−0.72	7.01	3.14	3.146	0.59	0.23	0
18	−1.76	6.56	2.72	4.019	0.00	−0.30	0
19	−0.87	6.56	2.72	3.129	0.00	−0.30	0
20	−1.44	7.01	3.14	3.866	0.00	−0.29	0

	log (rel IC_{50}): liver		log (rel IC_{50}): heart		log S	
no.	obsd[g]	calcd[h]	obsd[g]	calcd[i]	obsd[g]	calcd[j]
1	1.176	1.083	1.114	1.185	1.230	0.647
2	1.230	1.599	1.748	1.752	1.434	1.059
3	1.114	0.802	0.813	0.945	1.443	1.342
4	1.778	1.568	1.633	1.694	1.057	0.593
5	1.301	1.258	1.806	1.415	1.140	1.322
6	−0.201	0.308	0.041	0.451	0.960	1.510
7	−0.092	−0.016	0.342	0.211	0.000	0.486
8	1.491	1.228	1.491	1.474	0.210	0.466
9	0.771	0.673	1.176	1.052	0.000	0.022
10	0.869	1.160	0.987	1.457	0.544	0.475
11	−0.174	0.085	0.643	0.567	0.000	0.054
12	−0.018	0.202	0.544	0.326	0.648	1.005
13	0.929	1.015	1.176	1.176	0.641	1.005
14	1.301	0.744	1.176	0.919	1.093	1.498
15	0.362	0.217	0.431	0.413	1.699	1.798
16	−0.119	−0.572	−0.237	−0.225	1.870	1.275
17	−1.377	−1.599	−1.347	−1.374	1.301	1.300
18	−0.824	−0.394	−0.237	−0.185	1.655	1.814
19	0.301	0.177	0.602	0.371	1.978	1.479
20	−0.602	−0.321	−0.398	−0.116	2.317	2.069

[a] 3′-Substituent hydrophobicity (π) and molar refractivity (MR) taken from: Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979; π for compound **19** calculated from measured values of thyronine derivatives.[22] [b] 3′-Substituent surface area ($cm^2\ mol^{-1} \times 10^9$) calculated from: Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441. [c] 3′-Substituent hydrophilicity, calculated according to eq 2.[8] [d] Length of substituents in angstroms at ortho and meta positions of the 3′-aryl ring, relative to H = 0. [e] Partial charge on para substituent or atom of 3′-aryl ring, derived from Gaussian 80 ab initio quantum chemical calculations. [f] Indicator variable = 1 for 3′-aryl rings containing para hydroxyl substitution (excludes compounds where the para oxo structure is the preferred tautomer in aqueous solution). [g] From Table II. [h] Using eq 3. [i] Using eq 4. [j] Using eq 11.

Table V. Cross-Correlation Matrix (r) for the QSAR Parameters in Table IV

	π	A	MR	λ	D-o,m	Q-p
A	0.560					
MR	0.554	0.971				
λ	−0.945	−0.259	−0.264			
D-o,m	0.428	0.799	0.777	−0.185		
Q-p	0.378	0.009	0.055	−0.437	0.204	
p-OH	0.442	0.488	0.450	−0.323	0.543	0.176

calculations.[19] The calculations were performed on model methyl-substituted aromatics, i.e. structures where the common thyronine moiety in **1**–**20** is deleted, and the net charge on the para substituent, Q-p, was determined. Where there is no para substituent, Q-p was set equal to the charge on the atom at the para position, i.e. the hydrogen in compounds **1**, **7**, **12**, and **17**, and the nitrogen in the pyridine **13**. In initial multiple regression studies, the affinities of those compounds containing a para hydroxyl group were consistently underestimated. Consequently an indicator variable, p-OH, was introduced, set equal to unity for these compounds. The parameters used are given in Table IV, and the cross-correlation matrix in Table V. The following equations describing liver and heart in vitro receptor binding were derived:

$$\log (\text{rel } IC_{50})_{\text{liver}} = -0.642\lambda - 0.710D\text{-o,m} - 2.54Q\text{-p} + 0.777\ p\text{-OH} + 1.42 \quad (3)$$

$$n = 20,\ r = 0.938,\ s = 0.337,\ F = 25.4$$

$$t_\lambda = 8.13\ (p < 0.0001);\ t_{D\text{-o,m}} = 4.51\ (p < 0.001)$$

$$t_{Q\text{-p}} = 4.48\ (p < 0.001);\ t_{p\text{-OH}} = 3.82\ (p = 0.0017)$$

$$\log (\text{rel } IC_{50})_{\text{heart}} = -0.625\lambda - 0.551D\text{-o,m} - 2.66Q\text{-p} + 0.794p\text{-OH} + 1.53 \quad (4)$$

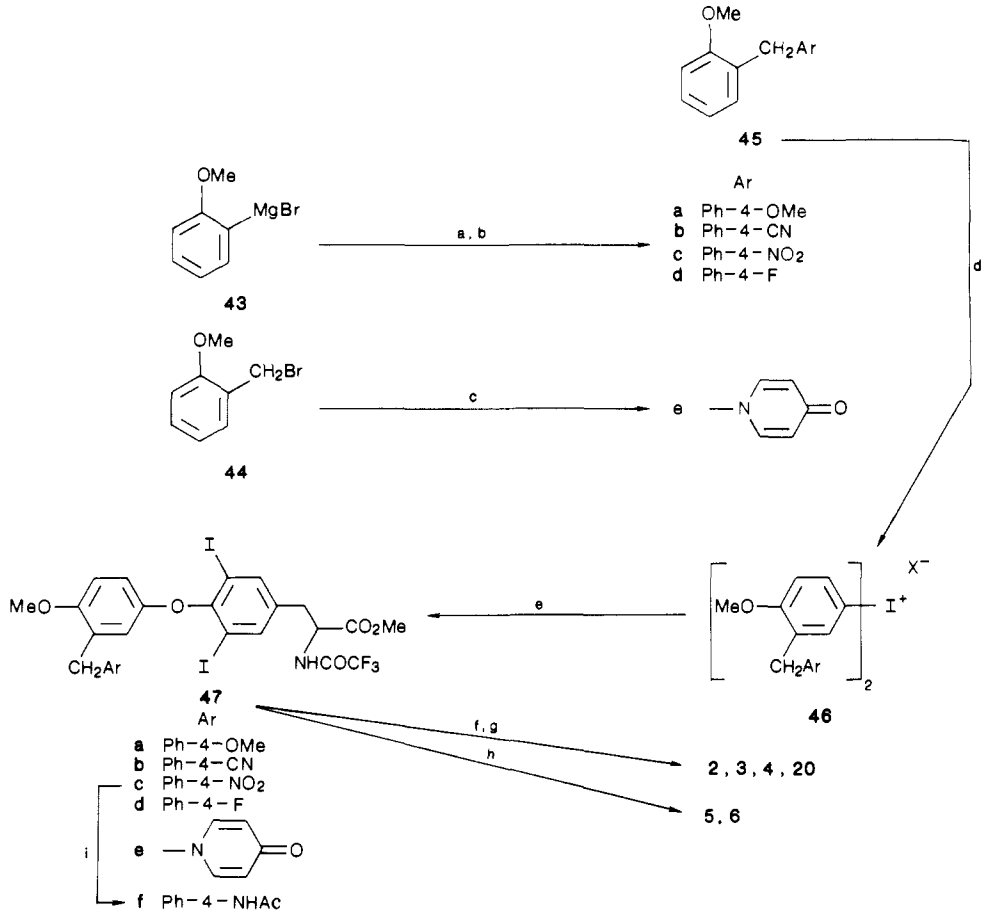
$$n = 20,\ r = 0.965,\ s = 0.239,\ F = 51.2$$

$$t_\lambda = 11.2\ (p < 0.0001);\ t_{D\text{-o,m}} = 4.93\ (p < 0.001)$$

$$t_{Q\text{-p}} = 6.60\ (p < 0.0001);\ t_{p\text{-OH}} = 5.50\ (p < 0.001)$$

(19) QCPE program used within CHEMX (Chemical Design Ltd.).

Scheme II[a]



[a] Reagents: (a) ArCHO; (b) Et_3SiH–CF_3CO_2H; (c) 4-hydroxypyridine/NaH; (d) (i) $I(OCOCF_3)_3$, (ii) MX; (e) *N*-trifluoroacetyl-3,5-diiodo-L-tyrosine methyl ester–Cu Bronze–Et_3N; (f) BBr_3; (g) NaOH; (h) HBr–HOAc–H_2O; (i) H_2-sulfided Pt/C.

The t values in these and subsequent equations are the ratios of the coefficient of the parameter to its standard deviation; the inclusion of each parameter is statistically significant at the level given (p). Observed and calculated affinities from eq 3 and 4 are given in Table IV; the equations are of similar statistical quality to eq 1, and there are no significant outliers. Comparison of eq 3 and 4 with eq 1 shows that the coefficients of λ and D-o,m in eq 3 and 4 are of the same sign as those found for the corresponding λ and D parameters in eq 1. However, the magnitudes of the coefficients of D-o,m are approximately 2-fold greater than that of D in eq 1; this is probably because D-o,m is directional whereas D is not.[9] The coefficient of the hydrophilicity parameter λ has essentially the same magnitude in all three equations and statistically makes the most significant contribution to affinity. The importance of electronic contributions of the para substituent to receptor binding is emphasized in eq 3 and 4 by the parameters Q-p and p-OH, which show that increasing negative charge or a hydroxyl group increases affinity. Inclusion of parameters describing the dimensions of the para substituent was not statistically significant.

