Synthesis of Halogenated Trimetoquinol Derivatives and Evaluation of Their β-Agonist and Thromboxane A_2 (TXA_2) Antagonist Activities

K. M. Markovich, V. Tantishaiyakul, A. Hamada, D. D. Miller,* K. J. Romstedt, G. Shams, Y. Shin, P. F. Fraundorfer, K. Doyle, and D. R. Feller

Divisions of Medicinal Chemistry and Pharmacognosy and Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210. *Received July 5, 1991*

The 5,8-difluoro (4), 5-iodo (5), 8-iodo (6), and 5-trifluoromethyl (7) derivatives of trimetoquinol (TMQ, 1) have been synthesized and evaluated for their ability to stimulate β_1 (guinea pig atria) and β_2 (guinea pig trachea) adrenoceptors as well as for their inhibitory activity against U46619 [a thromboxane A_2 (TXA_2) mimetic]-mediated contraction of rat thoracic aorta and human platelet aggregation. Both 5 and 6 were considerably less active than TMQ on both β-adrenergic systems and gave a rank order of stimulatory potency of $1 \gg 6 \geq 5$. Similarly, iodine substitution at either position also caused a reduction in TXA_2 antagonist activity with a rank order potency of $1 > 6 \gg 5$. Compared to 1, however, 5-iodo-TMQ (5) showed a marked selectivity for blockade of U46619 responses in rat aorta over human platelets. On β-systems, 4 had reduced potency compared to TMQ and was similarly nonselective. Introduction of a trifluoromethyl group at the 5-position of TMQ completely abolished both β_1- and β_2-adrenergic agonist activities while imparting weak antagonist activity on β_1 receptors. On TXA_2 systems, both 4 and 7 possessed significantly decreased inhibitory activity compared to TMQ. The synthetic approaches to the synthesis of 8-(trifluoromethyl)-TMQ (8) are also described. The enantiomers of the 8-fluoro derivative (3) of TMQ were separated on a preparative Chiralcel OD column and evaluated on β-adrenergic systems and TXA_2 systems. On β-adrenergic systems, (S)-(+)-8-fluoro-TMQ was at least 10-fold more potent than (R)-(-)-8-fluoro-TMQ. Conversely, (R)-(-)-8-fluoro-TMQ was approximately 14-fold more potent as an antagonist of TXA_2-mediated aggregation in human platelets than (S)-(+)-8-fluoro-TMQ. In contrast to platelets, (S)-(+)-8-fluoro-TMQ was an agonist in rat aorta whereas (R)-(-)-8-fluoro-TMQ was an antagonist.

Introduction

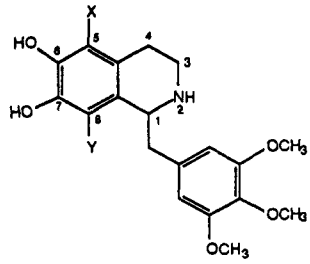
Trimetoquinol (TMQ, 1) is a nonspecific β-adrenergic agonist,[1,2] as well as a competitive inhibitor of human platelet aggregation and rat thoracic aortic contraction induced by the TXA_2 mimetic, U46619.[3] In β-adrenergic systems, (S)-(-)-TMQ is more potent than (R)-(+)-TMQ.[4] Currently, the S-(-) isomer of TMQ is marketed in Japan for its bronchodilating effects. The antiaggregatory activity of TMQ was first reported by Shtacher and co-workers[5] when TMQ was found to inhibit platelet aggregation induced by collagen, ADP, and epinephrine. This activity is unrelated to α- or β-adrenergic mechanisms and is independent of prostaglandin biosynthesis or cAMP.[5] Subsequently, our laboratory has shown that TMQ is an antagonist of PGH_2/TXA_2-mediated responses in rat aorta and human platelets.[3] The stereoselectivity for TMQ antagonism of putative PGH_2/TXA_2 receptors is opposite to that required for β-adrenergic activity.[6,7] The R-(+) isomer of TMQ was found to be 40- and 22-fold more potent than (S)-(-)-TMQ as an inhibitor of U46619-induced platelet aggregation and serotonin secretion, respectively.[8] Although there was early controversy as to whether TMQ was a PGH_2/TXA_2 antagonist,[9,10] work in our laboratory[8,11] has proved that (R)-(+)-TMQ acts as a selective and highly stereospecific PGH_2/TXA_2 receptor antagonist in platelets.