Overall, these quantitative structure–activity relationship (QSAR) studies using the in vitro receptor affinities of the 3′-arylmethyl compounds **1–20** provide good support for the previously proposed model of a hydrophobic, specifically size-limited binding pocket for the 3′-substituent on the T_3 receptor.[9] In addition, the analysis indicates that the binding pocket contains a specifically located dipolar element capable of enhancing the affinities of compounds having electronegative groups at the para position of the

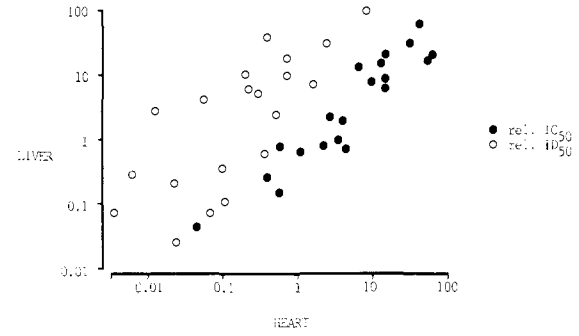
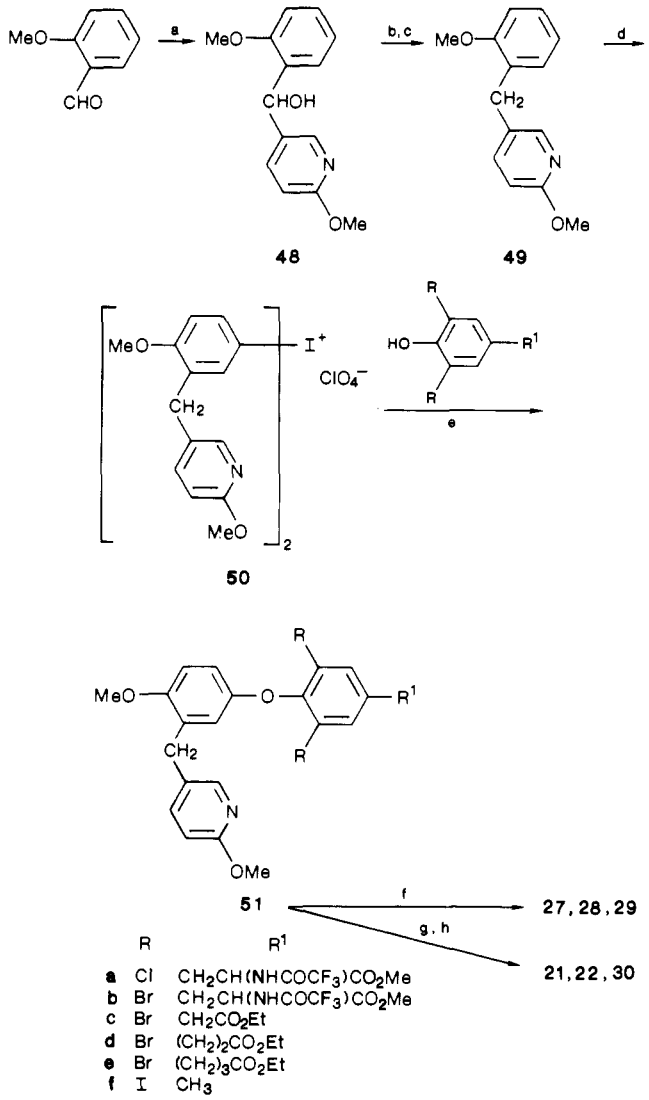


Figure 2. Relationships between liver and heart receptor affinities in vitro (rel IC_{50}) and in vivo (rel ID_{50}) for the 20 3′-(arylmethyl)-3,5-diiodothyronines in Table II. The correlation coefficients (r) are 0.938 ($p < 0.0001$) and 0.758 ($p < 0.001$), respectively (see eq 5 and 6).

3′-arylmethyl substituent. The QSAR study suggests that this interaction could be electrostatic, involving a partial positive charge on the receptor. With p-hydroxyl substitution, additional stabilization of receptor binding by hydrogen-bond donation may be involved. The beneficial effect of p-hydroxyl substitution may be confined to this group, since the affinity of the amino analogue (**6**), which can also donate a hydrogen bond, is well predicted by hydrophilicity and substituent charge alone (Table IV). The very similar equations for liver (3) and heart (4) affinities provide further evidence that the receptors in the two tissues recognize these analogues similarly.[9,11]

Tissue Selectivity and Access. The relationships

Scheme III[a]



[a] Reagents: (a) 5-bromo-2-methoxypyridine/*n*-BuLi, −78 °C; (b) Ac_2O–pyridine; (c) H_2–Pd/C; (d) (i) $I(OCOCF_3)_3$, (ii) $NaClO_4$; (e) Cu Bronze–Et_3N; (f) HBr–HOAc–H_2O; (g) BBr_3; (h) HCl–HOAc–H_2O.

between liver and heart receptor binding in vitro and in vivo are shown in Figure 2 and eq 5 and 6. It is evident

in vitro:

$$\log (\text{rel IC}_{50})_{\text{liver}} = 0.994 \log (\text{rel IC}_{50})_{\text{heart}} - 0.210 \quad (5)$$

$$n = 20,\ r = 0.938,\ s = 0.307,\ F = 131.6$$

$$t = 11.47\ (p < 0.0001)$$

in vivo:

$$\log (\text{rel ID}_{50})_{\text{liver}} = 0.901 \log (\text{rel ID}_{50})_{\text{heart}} + 0.982 \quad (6)$$

$$n = 20,\ r = 0.758,\ s = 0.699,\ F = 24.3$$

$$t = 4.93\ (p < 0.001);\ t_{\text{constant}} = 4.80\ (p < 0.001)$$

from Figure 2 and eq 5 and 6 that no receptor selectivity exists in vitro, but there is significant overall liver selectivity in vivo. The constant term in eq 6 is highly significant, showing that the compounds bind on average 10-fold more potently to liver receptors in vivo than to heart receptors. However, eq 6 is statistically poorer than is eq 5, which is to be expected since there is wider scatter in the data with several compounds (e.g. the phenols **7**–**11**)

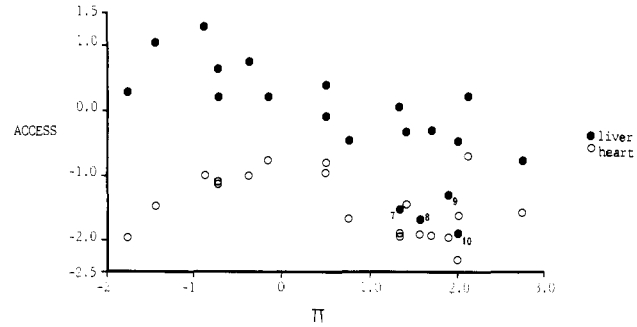


Figure 3. Relationships between tissue access (log [rel ID_{50}/rel IC_{50}]) and 3′-substituent hydrophobicity (π, Table IV) for the 3′-(arylmethyl)-3,5-diiodothyronines in Table II. Liver access shows a significant correlation (eq 9), with the indicated 3′-benzyl compounds being outliers. There is no correlation for the heart data (see text and eq 10).

showing no selectivity in vivo. Addition of 3′-substituent hydrophobicity (π, Table IV) to eq 6 results in a highly significant improvemment. Equation 7 can be rearranged

$$\log (\text{rel ID}_{50})_{\text{liver}} = 1.16 \log (\text{rel ID}_{50})_{\text{heart}} - 0.417\pi + 1.48 \quad (7)$$

$$n = 20,\ r = 0.898,\ s = 0.486,\ F = 35.2$$

$$t = 8.32\ (p < 0.0001);\ t_{\pi} = 4.49\ (p < 0.001)$$

to described overall selectivity (*S*, Table II). Equation

$$\log S = \log [(\text{rel ID}_{50})_{\text{liver}}/(\text{rel ID}_{50})_{\text{heart}}] = -0.371\pi + 1.32 \quad (8)$$

$$n = 20,\ r = 0.717,\ s = 0.491,\ F = 19.0$$

$$t = 4.36\ (p < 0.001)$$

8 shows that tissue selectivity, as assessed by differential in vivo receptor binding, increases as 3′-substituent hydrophobicity is reduced. This effect of substituent hydrophobicity on selectivity appears to be confined to the liver, as shown by the relationship between tissue access and hydrophobicity (Figure 3). Thus, access to heart receptors for this group of compounds does not change significantly, but access to liver receptors increases as 3′-substituent hydrophobicity is decreased. The relationships illustrated in Figure 3 are expressed quantitatively in eq 9 and 10.