Recently, our laboratory reported the synthesis and biological evaluation of the 5-fluoro (2) and the 8-fluoro (3) derivatives of TMQ.[12] In producing tracheal relaxation (β_2), TMQ and the fluoro analogues are essentially equal in activity.[12] However, on guinea pig atria (β_1) the order of chronotropic activity is TMQ > 5-FTMQ > 8-FTMQ.[12] The changes in β_1 activity were correlated to differences in phenolic pK_a attributed to the electronic influence of fluorine.[12] As the pK_a decreases from 8.77 for TMQ to 7.86 for 8-FTMQ, the β_1 activity decreases.[12] This converts to an enhanced β_2/β_1 selectivity as follows: 8-FTMQ > 5-FTMQ > TMQ.[12]

The PGH_2/TXA_2 receptors have been subclassified as the platelet α receptor (aggregation) and the vascular τ receptor (contractile responses of tone).[13] With respect

(1) Yamato, E.; Hirakura, M.; Sugasawa, S. Synthesis of 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline Derivatives. *Tetrahedron Suppl.* **1966**, *8*(Part 1), 129-134.
(2) Iwasawa, Y.; Kiyomoto, A. Studies on Tetrahydroisoquinoline (THI). I. Bronchodilator Activity and Structure Activity Relationship. *Jpn. J. Pharmacol.* **1967**, *17*, 143-152.
(3) Mukhopadhyay, A.; Navran, S. S.; Amin, H. M.; Abdel-Aziz, S. A.; Chang, J.; Sober, D. J.; Miller, D. D.; Feller, D. R. Effect of Trimetoquinol Analogs for Antagonism of Endoperoxide/Thromboxane A_2-Mediated Responses in Human Platelets and Rat Aorta. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 1-9.
(4) Feller, D. R.; Venkatramen, R.; Miller, D. D. Comparative Actions of the Trimetoquinol, Tetrahydropapaverine and Salsinol Isomers in Beta-Adrenoceptors. *Biochem. Pharmacol.* **1975**, *24*, 1357-1359.
(5) Shtacher, G.; Crowley, H. J.; Dalton, C. Inhibition of Platelet Aggregation by Papaverine-Like Drugs: Evidence for a Novel Mechanism of Action. *Biochem. Pharmacol.* **1976**, *25*, 1045-1050.
(6) Dalton, C.; Crowley, H. J.; Czyzewski, L. B. Trimetoquinol-Different Pharmacological Properties of Optical Isomers. *Biochem. Pharmacol.* **1976**, *25*, 2209-2210.
(7) Mayo, J. R.; Navran, S. S.; Akbar, H.; Miller, D. D.; Feller, D. R. Stereo-Dependent Inhibition of Human Platelet Function by the Optical Isomers of Trimetoquinol. *Biochem. Pharmacol.* **1981**, *30*, 2237-2241.
(8) Ahn, C.; Romstedt, K. J.; Wallace, L. J.; Miller, D. D.; Feller, D. R. Characterization of the Inhibition of U46619-Mediated Human Platelet Activation by the Trimetoquinol Isomers. *Biochem. Pharmacol.* **1988**, *37*, 3023-3033.
(9) Armstrong, R. A.; Jones, R. L.; Wilson, N. H. Ligand Binding to Thromboxane Receptors on Human Platelets: Correlation with Biological Activity. *Br. J. Pharmacol.* **1983**, *79*, 953-964.
(10) MacIntyre, D. E.; Pollock, W. K. Does Trimetoquinol Inhibit Thromboxane A_2-Induced Human Platelet Activation by Blocking a Specific Transduction Mechanism? *Br. J. Pharmacol.* **1983**, *78*, 600P.
(11) Ahn, C.; Wallace, L. J.; Miller, D. D.; Feller, D. R. Use of [^{3}H]Trimetoquinol as a Radioligand in Human Platelets: Interaction with Putative Endoperoxide/Thromboxane A_2 Receptor Sites. *Thromb. Res.* **1988**, *50*, 387-399.
(12) Clark, M. T.; Adejare, A.; Shams, G.; Feller, D. R.; Miller, D. D. 5-Fluoro- and 8-Fluorotrimetoquinol: Selective β_2-Adrenergic Agonists. *J. Med. Chem.* **1987**, *30*, 86-90.
(13) Mais, D. E.; Saussey, Jr., D. L.; Chaikhouni, A.; Kochel, P. J.; Knapp, D. R.; Hamanaka, N.; Halushka, P. V. Pharmacological Characterization of Human and Canine Thromboxane A_2/Prostaglandin H_2 Receptors in Platelets and Blood Vessels: Evidence for Different Receptors. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 418-424.

0022-2623/92/1835-0466$03.00/0



1 X = Y = H
2 X = F, Y = H
3 X = H, Y = F
4 X = Y = F
5 X = I, Y = H
6 X = H, Y = I
7 X = CF_3, Y = H
8 X = H, Y = CF_3

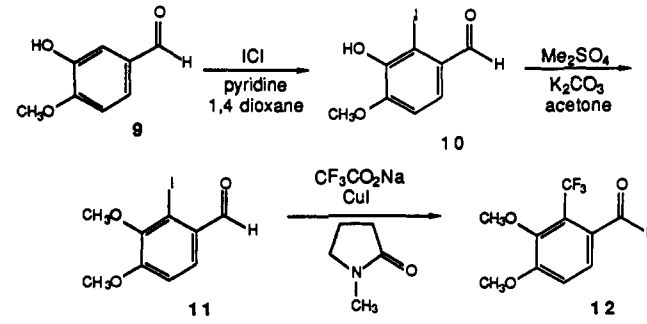
to PGH_2/TXA_2 receptor activity, the fluoro analogues are also selective relative to TMQ for inhibition of U46619 actions on rat thoracic aorta (τ receptors) versus human platelets (α receptors).[14] Although 8-FTMQ is less active than TMQ on human platelets, it is of interest to note that 8-FTMQ is more active than TMQ on aorta.[14] This corresponds to a τ/α selectivity ratio of 35 for 8-FTMQ when compared to TMQ.[14]