$$\log (\text{rel ID}_{50})_{\text{liver}} = 1.22 \log (\text{rel IC}_{50})_{\text{liver}} - 0.554\pi + 0.101 \quad (9)$$

$$n = 20,\ r = 0.814,\ s = 0.640,\ F = 16.7$$

$$t = 5.72\ (p < 0.001);\ t_{\pi} = 4.12\ (p < 0.001)$$

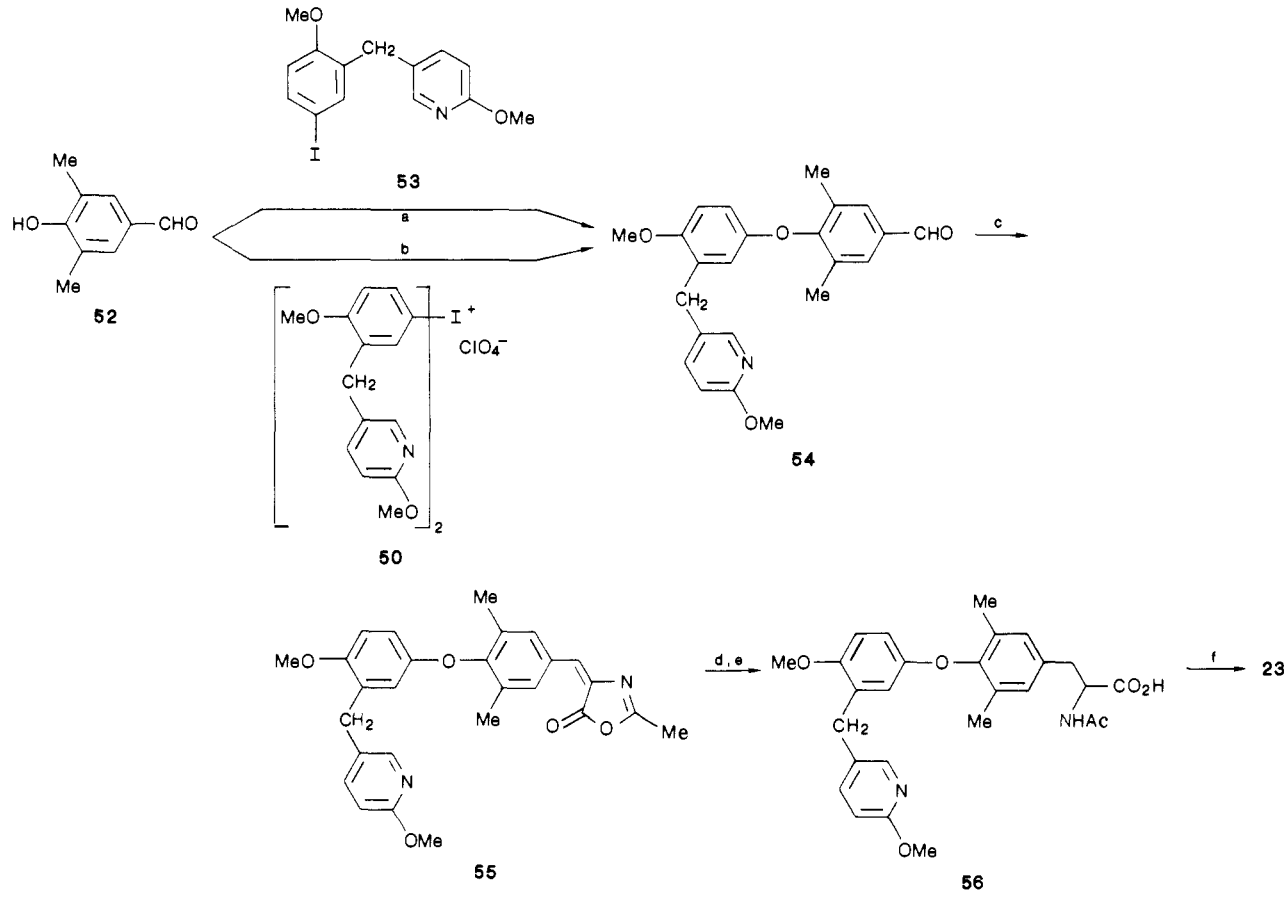
$$\log (\text{rel ID}_{50})_{\text{heart}} = 0.897 \log (\text{rel IC}_{50})_{\text{heart}} - 1.40 \quad (10)$$

$$n = 20,\ r = 0.831,\ s = 0.502,\ F = 40.2$$

$$t = 6.34\ (p < 0.0001);\ t_{\text{constant}} = 9.51\ (p < 0.0001)$$

Equation 9 shows that there is a hydrophobicity-dependent modulation of in vivo binding, relative to in vitro binding, in the liver. In contrast, eq 10 shows that in vivo binding to heart receptors is reduced on average by 25-fold relative to in vitro binding. Equation 10 could not be improved by the addition of 3′-substituent properties. Hydrophilic 3′-substituted analogues such as the pyridones **15** and **20** and the pyridazinone **19** have enhanced in vivo affinity in the liver relative to their in vitro affinity (i.e. increased access). The combination of this enhanced in vivo liver binding with reduced in vivo heart binding is consistent with the liver-selective thyromimetic activity

Scheme IV[a]



[a] Reagents: (a) Cu_2O–K_2CO_3–pyridine; (b) KOBu-t–Cu bronze–18-crown-6; (c) $AcNHCH_2CO_2H$–NaOAc–H_2O; (d) NaOH; (e) H_2–Pd/C; (f) HBr–HOAc–H_2O.

possessed by these compounds.

Although 3′-substituent hydrophobicity provides significant correlations with selectivity and liver access, this property may not represent a differential partitioning event in vivo, since compounds containing 3′-substituents of widely differing hydrophobicity are not selective thyromimetics.[9] Potent selective thyromimetic activity is at present confined to 3′-arylmethyl analogues, suggesting the importance for selectivity of specific local 3′-substituent hydrophobicity or dipolar properties. This is emphasized by the large loss of selectivity (S, Table II) seen with the 2-hydroxy analogue **7** relative to the 4-isomer **2** and in the meta substituted analogues **8**–**11**; these changes in selectivity cannot be accounted for by hydrophobicity alone, and consequently these compounds are outliers in eq 8 and 9 (see Figure 3). We therefore sought to improve eq 8 by the addition of other substituent properties. Inclusion of molar refractivity (MR) and D-o,m (Table IV) proved to be statistically significant:

$$\log S = -0.375\pi + 0.893\text{MR} - 0.844D\text{-o,m} - 1.28 \quad (11)$$

$$n = 20,\ r = 0.850,\ s = 0.393,\ F = 13.9$$

$$t_\pi = 4.59\ (p < 0.001);\ t_{\text{MR}} = 2.55\ (p = 0.022);\ t_{D\text{-o,m}} = 3.48\ (p = 0.0031)$$

Calculated selectivities (log S) from this equation are given in Table IV. Although the inclusion of MR in eq 11 is statistically significant, this may not represent an increase in selectivity with increasing substituent bulk, since the combination of a negative coefficient in hydrophobicity with a positive coefficient in a volume descriptor, as found in eq 11, probably reflects the importance of substituent hydrophilicity.[9] Consequently, hydrophobicity and molar refractivity in eq 1 can be replaced by 3′-substituent hydrophilicity (λ):

$$\log S = 0.350\lambda - 0.559D\text{-o,m} + 0.586 \quad (12)$$

$$n = 20,\ r = 0.819,\ s = 0.416,\ F = 17.3$$

$$t_\lambda = 4.09\ (p < 0.001);\ t_{D\text{-o,m}} = 3.40\ (p = 0.0034)$$

Equations 11 and 12 are of similar quality and show that liver selectivity increases with increasing 3′-substituent hydrophilicity and is reduced by substitution at the ortho and meta positions of the aryl ring. In contrast to the loss of selective in vivo binding seen with substitution of **2**, N-methylation of the pyridone **15** results in retention of selectivity (compound **16**). Thus the effects of ring substitution are not strictly additive and consequently compound **16** is an outlier in eq 11 and 12 (Table IV). If the 4-pyridyl **13** and meta pyridone **17** analogues are excluded, overall substituent hydrophilicity (λ) in eq 12 can be replaced by Q-p, the specific partial charge on the aryl para substituent:

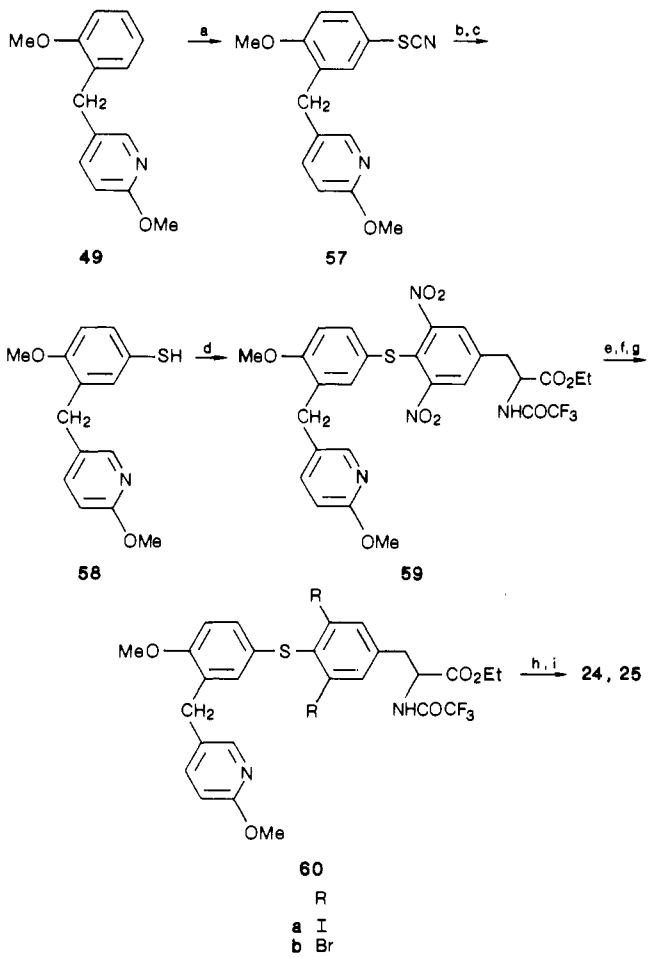
$$\log S = -3.33Q\text{-p} - 0.618D\text{-o,m} + 0.859 \quad (13)$$

$$n = 18,\ r = 0.882,\ s = 0.337,\ F = 26.2$$

$$t_{Q\text{-p}} = 5.04\ (p < 0.001);\ t_{D\text{-o,m}} = 4.31\ (p < 0.001)$$

Inclusion of the 4-pyridyl **13** and meta pyridone **17** analogues results in a significantly poorer equation [$r = 0.710$, $s = 0.511$, $F = 8.62$ ($p = 0.0026$)] with these compounds being outliers. The Q-p term for pyridine **13** refers to the partial negative charge on the nitrogen atom (–0.25, Table IV), and consequently eq 13 predicts that this compound will be selective. The relatively weak selectivity of **13** suggests that the partial negative charge on the aryl ring