The first objective of this research was to design and synthesize selective β_2 agonists, which retain the bronchodilating actions but lack the β_1-attributed side effects. The second goal was to study the structural requirements for optimum activity at α and τ PGH_2/TXA_2 receptors with TMQ analogues, one of the few classes of nonprostanoid TXA_2 antagonists. To this end, we prepared and will describe the biological activities of 5,8-difluoro-TMQ (4), 5-iodo-TMQ (5), 8-iodo-TMQ (6) and 5-(trifluoromethyl)-TMQ (7). Also, the synthetic approaches to 8-(trifluoromethyl)-TMQ (8) will be discussed. Changing the halogen substituent at the 5- and 8-positions of TMQ from fluorine to iodine provides a similar electronic effect (σ_m and σ_p values for iodine are 0.35 and 0.18, respectively, versus the σ_m and σ_p values for fluorine which are 0.34 and 0.06, respectively),[15] while greatly enhancing the lipophilic effect (the π value for iodine is 1.12 versus the π value for fluorine which is 0.14).[16] Because introduction of an electron-withdrawing fluorine atom at the 5- and 8-position of TMQ provided very desirable profiles on both β-systems and TXA_2 systems, we chose to introduce the highly electron withdrawing trifluoromethyl group. For the trifluoromethyl group, σ_m equals 0.43 and σ_p equals 0.54,[15] which corresponds to significantly greater electron withdrawing ability of the trifluoromethyl group compared to fluorine. The trifluoromethyl group also contributes a greater lipophilic effect ($\pi_{CF_3} = 0.88$).[16] Since 8-FTMQ retained potent activity as well as showed a marked separation of activities on β-adrenergic systems and TXA_2 systems, the enantiomers of 8-FTMQ were separated and the biological activities of (*R*)-(−)-8-FTMQ and (*S*)-(+)-8-FTMQ will be described.

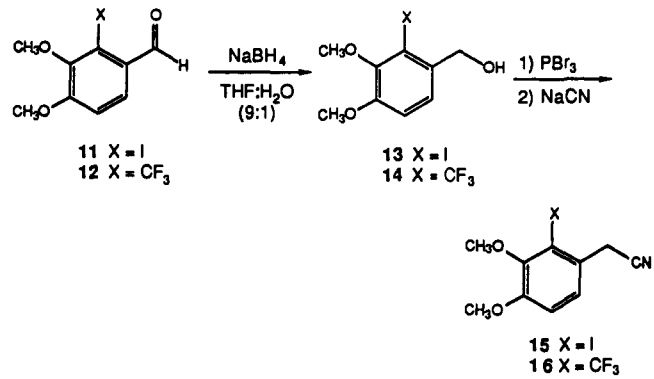
Chemistry

The synthesis of compounds 5, 6, and 7 is outlined in Scheme III. The starting materials required for compounds 5 and 7 are 2-iodo-3,4-dimethoxybenzaldehyde (11) and 2-(trifluoromethyl)-3,4-dimethoxybenzaldehyde (12), respectively, and were synthesized as outlined in Scheme I. 2-Iodovanillin, one of the possible starting materials for 11, was previously synthesized in a 6-step reaction by Freudenberg et al.[17] An alternate starting material is 2-iodopiperonal, which was synthesized from piperonal in a three-step reaction with an overall yield of only 25%.[18] We have found a more efficient method for the preparation of 2-iodoisovanillin (10) via the reaction of isovanillin (9) with iodine monochloride which proceeds in 73% yield. 2-Iodoisovanillin (10) was methylated with dimethyl sulfate to afford dimethoxybenzaldehyde 11. The synthesis of trifluoromethylated benazaldehyde 12 was achieved using iodobenzaldehyde 11 by applying the method of Matsui and co-workers[19] in which aromatic halides are trifluoromethylated. Thus, heating a mixture of 11, 2 equiv of cuprous iodide and 4 equiv of sodium trifluoroacetate in *N*-methyl-2-pyrrolidinone (5% w/v) to 175 °C for 4 h gave 12 in 40% yield. It is essential that this reaction be carried out under strict anhydrous conditions as the inclusion of moisture favors the formation of the reduced dehalogenated derivative of 11.[18] The trifluoromethylated product was confirmed first by the ^1H NMR spectrum of 12 in which the aldehyde and aromatic peaks were shifted downfield compared to the starting material 11. Furthermore, the aldehydic proton of the starting material 11 appeared as a sharp singlet at 10.0 ppm, whereas in the ^1H NMR spectrum of 12, the aldehydic peak appeared as a broad quartet at 10.2 ppm as a result of splitting by the three fluorine atoms. Also, a single peak at −52.5 ppm was