Scheme V[a]



[a] Reagents: (a) $Pb(SCN)_2$–Cl_2–AcOH; (b) NaOH; (c) PPh_3–HCl; (d) *N*-trifluoroacetyl-3,5-dinitro-L-tyrosine ethyl ester–$MeSO_2Cl$–pyridine; (e) H_2–Pd/C; (f) $NaNO_2$–H_2SO_4–H_2O; (g) KI–I_2 or Cu–Br_2–HBr; (h) BBr_3; (i) HCl–HOAc–H_2O.

para *substituent* is more important for selectivity than negative charge on particular ring atoms. This contrasts with the effect of ring atomic charge on receptor affinity, which in eq 3 and 4 accounts for the higher binding of **13** relative to the 2-pyridyl isomer **12**. In the case of the meta pyridone **17**, *Q*-p is the charge on the hydrogen atom attached to nitrogen; since this is positive (0.23, Table IV), eq 13 predicts that compound **17** will not be selective. Thus, although the *Q*-p parameter explains the weak receptor affinity of **17** relative to its isomer **15** (eq 3 and **4**), it does not predict the observed retention of moderate selectivity with pyridone **17**. This indicates that the partial charge on the meta oxo substituent in **17** may be selectivity conferring. Equation 13, together with the analysis of outliers, reveals the possibility that a directional electrostatic interaction, requiring 3′-substituent negative potential and being sensitive to steric effects of ring substituents, may be involved in determination of selectivity in vivo. The analysis presented in Figure 3 implies that this interaction may occur principally in the liver.

Comparison of the 3′-substituent properties necessary for selectivity (eq 8, 11–13) and receptor binding (eq 3 and 4) shows that increasing substituent hydrophilicity has opposite effects, resulting in improved selectivity but reduced binding. Substitution at ortho and meta positions of the aryl ring is deleterious to both receptor affinity and selectivity. The contributions of polar para substituents on the 3′-aryl ring are critical in this series, since partial negative charge on this group (*Q*-p, Table IV) can be used to account for both the enhanced receptor binding (eq 3) and tissue selectivity (eq 13) of the majority of the analogues studied. However, the appearance of outliers in eq 13 suggests that the *Q*-p terms in eq 3 and 13 reflect different electrical interactions operative in receptor recognition and in vivo receptor access.

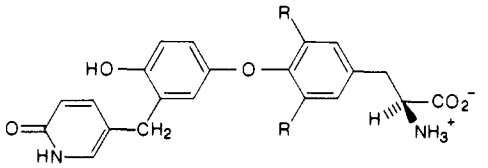
Optimization of Selective Activity

Compounds **1**–**20** serve to define the structural requirements for both selectivity and activity of the key selectivity inducing 3′-arylmethyl substituent. The most active selective compounds, for example the pyridone **15** and pyridazinone **19**, however, have only about 2% of the activity of T_3 in the liver and were found to possess very weak activity after oral administration (rel ED_{50} < 0.1% of T_3). In order to develop a potential hypocholesterolemic agent, increased potency and oral bioavailability were both required. Since the QSAR analysis presented above showed that hormonal activity and receptor affinity are positively correlated with 3′-substituent hydrophobicity and liver selectivity is negatively correlated with this property, improvement of both potency and oral bioavailability (while retaining selectivity) by modification of the 3′-substituent seemed unpromising. Consequently we synthesized a series of 3,5-substituent, ether link and alanyl side chain analogues retaining the selectivity conferring 3′-substituents found in **15** and **19** (Table III). The analogues were chosen on the basis of the known effects on receptor affinity or thyromimetic activity of the corresponding modifications in nonselective 3′-iodo and 3′-isopropyl thyromimetics.[16,20] These previous studies, however, provide no clear analogy for increasing either potency or oral bioavailability.

3,5-Substituents. In nonselective 3′-iodo and isopropyl T_3 analogues, only chloro, bromo, and methyl groups have been reported as effective replacements of the 3,5-iodo substituents. However, reduced activity is observed in all such analogues.[16,20] Unexpectedly, with the selective thyromimetics **15** and **19**, the corresponding 3,5-dibromo analogues **21** and **32** proved to be 4–8 times more potent as liver thyromimetics (rel ED_{50}, Table III). In addition, these dibromo compounds retain selective thyromimetic activity, and display selective in vivo liver receptor binding. As well as increased activity, the 3,5-dibromo compounds were found to possess enhanced oral bioavailability, being equipotent in increasing metabolic rate after either oral or intramuscular administration (Table III). The 3,5-dichloro compounds **22** and **33** also retain selective thyromimetic activity and are orally active. The 3,5-dichloro pyridazinone **33** has enhanced agonist activity relative to the diiodo analogue **19**, but in contrast the corresponding pyridones **22** and **15** are equiactive. The increased intrinsic activity and bioavailability of the 3,5-dibromo and -dichloro analogues could be a consequence of the inertness of these substances to deactivation by liver deiodinase enzymes.[21] The 3,5-dibromo and 3,5-dichloro pyridones **21** and **22** also have reduced octanol/water partition coefficients (log *P*) and increased aqueous solubility relative to the 3,5-diiodo compound **15** (Table VI). Although the differences in log *P* between these compounds are less than would be predicted from accepted π values,[22] the changes in physical properties may result in reduced

(20) Ellis, D.; Emmett, J. C.; Leeson, P. D.; Underwood, A. H. In *Handbook of Hormones, Vitamins and Radiopaques*; Verderame, M., Ed.; CRC: Boca Raton, FL, 1986; p 93.
(21) Koehrle, J.; Auf'mkolk, M.; Rokos, H.; Hesch, R.-D.; Cody, V. *J. Biol. Chem.* **1986**, *261*, 11613.
(22) Mitchell, R. C.; Leeson, P. D., unpublished results.

Table VI. Solubility and Partition Coefficients of 3,5-Dihalo-Substituted Selective Thyromimetics



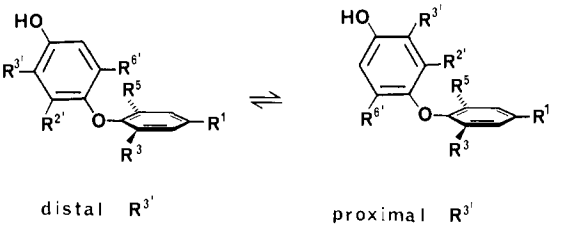
no.	R	aqueous solubility at 37 °C, pH 7.38 ($\times 10^5$ mol L^{-1})	log P at 25 °C, octanol/aqueous buffer, pH 6.02
(15)	I	1.0	1.01
(21)	Br	3.3	0.70
(22)	Cl	10	0.60

binding to plasma proteins[23] and consequently increased free concentrations of the noniodinated analogues being available for tissue uptake. The 3,5-dibromo-3′-pyridazinone analogue **32** (SK&F L-94901) was selected for further studies and was shown to be as active as T_3 as a liver thyromimetic and hypocholesterolemic after chronic oral administration.[10]

The 3,5-dimethyl compound **23** also shows good in vivo liver activity, being equipotent with the 3,5-dichloro analogue **22**, despite having reduced in vitro receptor binding. The nonselective 3′-isopropyl-3,5-dimethyl-L-thyronine (DIMIT[24]) shows a similar discrepancy between in vivo activity and in vitro affinity,[20] but the in vivo receptor affinity of this compound is consistent with the observed thyromimetic activity.[25] Liver selectivity and oral bioavailability are retained in **23**, showing that 3,5-dihalogen substitution is not necessary for selectivity in these thyromimetics.

Diphenyl Ether Oxygen. The ether oxygen atom in thyromimetics can be replaced by groups such as sulfur or methylene, which maintain the orthogonal arrangement of the aromatic rings in the preferred conformation of the hormone analogues.[16] The 3,5-diiodo thioether analogue **24** has 6-fold improved receptor affinity and liver thyromimetic activity (rel ED_{50}, Table III) compared with the ether **15** and retains selectivity. The corresponding nonselective 3′-isopropyl thioether is also more potent than its ether analogue.[20] The 3,5-dibromo thioether **25** is equipotent with the diiodo analogue, in contrast to the ether compounds, where 3,5-dibromo substitution enhances activity. The reduced oral activity of the 3,5-dibromo thioether **25** relative to its ether analogue **21** suggests that bioavailability of thioether analogues may be limited.