Scheme I



Scheme II



15 X = I
16 X = CF_3

(14) Miller, D. D.; Clark, M. T.; Adejare, A.; Neidert, K.; Hamada, A.; Shams, G.; Romstedt, K. J.; Feller, D. R. *Fluorotetrahydroisoquinolines* as Adrenergic and Antithrombotic Agents. In *Progress in Catecholamine Research, Part A: Basic Aspects and Peripheral Mechanisms*; Dahlstrom, A., Belmaker, R. H., Sandler, M., Eds.; Alan R. Liss, Inc.: New York, 1988; pp 403–407.

(15) Bowden, K. Electronic Effects in Drugs. In *Comprehensive Medicinal Chemistry, Vol. 4*; Hansch, C., Sammes, P. G., Taylor, J. B., Ramsden, C. A., Eds.; Pergamon Press: New York, 1990; pp 234–235.

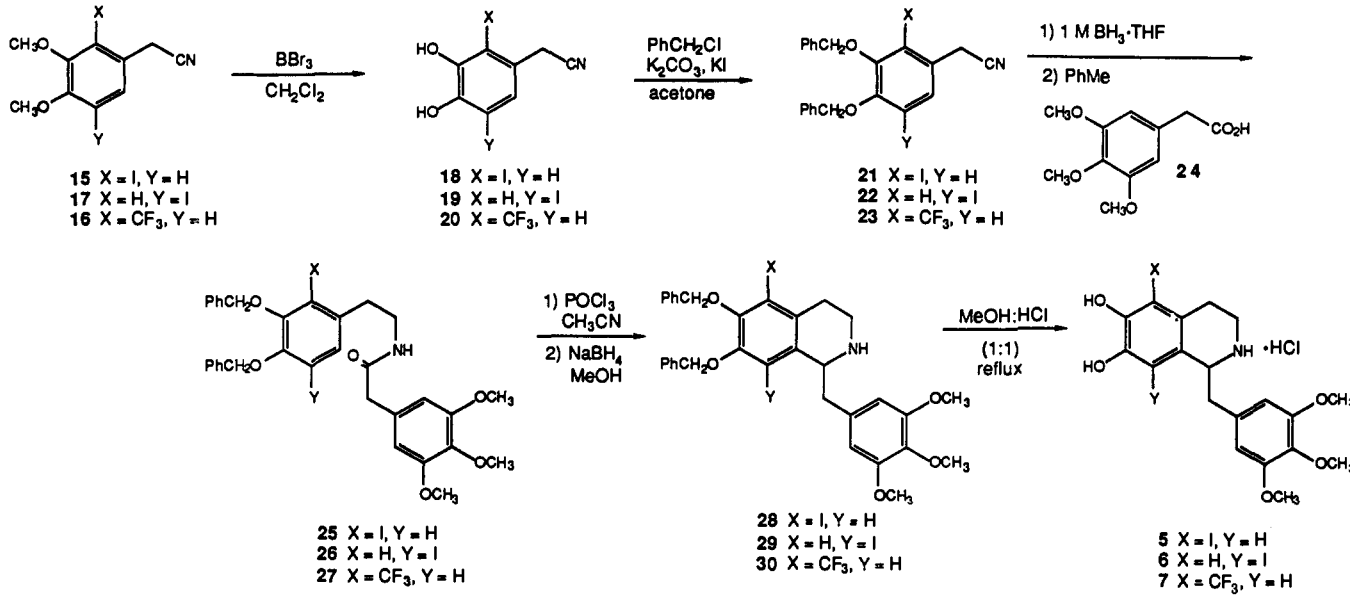
(16) Leo, A. J. Methods of Calculating Partition Coefficients. In *Comprehensive Medicinal Chemistry, Vol. 4*; Hansch, C., Sammes, P. G., Taylor, J. B., Ramsden, C. A., Eds.; Pergamon Press: New York, 1990; p 298.

(17) Freudenberg, K.; Jovanovic, V. Deuterated Coniferyl Alcohol. *Chem. Ber.* **1963**, *96*, 2178–2181.

(18) Ziegler, F. E.; Fowler, K. W. Substitution Reactions of Specifically Ortho-Metallated Piperonal Cyclohexylimine. *J. Org. Chem.* **1976**, *41*, 1564–1566.

(19) Matsui, K.; Tobita, E.; Ando, M.; Kondo, K. A Convenient Trifluoromethylation of Aromatic Halides with Sodium Trifluoroacetate. *Chem. Lett.* **1981**, 1719–1720.

Scheme III



15 X = I, Y = H
17 X = H, Y = I
16 X = CF_3, Y = H

18 X = I, Y = H
19 X = H, Y = I
20 X = CF_3, Y = H

21 X = I, Y = H
22 X = H, Y = I
23 X = CF_3, Y = H

24

25 X = I, Y = H
26 X = H, Y = I
27 X = CF_3, Y = H

28 X = I, Y = H
29 X = H, Y = I
30 X = CF_3, Y = H

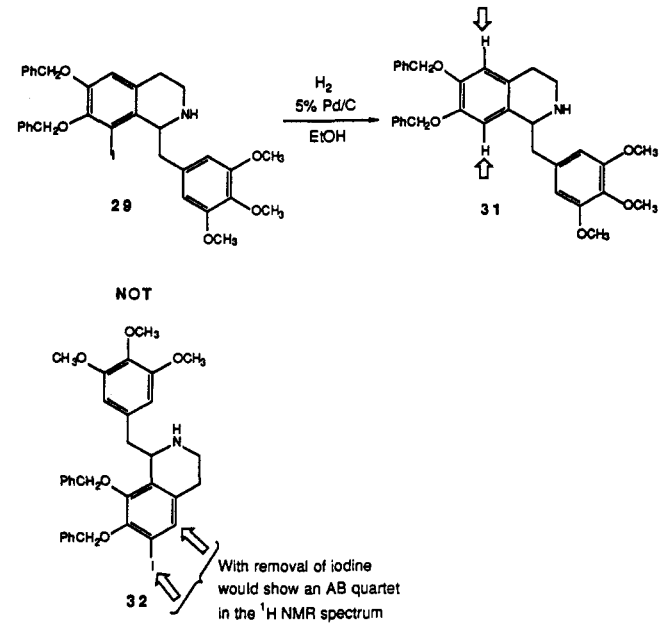
5 X = I, Y = H
6 X = H, Y = I
7 X = CF_3, Y = H

observed in the ^{19}F NMR spectrum of **12** which is indicative of an aromatic trifluoromethyl group.[20]

Aldehydes **11** and **12** were reduced with sodium borohydride to afford the benzyl alcohols **13** and **14**, respectively (Scheme II). Alcohols **13** and **14** were converted to their respective benzyl bromides via phosphorus tribromide in dichloromethane, and subsequent displacement with sodium cyanide in dimethylformamide gave phenylacetonitriles **15** and **16**, respectively. The starting material for compound **6** was 3-iodo-4,5-dimethoxyphenylacetonitrile **22**, previously synthesized in our laboratory.[21]

Beginning with the respective phenylacetonitrile, the remaining synthesis of 5-iodo-TMQ (**5**), 8-iodo-TMQ (**6**), and 5-(trifluoromethyl)-TMQ (**7**) is outlined in Scheme III. Demethylation using boron tribromide in dichloromethane liberated catechols **18**, **19**, and **20**, which were reprotected by reaction with benzyl chloride to produce the bis(benzyloxy)-protected benzyl nitriles **21**, **22**, and **23**, respectively. The cyano moieties of **21**, **22**, and **23** were reduced to the amines using diborane in tetrahydrofuran (THF). Without purification, the resultant phenethylamines were condensed with trimethoxyphenylacetic acid **24** by heating to reflux in toluene with azeotropic removal of water via a Dean–Stark trap to afford the corresponding phenylacetamides **25**, **26**, and **27**. Applying Bischler–Napieralski conditions of phosphorus oxychloride in acetonitrile to the phenylacetamides resulted in the dihydroisoquinolines which were immediately reduced with sodium borohydride to afford the bis(benzyloxy)-protected tetrahydroisoquinolines **28**, **29**, and **30**. Deprotection of the catechol moiety with a refluxing equivolume mixture of concentrated hydrochloric acid and methanol gave the desired 5-iodo-TMQ (**5**), 8-iodo-TMQ (**6**), and 5-(trifluoromethyl)-TMQ (**7**). Structure elucidation of **29** was achieved by catalytic hydrogenation (5% palladium on carbon in ethanol) of **29** resulting in removal of the iodo moiety to afford **31** (Scheme IV). The structure of **31** was