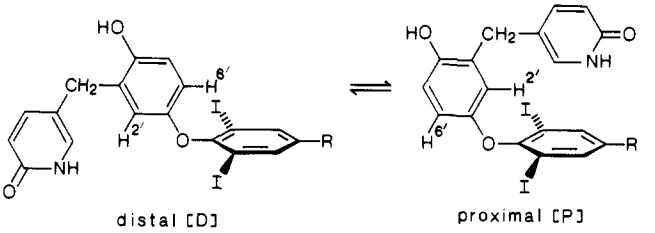
L-Alanyl Side Chain. Replacement of the L-alanyl side chain of **15** by D-alanyl to give **26** results in maintenance of selectivity and only a small loss of thyromimetic activity, in agreement with the well-established lack of marked enantioselectivity in thyroid hormone analogues.[16] Alkanoic acid analogues of T_3 and other nonselective thyromimetics generally show improved in vitro receptor binding[26] but reduced hormonal activity in vivo as a consequence of enhanced elimination.[27] The same trend is observed with the alkanoic acid derivatives (**27–29**) of the 3,5-dibromo-substituted selective thyromimetic **21** (Table III).



distal $R^{3'}$ proximal $R^{3'}$

Figure 4. Conformations of thyroid hormone structures which place the 3′-substituent *distal* or *proximal* to the side chain (R^1) bearing ring. These conformations have near equal energy when both $R^{2'}$ and $R^{6'}$ are hydrogen.[30]

Table VII. Conformational Study Using ^1H NMR Spectroscopy



distal [D] proximal [P]

no.	R	solvent	temp, °C	[D/P] ratio[a]
(15)	$CH_2CH(NH_2)CO_2H$	DMSO	25	2.0
		H_2O	25	0.5
(30)	CH_3	THF	25	1.5
			−20	1.8
			−80	2.6

[a] Determined from the relative chemical shifts of the 2′,6′-protons.[30,32]

Notably, the acetic acid **27** and butyric acid **29** retain selective thyromimetic activity, but in contrast the propionic acid **28** has full agonist activity in the heart. The in vivo receptor affinities (rel ID_{50}) of these carboxylic acid derivatives are, however, fully consistent with the observed thyromimetic activities. Thus, the propionic acid **27** has considerably greater in vivo affinity for heart receptors than either of the homologous acids and possesses negligible liver/heart selectivity for receptors in vivo. These data suggest that the 1-substituent in selective thyromimetics can influence selective tissue access or uptake to nuclear receptors. In particular, the amino group in the alanyl analogue **21** seems to be critical for selectivity, since its removal to give **28** results in reduced selective thyromimetic activity.

Conformational Study. 3,5-Halo[28] or -methyl[29] substituents cause the diphenyl ether to adopt conformations in which the aromatic rings are mutually perpendicular. This results in overall conformations where the 3′-substituent is either distal or proximal to the alanine-bearing ring (Figure 4). For 3′-alkyl and -halo analogues, distal and proximal conformers have near equal energy, both conformations being found in crystal structures[28] and in solutions.[30] Introduction of 2′-methyl substitution results in a single low-energy conformation, where the 2′-methyl group is distal.[30,31] Thus, in the preferred conformation of the 2′-methyl analogue **31** the arylmethyl group is proximal to the alanine-bearing ring. Since 2′-methyl substitution alone does not influence binding,[20] the weak receptor affinity of **31** (0.028% of T_3) relative to **15** (2.3% of T_3) must be due to the proximal oriented arylmethyl

(23) Andrea, T. A.; Cavieri, R. R.; Goldfine, I. D.; Jorgensen, E. C. *Biochemistry* **1980**, *19*, 55.
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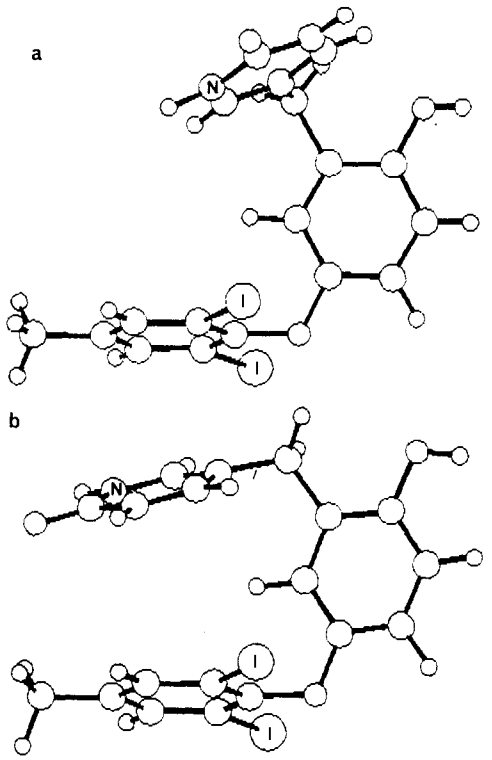

Figure 5. (a) Structure of compound (**30**) as determined by X-ray crystallography.[34] (Crystal data: $C_{19}H_{15}I_2NO_3$, MW = 559.1; triclinic; a = 9.1892, b = 9.6547, and c = 12.7186 Å; α = 101.01, β = 102.66, and γ = 112.61°; Z = 2; D_c = 1.915 g cm^{-3}). (b) The global minimum energy conformation of **30** determined by molecular mechanics procedures.[35]

group. This is consistent with other studies, using nonselective analogues, which have shown that a distally oriented 3′-substituent is required for receptor recognition.[20,31]

The solution conformations of the amino acid **15** and its desglycyl derivative **30** have been studied by ^{1}H NMR spectroscopy.[32] Distal:proximal conformer ratios were derived from the relative chemical shifts of the 2′- and 6′-protons, by the method previously described.[30] The results (Table VII) show that in each compound, both conformers are observed in aqueous and organic solvents. Similar results have been obtained for 3′-iodo[30] and 3′-alkyl[33] derivatives, showing that the nature of the 3′-substituent does not influence overall diphenyl ether conformation in solution. However, the structure of **30** as found in the crystal shows a proximal overall conformation (Figure 5a) in which C2 and N3 of the pyridone ring are located 4.43 and 4.57 Å, respectively, from I2 of the diiodo ring.[34] In addition, molecular modelling studies[35] with **30** identified the proximal conformation shown in Figure 5b as the global minimum, in which intramolecular van der Waals attraction or π–π interaction between the pyridone and diiodophenyl rings may occur. In selective thyromimetics this conformation also places the key electronegative para group on the 3′-aryl ring within hydrogen bonding distance of cisoid conformations[28] of the alanine side chain. These crystal and calculated conformations of **30** suggest the possibility of weak intramolecular interactions between the 3′-arylmethyl group and the 3,5-diiodo aromatic ring in proximal conformations of selective thyromimetics. The relationship between 3′-aryl partial charge and selectivity (eq 13) may reflect a requirement for the proximal conformation in tissue access, this conformation being increasingly populated by increased intramolecular attraction between the partial negative charge on the 3′-aryl ring and the electron deficient diiodo ring or ammonium group of the alanyl side chain. The solvent-dependent change in preferred conformation of **15** (Table VII), although small, may be significant in this respect, since the proximal conformation may be favored in a structured hydrophilic environment that a specific binding site (required for receptor access) may present. These tentative arguments are supported by the relative in vivo affinities of the proximally fixed compound **31**, which despite being weak, do indicate that this derivative has similar liver/heart selectivity (S = 40) to the conformationally mobile analogue **15** (S = 50).

Conclusions

These studies have shown that replacement of the 3′-iodo substituent in T_3 and its derivatives by specific arylmethyl groups provides compounds that possess selective, cardiac sparing activity in rats. QSAR studies of in vitro receptor affinities suggest that the 3′-arylmethyl group interacts with the same size-limited, hydrophobic pocket that recognizes the 3′-halo and -alkyl groups present in nonselective thyromimetics.[9] In addition, affinity and activity are enhanced by electronegative substituents at the para position of the 3′-arylmethyl ring. In contrast to other transmitter- and hormone-selective agonists and antagonists, the selectivity of these thyromimetics does not depend on selective receptor recognition, since the in vitro affinities for receptors for heart and liver are essentially the same. However selective activity is fully consistent with the observed differential liver/heart in vivo binding, indicating that selectivity depends on selective access or uptake to the nuclear receptors in vivo. Selective in vivo binding correlates positively with 3′-arylmethyl hydrophilicity and is reduced by ortho or meta substituents on the aryl ring. Analysis of the relationships between in vitro and in vivo receptor affinities show that the effect of these 3′-substituent properties is essentially confined to the liver, all compounds having diminished relative in vivo binding in the heart (Figure 3). The QSAR studies also suggest that a 6-membered 3′-arylmethyl substituent may not be uniquely required as a selectivity conferring group, since alternative 3′-substituents, which place an electronegative group in the same spatial region as the 3′-aryl para substituent, would be predicted to maintain receptor affinity and to induce selective binding in vivo (eq 13). For example, a thiazolone ring can replace the pyridone in **15** with retention of selectivity and in vitro receptor affinity.[11] Substitution of alternative high affinity, semirigid 3′-substituted analogues, for example 3′-cyclohexylmethyl T_2,[9] with polar groups in the 3′-substituent may provide alternative means of inducing selectivity.

Further modifications to the 1,3,5-substituents and ether link substituents were necessary to increase potency and gain oral bioavailability. Replacement of the 3,5-iodo groups by bromo proved to be crucial in meeting both objectives, with the pyridazinone analogue **32** (SK&F L-94901) being equipotent with T_3 as a liver thyromimetic and hypocholesterolemic, while possessing only one-thousandth of the activity of T_3 in the heart.[10] Replacement of the diphenyl ether oxygen with sulfur also increased potency, but oral activity was reduced. Studies with 1-substituted analogues showed that the α-amino group in L- and D-alanyl analogues is essential for selective

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(33) Pepper, E. S.; Emmett, J. C.; Leeson, P. D., unpublished results.
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in vivo binding and activity. However, introduction of acetic acid and butyric acid substituents at the 1-position resulted in retention of selectivity but reduced potency.