Scheme IV

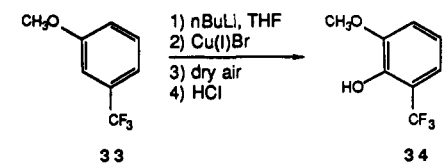


29

31

32

Scheme V



33

34

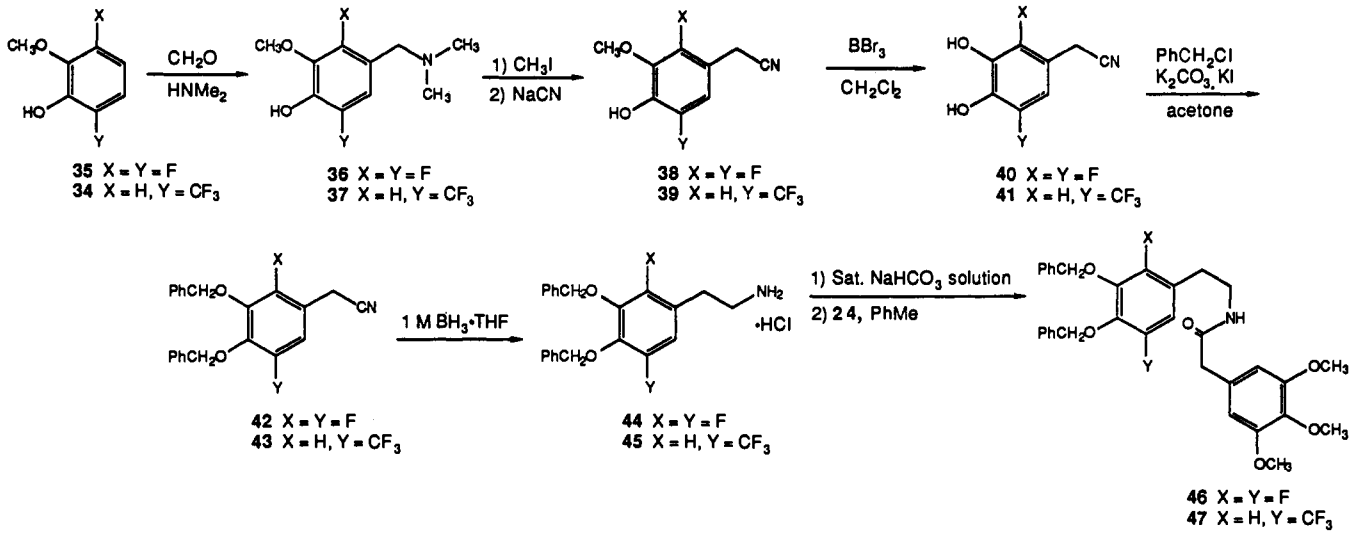
assigned by comparison of the ^1H NMR spectrum of **31** with that of authentic compound previously prepared in our laboratory.[22] The ^1H NMR spectrum of the deiodinated product showed two singlets for the aromatic protons of the tetrahydroisoquinoline ring at 6.89 and 6.62 ppm, confirming that **26** underwent cyclization to **29** and not to **32**, which upon removal of iodine would be expected to

(20) Bovey, F. A. Less Common Nuclei. In *Nuclear Magnetic Resonance Spectroscopy*, 2nd ed.; Bovey, F. A., Ed.; Academic Press: San Diego, CA, 1988; pp 437–489.

(21) Harrold, M. W.; Gerhardt, M. A.; Romstedt, K.; Feller, D. R.; Miller, D. D. Synthesis and Platelet Antiaggregatory Activity of Trimetoquinol Analogs as Endoperoxide/Thromboxane A_2 Antagonists. *Drug Design Delivery* **1987**, *1*, 193–207.

(22) Miller, D. D.; Osei-Gyimah, P.; Bardin, J.; Feller, D. R. Synthesis and Biological Evaluation of Fragmented Derivatives of Tetrahydroisoquinoline. 2. Trimetoquinol Studies. *J. Med. Chem.* **1975**, *18*, 454–457.

Scheme VI

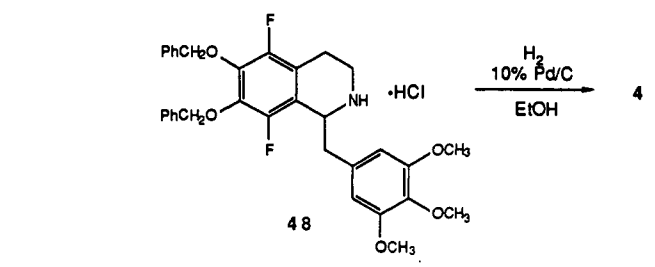


show an AB quartet for the two ortho protons.

Initial retrosynthetic analysis of 8-(trifluoromethyl)-TMQ (8) led to 2-hydroxy-3-(trifluoromethyl)anisole (34). Bäckstrom and co-workers[23] reported the synthesis of phenol 34 in 18% yield by treatment of commercially available 3-(trifluoromethyl)anisole (33) with a typical aromatic hydroxylation method *n*-butyllithium and *N,N,N′,N′*-tetramethyleneethylenediamine (TMEDA), followed by trimethyl borate, then hydrogen peroxide and basic then acidic workup. We report a more efficient synthesis of trifluoromethylated phenol 34 achieved by applying an alternate aromatic hydroxylation method described by Lambert and co-workers.[24] 3-(Trifluoromethyl)anisole (33) was lithiated using *n*-butyllithium in THF, followed by transmetalation by addition of 1 equiv cuprous bromide (Scheme V). Dry air was then bubbled into the reaction mixture, and the reaction was quenched with HCl. Purification via column chromatography afforded the desired phenol (34) in 59% yield.