The observed in vitro structure–affinity relationships of the 1,3,5-substituents and ether link modified selective thyromimetics (Table III) show the same trends as found in nonselective 3′-iodo and isopropyl compounds.[16,20] This additivity suggests that in the analogues studied, the essential ligand–receptor interactions of the diphenyl ether moiety and 1- and 4′-substituents do not depend on the nature of the 3′-substituent. Additionally, the reduced receptor affinity of a conformationally fixed 2′-methyl selective thyromimetic **31** shows that a distally oriented 3′-substituent is necessary for receptor recognition, as found in nonselective thyromimetics. Unambiguous identification of the conformations required for selective tissue access in vivo awaits the synthesis of more potent conformationally restricted selective thyromimetics.

The mechanism of selectivity is not yet known. Differential tissue transport mechanisms may operate at several of the barriers between plasma and nuclear receptor, including the plasma membrane[36] and the nucleus.[37] Since the observed selectivity dependency on structure is confined to the liver, the possibility of conversion in this tissue to receptor-differentiating metabolites cannot be excluded.

Experimental Section

General directions and methods for preparation of 3′-substituted thyroid hormone analogues[9] and selective thyromimetics[11-13] have been reported.

2-Formyl-4-methoxy-5-[(2-methoxypyrid-5-yl)methyl]phenol (37). A slurry of phenol **36**[11] (2.0 g, 0.0082 mol) in ethylene glycol (15 mL) was added dropwise to a stirred solution of boric acid (2.83 g) and hexamine (2.02 g) in ethylene glycol at 130 °C. After 0.5 h the mixture was cooled to 80 °C, 30% sulfuric acid (10 mL) was added, and then the mixture was cooled to room temperature and poured into water (120 mL). Sodium acetate was added to pH 5, and the mixture was extracted with chloroform. The organic extracts were washed with water, dried, and evaporated, and the residue was purified by column chromatography on silica gel, eluting with 15% EtOAc/petroleum ether to give the phenol **37** (0.56 g, 25%): mp 128–29 °C; IR ν_{max} (Nujol) 3350–2500 (OH) and 1665 cm^{-1} (CHO); ^1H NMR ($CDCl_3$) δ 3.86 (3 H, s, OMe), 3.89 (2 H, s, CH_2), 3.92 (3 H, s, OMe), 6.68 (1 H, d, Py-5H), 6.73 (1 H, s, Ar-6H), 6.92 (1 H, s, Ar-3H), 7.41 (1 H, d of d, Py-4H), 8.08 (1 H, d, Py-2H), 9.82 (1 H, s, OH), and 10.74 (1 H, s, CHO). Anal. ($C_{15}H_{15}NO_4$) C, H, N.

2-(Acetoxymethyl)-4-methoxy-5-[(2-methoxypyrid-5-yl)methyl]-1-acetoxybenzene (38). Sodium borohydride (0.78 g) was added in portions to a stirred solution of aldehyde **37** (5.6 g, 0.0205 mol) in methanol (25 mL) and 1,4-dioxane (10 mL). After 0.5 h at room temperature, the solvents were evaporated, and the residue was partitioned between EtOAc and dilute aqueous acetic acid (pH 6). The organic layer was removed, dried, and evaporated to give 2-(hydroxymethyl)-4-methoxy-5-[(2-methoxypyrid-5-yl)-methyl]phenol (4.22 g, 75%), mp 145–46 °C. Anal ($C_{15}H_{17}NO_4$) C, H, N. A solution of this phenol (4.10 g, 0.015 mol) in pyridine (12 mL) and acetic anhydride (7.1 mL) was heated on a steam bath for 1 h and then evaporated to dryness. The residue was dissolved in chloroform, washed successively with dilute HCl and water, dried, and evaporated to give the diacetate **38** as a pale yellow gum. MS, m/z = 359 (M^+). Anal. ($C_{19}H_{21}NO_6$) H, N; C: Calcd 63.5, found 62.36.

4-Methoxy-5-[(2-methoxypyrid-5-yl)methyl]-2-methylphenol (39). To a stirred solution of diacetate **38** (3.32 g, 0.0092 mol) in 1,2-dimethoxyethane (20 mL) at 45–50 °C was added sodium borohydride (1.75 g).[17] After 17 h, the mixture was cooled and quenched with saturated NH_4Cl and then extracted with chloroform. The organic layer was washed with saturated NH_4Cl and then with brine, dried, and evaporated. The residue was purified by chromatography on silica gel, eluting with 10% EtOAc/petroleum ether, to give the phenol **39** (1.20 g, 50%): mp 164–67 °C; ^1H NMR δ (DMSO-d_6) 2.11 (3 H, s, ArMe), 3.70 (3 H, s, OMe), 3.72 (2 H, s, CH_2), 3.83 (3 H, s, OMe), 6.49 (1 H, s, Ar-3H), 6.71 (1 H, s, Ar-6H), 6.73 (1 H, d, Py-5H), 7.44 (1 H, d of d, Py-4H), 7.98 (1 H, d, Py-2H) and 8.48 (1 H, s, OH); MS, m/z 259 (M^+). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

3-(3-Chloro-4-methoxybenzyl)-4-methoxyphenol (40e). (a) To a cooled (10 °C) stirred solution of the Grignard reagent prepared from 4-bromo-2-chloroanisole (74.26 g, 0.335 mol) and magnesium turnings (8.27 g, 0.34 g-atom) in dry THF (170 mL) was added, dropwise, a solution of the aldehyde **34**[15] (20.39 g, 0.134 mol) in dry THF (100 mL). Additional THF (150 mL) was added to facilitate stirring, and the mixture was refluxed for 2 h and then cooled to room temperature and quenched with excess saturated NH_4Cl solution. The mixture was extracted with ethyl acetate, the organic extracts were dried and evaporated, and the residue was recrystallized from dichloromethane/petroleum ether to give the intermediate carbinol (34.85 g, 88%), mp 134 °C. Anal. ($C_{15}H_{15}ClO_4$) C, H, Cl. (b) This carbinol (32.19 g) was acetylated by heating at 50 °C with pyridine (100 mL) and acetic anhydride (100 mL). To a stirred solution of the diacetate (39.5 g, 0.104 mol) and triethylsilane (12.73 g, 0.110 mol) in dichloromethane (100 mL) was added, dropwise, a solution of trifluoroacetic acid (12.49 g, 0.110 mol) in dichloromethane (50 mL). The mixture was warmed to 35 °C for 4 h and then evaporated, and the residue was triturated with petroleum ether to give (5-acetoxy-2-methoxyphenyl)(3-chloro-4-methoxyphenyl)methane (30.69 g, 92%). (c) A mixture of this acetate (30.59 g), methanol (200 mL), and 2 N aqueous sodium hydroxide (150 mL) was heated on a steam bath until a homogeneous solution was obtained. The cooled solution was acidified with concentrated HCl, the mixture was extracted with dichloromethane, the extracts were dried and evaporated, and the residue was crystallized from chloroform/petroleum ether to give the phenol **40e** (26.27 g, 99%; overall yield from **34**, 77%): mp 74–5 °C; ^1H NMR ($CDCl_3$) δ 3.76 (3 H, s, OMe), 3.82 (2 H, s, CH_2), 3.86 (3 H, s, OMe), 4.70 (1 H, s, OH), and 6.5–7.3 (6 H, m, Ar H). Anal. ($C_{15}H_{15}ClO_3$) C, H, Cl.

(2-Methoxyphenyl)(2-methoxypyrid-5-yl)methanol (48). *n*-Butyllithium (137 mL of a 1.6 M solution in hexane, 0.219 mol) in dry THF (50 mL) was added dropwise, under N_2, to a stirred solution of 5-bromo-2-methoxypyridine[38] (41.36 g, 0.220 mol) in dry THF (50 mL) at −85 °C. After 5 min, a solution of 2-methoxybenzaldehyde (25.0 g, 0.184 mol) in THF (150 mL) was added, dropwise, with the temperature being maintained below −70 °C. The mixture was warmed to room temperature, quenched with excess saturated NH_4Cl solution, and then extracted with EtOAc. The organic solution was dried and evaporated, and the residue was recrystallized from dichloromethane/petroleum ether to give the carbinol **48** (28.39 g, 63%), mp 83–4 °C. Anal. ($C_{14}H_{15}NO_3$) C, H, N.

2-[(2-Methoxypyrid-5-yl)methyl]anisole (49). The carbinol **48** was acetylated as described above, and the product (22.0 g) was hydrogenated in methanol (180 mL) over 10% palladium on charcoal (2.0 g) with using a Parr apparatus. After filtration and evaporation to dryness, the residue was purified by filtration through a short silica gel column by elution with EtOAc/petroleum ether to give anisole **49** as a colorless oil (15.89 g, 91%): ^1H NMR ($CDCl_3$) δ 3.78 (3 H, s, OMe), 3.82 (2 H, s, CH_2), 3.87 (3 H, s, OMe), 6.61 (1 H, d, Py-5H), 7.1 (5 H, m, Ar H), and 8.02 (1 H, d, Py-2H). Anal. ($C_{14}H_{15}NO_2$) C, H, N.

4,4′-Dimethoxy-3,3′-bis[(2-methoxypyrid-5-yl)methyl]diphenyliodonium Perchlorate (50). Anisole **49** (72.9 g, 0.318 mol) was slowly dissolved in cold mixture of trifluoroacetic acid (100 mL) and trifluoroacetic anhydride (100 mL), and the resulting solution was added dropwise to a stirred solution of iodine tris(trifluoroacetate)[39] (74.1 g, 0.309 mol) in trifluoroacetic anhydride

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(120 mL) at −12 to −8 °C. The mixture was kept at room temperature overnight and then evaporated to dryness, keeping the temperature below 25 °C. The residue was dissolved in dichloromethane (500 mL) and added to a stirred solution of sodium perchlorate (100 g) and sodium acetate (200 g) in water (800 mL). The crystalline product that separated was collected (24.0 g) and recrystallized from ether–THF to give the iodonium salt **50**: mp 168–69 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (3 H, s, OMe), 3.83 (3 H, s, OMe), 3.8 (2 H, s, CH_2), and 6.69–7.75 (6 H, m, Ar H); ^{13}C NMR (DMSO-d_6) δ 105.5 (s, C − I$^+$).[9] Anal. ($[C_{28}H_{28}IN_2O_4]ClO_4$) C, H, N, Cl, I. A second crop of product (55 g, total yield 73%) was obtained after addition of the dichloromethane to ether (2 L). Both crops were suitable for further transformations (Scheme III).

3,5-Dibromo-3′-[(2-methoxypyrid-5-yl)methyl]-*O*-methyl-*N*-(trifluoroacetyl)-L-thyronine Methyl Ester (51b). To a stirred solution of the iodonium perchlorate **50** (91.3 g, 0.134 mol), 3,5-dibromo-*N*-(trifluoroacetyl)-L-tyrosine methyl ester[13] (72.0 g, 0.160 mol), and triethylamine (25 mL) in dry dichloromethane (2 L) was added copper bronze (10 g). The mixture was stirred at room temperature for 19 h, filtered, and washed with aqueous acetic acid, 0.2 N NaOH, and then brine. The dried solution was evaporated, and the residue was chromatographed on silica gel, eluting with dichloromethane, to give initially the *iodoanisole* **53** (37 g, 78%) [mp 68–70 °C. Anal. ($C_{14}H_{14}INO_2$) C, H, N, I] and then the coupled product, which was recrystallized from dichloromethane/petroleum ether to give thyronine **51b** (39.5 g, 44%): mp 125–25 °C; IR ν_{max} 3300, 3270 (NH), 1744 (COO), and 1709 cm^{-1} (CON); ^{1}H NMR ($CDCl_3$) δ 3.16 (2 H, CH_2CH), 3.73–3.90 (11 H, 4 s, CH_2 and OMe), 4.84 (1 H, m, CH_2C*H*), 6.49–6.74 (4 H, m, Ar H), 7.03 (1 H, m, NH), 7.31 (2 H, s, Ar H), 7.51 (1 H, d of d, Py-4H), and 8.00 (1 H, m, Py-2H). Anal. ($C_{26}H_{23}Br_2F_3N_2O_6$) C, H, N, Br.

3,5-Dibromo-3′-[(2-oxo-1,2-dihydropyrid-5-yl)methyl]-L-thyronine (21). To a stirred cooled (−55 °C) solution of thyronine **51b** (25.77 g) in dichloromethane (225 mL) was added, dropwise, a solution of boron tribromide (23 mL) in dichloromethane (50 mL). The mixture was allowed to warm to room temperature and after 2 h was poured into an ice-cold solution of sodium acetate (100 g) in water (400 mL). The mixture was extracted with ethyl acetate, the organic extracts were evaporated, and the residue was refluxed for 16.5 h in glacial acetic acid (1000 mL) containing concentrated hydrochloric acid (500 mL). The solvents were removed in vacuo, and the residue was recrystallized from aqueous ethanolic sodium hydroxide on addition of acetic acid to pH 5 to give compound **21** (19.0 g, 93%) (see Table I): IR ν_{max} (Nujol) 3700–2100 (OH, NH), 1682, 1660 (CON), and 1610 cm^{-1} (COO$^-$); ^{1}H NMR (1 N NaOD) δ 2.80 (2 H, m, CH_2CH), 3.45 (1 H, m, CH_2C*H*), 3.59 (2 H, s, CH_2), 6.30–6.55 (4 H, m, Ar H), 7.22 (1 H, d of d, Py-4H), 7.48 (2 H, s, Ar H), and 7.59 (1 H, d, Py-2H).

4-Formyl-4′-methoxy-3′-[(2-methoxypyrid-5-yl)methyl]-2,6-dimethyl-1,1-diphenyl Ether (54). To a stirred solution of iodoanisole **53** (7.20 g, 0.200 mol) and phenol **52** (3.35 g, 0.022 mol) in dry pyridine (25 mL) containing potassium carbonate (1.56 g) was added cupric oxide (2.0 g), and the mixture was heated at 150 °C for 6 h. The mixture was cooled, combined with a second reaction (having used 4.0 g of **53**), poured into water, and extracted with chloroform. The organic extracts were washed with water, 2 N HCl, and 2 N NaOH, dried, and evaporated. Purification of the residue by column chromatography on silica gel, eluting with EtOAc/petroleum ether gave diphenyl ether **54** (2.32 g, 19%), mp 104–5 °C. Anal. ($C_{23}H_{23}NO_4$) C, H, N. Alternatively, phenol **52** (0.61 g), iodonium salt **50** (2.80 g), potassium *tert*-butoxide (0.45 g), dicyclohexyl-18-crown-6 (10 mg), and copper bronze (50 mg) were stirred in dry dichloromethane (10 mL) for 4 h. Workup as described for **51b** gave crude product (2.25 g), which after purification proved to be identical to **54**.

Azlactone 55. Aldehyde **54** (10.84 g, 0.0288 mol), *N*-acetylglycine (5.38 g, 0.046 mol), sodium acetate (3.77 g, 0.057 mol), and acetic anhydride (70 mL) were heated with stirring at 100 °C for 24 h. The solution was cooled and evaporated to dryness, and the residue was succesively triturated with water and then methanol to give azlactone **55** (8.27 g, 64%), mp 164–65 °C. Anal. ($C_{27}H_{26}N_2O_5$) C, H, N.

***N*-Acetyl-3,5-dimethyl-*O*-methyl-3′-[(2-methoxypyrid-5-yl)methyl]-DL-thyronine (56).** (a) A solution of azlactone **55** (8.20 g) in ethanol (50 mL) and 2 N NaOH (50 mL) was heated with stirring at 65 °C for 0.5 h. The solvents were removed, and the residue was recrystallized from aqueous acetic acid to give the intermediate acetamidopropenoic acid (6.84 g, 80%) [mp 200–202 °C. Anal. ($C_{27}H_{28}N_2O_6$·0.4CH_3CO_2H) C, H, N]. (b) This acid (5.92 g) was hydrogenated over 10% palladium on charcoal (0.5 g) in glacial acetic acid (80 mL) in a Parr apparatus at 3 atm/45 °C for 8 h. The mixture was filtered and evaporated, and the residue was chromatographed on silica gel to give recovered starting material (3.23 g) and the thyronine **56** (0.94 g): mp 186–88 °C; IR ν_{max} (Nujol) 3285 (NH), 2800–1800 (OH), 1718 (COOH), and 1655 cm^{-1}; (CON); ^{1}H NMR (DMSO-d_6) δ 1.81 (3 H, s, COMe), 1.99 (6 H, s, ArMe), 2.90 (2 H, m, CH_2CH), 3.72 (3 H, s, OMe), 3.78 (2 H, s, CH_2), 3.81 (3 H, s, OMe), 4.40 (1 H, m, CH_2C*H*), 6.41 (1 H, d of d, Ar-6′H), 6.64 (1 H, d, Ar-2′H), 6.70 (1 H, d, Py-5H), 6.85 (1 H, d, Ar-5′H), 6.98 (2 H, s, Ar H), 7.46 (1 H, d of d, Py-4H), 7.98 (1 H, d, Py-2H), and 8.00 (1 H, d, NHCO); MS, *m/z* 478 (M$^+$). Anal. ($C_{27}H_{30}N_2O_6$) C, H, N.

3,5-Dimethyl-3′-[(2-oxo-1,2-dihydropyrid-5-yl)methyl]-DL-thyronine (23). A solution of the acid **56** (0.88 g) in 48% aqueous hydrobromic acid (16 mL) and glacial acetic acid (8 mL) was refluxed for 5 h and then evaporated to dryness. The residue was combined with an additional batch and recrystallized from aqueous ethanolic sodium hydroxide to give the thyronine **23** (1.11 g) (see Table I): IR ν_{max} (Nujol) 3680–2120 (OH, NH, NH_3^+), 1685 and 1665 (CON), 1620 and 1590 cm^{-1} (COO$^-$, Ar); ^{1}H NMR (1 N NaOD) δ 2.03 (6 H, s, $ArCH_3$), 2.70 and 2.95 (2 H, m, CH_2CH), 3.62 (2 H, s, CH_2), 6.28 (1 H, d, Ar-2′H), 6.35 (1 H, d, Py-5H), 6.48 (1 H, d of d, Ar-6′H), 6.55, (1 H, d of d, Py-4H) and 7.62 (1 H, d, Py-2H).

4-Methoxy-3-[(2-methoxypyrid-5-yl)methyl]phenyl Thiocyanate (57). Acetic acid containing 5% acetic anhydride (250 mL) was refluxed for 4.5 h, and then to the cooled solution was added dry chlorine (16.0 g, 0.225 mol) and then lead(II) thiocyanate (36.48 g, 0.113 mol) in small portions with rapid stirring. After 40 min a solution of anisole **49** (45.85 g, 0.200 mol) in dry acetic acid (175 mL) was slowly added. The mixture was stirred at room temperature for 20 h, filtered, added to water (2 L), and extracted with chloroform. The combined extracts were washed with water and 2 N NaOH, dried, and evaporated to give an orange gum, which crystallized from chloroform–petroleum ether to give the thiocyanate **57** (39.89 g, 55%), mp 56–58 °C. Anal. ($C_{15}H_{14}N_2O_2S$) C, H, N, S.