The synthesis of the Bischler–Napieralski precursor phenylacetamides 46 and 47 for the synthesis of difluoro-TMQ (4) and 8-(trifluoromethyl)-TMQ (8) is outlined in Scheme VI. The starting material for difluoro-TMQ (4) was 3,6-difluoro-2-hydroxyanisole (35) prepared by the method of Ladd and Weinstock.[25] Aminomethylation of phenols 35 and 34 was achieved using formaldehyde and *N,N*-dimethylamine to yield benzylamines 36 and 37, respectively. The corresponding benzyl nitriles, 38 and 39, were prepared via formation of the quaternary ammonium salts with iodomethane followed by displacement with sodium cyanide. Deprotection of 38 and 39 with boron tribromide in dichloromethane gave catechols 40 and 41, which were protected with benzyl chloride to afford the corresponding bis(benzyloxy) nitriles 42 and 43. Diborane reduction of benzyl nitriles 42 and 43 gave phenethylamines 44 and 45, which were isolated as their hydrochloride salts. The free base of phenethylamines 44 and 45 were condensed with trimethoxyphenylacetic acid 24 to give phenylacetamides 46 and 47.

Scheme VII



Applying Bischler–Napieralski conditions to phenylacetamide 46 using phosphorus oxychloride in acetonitrile did not afford the expected dihydroisoquinoline but resulted in an unusual 3-aminoisoquinoline side product which we have reported elsewhere.[26] Changing the solvent to benzene eliminated the side product and gave the desired dihydroisoquinoline which was reduced with sodium borohydride to yield the protected tetrahydroisoquinoline 48, isolated as the hydrochloride salt (Scheme VII). Deprotection of the catechol via catalytic hydrogenation with palladium on carbon completed the synthesis of the desired compound 4.

Applying Bischler–Napieralski conditions of phosphorus oxychloride in acetonitrile to phenylacetamide 47 did not give the desired dihydroisoquinoline 49, but again gave an unusual 3-aminoisoquinoline side product (50)[26] (Scheme VIII). Performing the Bischler–Napieralski reaction on 47 with phosphorus oxychloride in toluene or benzene completely eliminated the presence of the aminoisoquinoline side product; however, the desired compound 49 could not be isolated from the complex mixture.

An alternate approach to 49 involved the trifluoromethylation of the 8-iodinated protected tetrahydroiso-

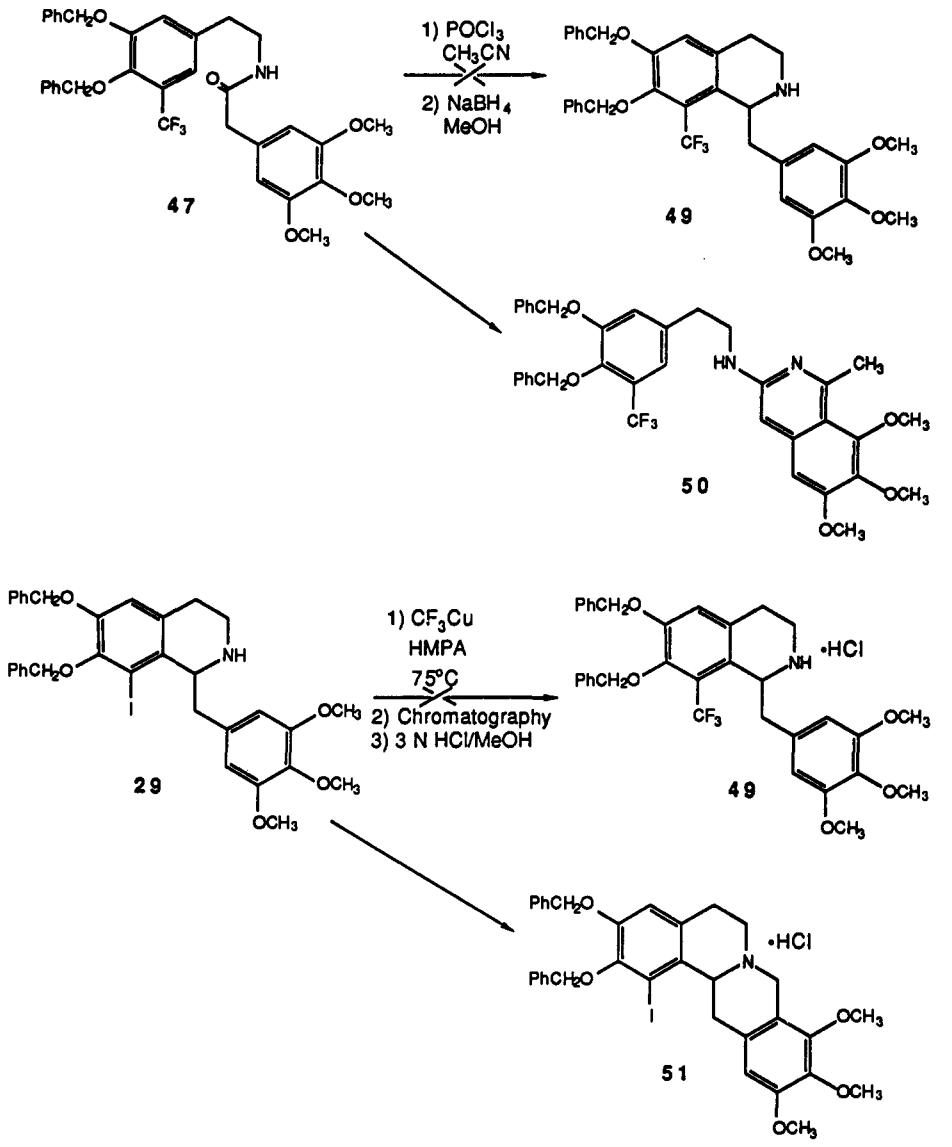
(23) Bäckstrom, R.; Honkanen, E.; Pippuri, A.; Kairisalo, P.; Pystenen, J.; Heinola, K.; Nissinen, E. A. O.; Linden, I. B.; Mannisto, P. T.; et al. Synthesis of Some Novel Potent and Selective Catechol-O-Methyltransferase Inhibitors. *J. Med. Chem.* **1989**, *32*, 841–846.