4-Methoxy-3-[(2-methoxypyrid-5-yl)methyl]thiophenol (58). To a stirred suspension of the thiocyanate **57** (31.71 g, 0.111 mol) in 1,4-dioxane (120 mL) was added a solution of sodium hydroxide (17.76 g, 0.444 mol) in water (120 mL), and the mixture was refluxed under N_2 for 5 h. The cooled mixture was acidified to pH 4 with concentrated HCl and then diluted with chloroform (300 mL) and water (300 mL). The organic layer was removed, washed with water, dried, and evaporated to give a mixture, which was separated by chromatography on silica gel, eluting with EtOAc–petroleum ether to give **58** (2.55 g) and its disulfide (23.04 g). The disulfide (19.44 g, 0.037 mol) was dissolved in 1,4-dioxane (100 mL) and water (25 mL) and treated with triphenylphosphine (9.79 g) and concentrated HCl (4 drops). The mixture was heated to 45 °C for 0.5 h, the solvents were removed, and the residue was dissolved in EtOAc, washed with water, dried, and evaporated to dryness. Chromatography on silica gel, eluting with 5% EtOAc–petroleum ether, gave pure thiophenol **58** (15.51 g, 80%) as a colorless oil: IR ν_{max} (film) 2550 cm^{-1} (SH); ^{1}H NMR ($CDCl_3$) δ 3.33 (1 H, s, SH), 3.78 (3 H, s, OMe), 3.80 (2 H, s, CH_2), 3.90 (3 H, s, OMe), 6.73 (1 H, d, Ar-5H), 7.04 (1 h, m, Ar-2H), 7.15 (1 H, m, Ar-6H), 7.38 (1 H, d of d, Py-4H), and 8.00 (1 H, m, Py-2H); MS, *m/z* 261 (M$^+$). Anal. ($C_{14}H_{15}NO_2S$) C, H, N, S.

Dinitrodiphenyl Thioether (59). To a rapidly stirred solution of 3,5-dinitro-*N*-trifluoroacetyl-L-tyrosine ethyl ester (14.33 g, 0.0363 mol) in dry pyridine (40 mL) was added methanesulfonyl chloride (2.8 mL), and the solution was refluxed for 10 min. Thiophenol **58** (8.67 g, 0.0332 mol) in dry pyridine (40 mL) was added, and the mixture was refluxed for 20 min and then evaporated to dryness. The residue was dissolved in chloroform, and the solution was washed successively with water, 2 N HCl, saturated $NaHCO_3$, 2 N NaOH, and water, dried, charcoaled, and evaporated. Purification by chromatography on silica gel, eluting with EtOAc–petroleum ether, followed by crystallization from EtOH–water, gave the thioether **59** (14.35 g, 68%), mp 115–19

°C. Anal. ($C_{27}H_{25}F_3N_4O_9S$) C, H, N, S.

Dibromodiphenyl Thioether (60b). The dinitro compound **59** (4.00 g, 0.0063 mol) was hydrogenated in glacial acetic acid (20 mL) over 10% palladium on charcoal (0.40 g) with a Parr apparatus. The mixture was filtered, and the solution was added to a well-stirred solution of sodium nitrite (1.31 g, 0.019 mol) in concentrated sulfuric acid (50 mL) and acetic acid, under N_2, with the temperature being kept at or below −15 °C. After 5 min, the mixture was added to a vigorously stirred mixture of cuprous bromide (14.35 g) and urea (1.5 g) in 47% aqueous hydrobromic acid (70 mL) and chloroform (70 mL). After 2 h, the organic layer was removed and washed successively with water, saturated $NaHCO_3$, 2 N NaOH, water, and then brine. The dried solution was evaporated, and the residue was purified by exhaustive chromatography on silica gel, eluting with EtOAc–petroleum ether, to give the thioether **60b** (0.75 g, 17%): mp 111–113 °C; ^1H NMR ($CDCl_3$) δ 1.30 (3 H, t, CH_2CH_3), 3.13 and 3.21 (2 H, m, CH_2CH), 3.81 (3 H, s, OMe), 3.82 (2 H, s, CH_2), 3.94 (3 H, s, OMe), 4.30 (2 H, q, CH_2CH_3), 4.80 (1 H, m, CH_2CH), 6.66 (1 H, d, Ar-5′H), 6.77 (1 H, d, Py-5H), 6.96 (1 H, d, Ar-2′H), 7.10 (1 H, d of d, Ar-6′H), 7.19 (1 H, d, NHCO), 7.38 (1 H, d of d, Py-4H), 7.42 (2 H, s, Ar H), and 7.98 (1 H, s, Py-2H). Anal. ($C_{27}H_{25}Br_2F_3N_2O_5S$) C, H, N, Br, S.

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Registry No. 1, 72469-00-2; **2**, 105170-31-8; **3**, 117653-10-8; 4, 117653-11-9; **5**, 117653-12-0; **6**, 117653-13-1; **7**, 117653-14-2; 8, 117653-15-3; **9**, 117653-16-4; **10**, 117653-17-5; 11, 117653-18-6; **12**, 117653-19-7; **13**, 117653-20-0; 14, 105170-26-1; **15**, 105189-53-5; **16**, 117653-21-1; **17**, 117653-22-2; 18, 117653-23-3; **19**, 105170-16-9; **20**, 117653-24-4; **21**, 105189-37-5; **22**, 105189-56-8; **23**, 105189-72-8; **24**, 105189-60-4; **25**, 105189-67-1; **26**, 105189-91-1; **27**, 105189-93-3; **28**, 105189-96-6; **29**, 105189-99-9; **30**, 117653-25-5; **31**, 117653-26-6; **32**, 105211-23-2; **33**, 105170-24-9; **34**, 35431-26-6; **36**, 105189-48-8; **37**, 117653-28-8; **37** (R′ = CH_2OH), 117653-27-7; **38**, 117653-29-9; **39**, 117653-30-2; **40e**, 117653-31-3; **40e** (acetate), 117653-33-5; **48**, 105189-38-6; **48** (acetate), 105189-39-7; **49**, 105189-40-0; **50**, 105189-42-2; **51b**, 105189-46-6; **52**, 2233-18-3; **53**, 105189-45-5; **54**, 105211-19-6; **55**, 105211-20-9; **56**, 105189-74-0; **56** (α,β-unsaturated), 105189-73-1; **57**, 105189-61-5; **58**, 105189-62-6; **58** (disulfide), 105189-63-7; **59**, 105189-64-8; **60b**, 105189-68-2; 2-$MeOC_6H_4CHO$, 135-02-4; $I(OCOCF_3)_3$, 14353-86-7; CF_3CO-(3,5-Br_2)Tyr-OMe, 105189-44-4; Ac-Gly-OH, 543-24-8; $Pb(SCN)_2$, 592-87-0; CF_3CO-[3,5-$(NO_2)_2$]Tyr-OEt, 105189-50-2; 4-bromo-2-chloroanisole, 50638-47-6; 5-bromo-2-methoxypyridine, 13472-85-0; (5-hydroxy-2-methoxyphenyl)(3-chloro-4-methoxyphenyl)methanol, 117653-32-4; (5-acetoxy-2-methoxyphenyl)(3-chloro-4-methoxyphenyl)methyl acetate, 117653-34-6.

Bifunctional Antitumor Compounds: Interaction of Adriamycin with Metallocene Dichlorides

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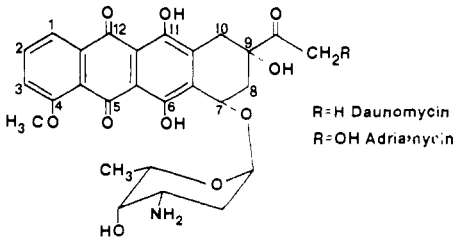
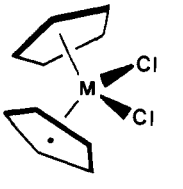
In order to synthesize bifunctional antitumor compounds, the interactions of adriamycin with metallocene dichlorides, Cp_2MCl_2, where M = Zr, Ti, V, have been studied. Using absorption, fluorescence, and circular dichroism measurements, we have shown that adriamycin is able to coordinate to the three metal ions. The interaction of Cp_2ZrCl_2 and Cp_2VCl_2 with adriamycin leads to compounds of 1:2 metal:drug stoichiometry, whereas the interaction of Cp_2TiCl_2 with adriamycin leads to two types of compounds of 1:2 and 1:1 stoichiometry. The Zr–adriamycin complex, which is unable to dissociate, even at a pH lower than 1, does not display antitumor activity against P-388 leukemia. However Ti–adriamycin complexes, which are more susceptible to dissociation in acidic media, exhibit antitumor activity that compares with that of the free drug. These complexes, unlike adriamycin, do not catalyze the flow of electrons from NADH to molecular oxygen through NADH dehydrogenase. In addition, the presence of metal ions promote the binding of the drug to DNA and erythrocyte ghosts.

The anthracycline antibiotic adriamycin (Adr) is an important antitumor agent with marked activity against a wide variety of human neoplasms. Unfortunately, an-



thracyclines exhibit secondary toxic effects, the most serious being cardiotoxicity. A great deal of research effort has been directed toward finding new anthracyclines that might retain the excellent broad-spectrum activity of adriamycin while eliminating its cardiotoxicity.[1-3]

On the other hand, over the past 7 years, Köpf and Köpf-Maier[4] have shown that metallocene dihalides of the constitution Cp_2MX_2, where Cp = η^5-C_5H_5; M = Ti, V, Nb,



Metallocene dichlorides

Mo; X = F, Cl, Br, I, NCS, and N_3, are highly active against Ehrlich ascites tumor cells, lymphoid leukemia L1210, and lymphocytic leukemia P-388. These compounds thus

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