(24) Lambert, G. J.; Duffley, R. P.; Dalzell, H. C.; Razden, R. K. Regioselective Aromatic Hydroxylation. An Oxidative Reaction of Arylcopper(I) and Lithium Diarylcopper(I) Ate Complexes. *J. Org. Chem.* **1982**, *47*, 3350–3353.

(25) Ladd, D. L.; Weinstock, J. Improved Synthesis of Fluoroveratroles and Fluorophenethylamines via Organolithium Reagents. *J. Org. Chem.* **1981**, *46*, 203–206.

(26) Markovich, K. M.; Hamada, A.; Miller, D. D. Unexpected Aminoisoquinoline Formed Under Bischler-Napieralski Conditions Provides for a New Synthesis of 3-Aminoisoquinolines. *J. Heterocyclic Chem.* **1990**, *27*, 1665–1667.

Scheme VIII



quinoline **29** (Scheme VIII). The trifluoromethylation method of Matsui and co-workers[19] on an N-protected iodinated precursor gave only the reduced product; therefore, the method of Kobayashi and co-workers[27] was attempted. In this method, the CF_3Cu complex is isolated before using, eliminating any excess copper which may mediate the reduction. Thus, the CF_3Cu complex was prepared heating a mixture of trifluoromethyl iodide and copper powder in hexamethylphosphoramide (HMPA) in a steel tube to 120 °C for 2.5 h and cooled. In an Atmosbag (Aldrich) purged with argon, the solution containing the CF_3Cu complex was filtered through the Celite to remove the excess copper. The filtrate and **29** were heated to 75 °C under argon (Scheme VIII). After 1 week, the absence of starting material was detected by TLC. Upon workup, the isolated compound was not the desired trifluoromethylated compound **49**. Elemental analysis and mass spectral analysis suggested the product was the iodinated protoberberine hydrochloride **51** isolated in 14% yield. The presence of the berberine system was also indicated by the ^1H NMR spectrum of **51** which showed the typical AB doublets at 4.64 and 4.45 ppm indicative of the C-8 protons and geminal coupling. The protoberberine structure was further confirmed by comparison to the protoberberine isolated from the reaction of the hydrochloride salt of the tetrahydroisoquinoline **29** with formaldehyde, ethanol and water, a typical method for protoberberine formation.[28] The ^1H NMR and mass spectra of the protoberberine free base isolated from this method were identical to the free base of the protoberberine structure obtained using CF_3Cu. At this time, the mechanism of this process is unknown. We speculate that the formaldehyde equivalent in this process is derived from the trifluoromethyl group. To date, the synthesis of 8-(trifluoromethyl)-TMQ (**8**) has not been achieved.

The approach to the separation of the enantiomers of 8-fluoro-TMQ (**3**) began with the free base of the dibenzyloxy protected 8-fluoro derivative **52** which was previously synthesized in our laboratory.[12] The free base of **52** was resolved using a preparative Chiralcel OD (Diacel) column as shown in Scheme IX. The Chiralcel

(27) Kobayashi, Y.; Yamamoto, K.; Asai, T.; Nakano, M.; Kumadaki, I. Studies on Organic Fluorine Compounds. Part 35. Trifluoromethylation of Pyrimidine- and Purine-Nucleosides with Trifluoromethyl-Copper Complexes. *J. Perkin Trans. 1* 1980, 2755–2761 and references within.

(28) Osei-Gyimah, P.; Piasek, M. T.; Fowble, J. W.; Feller, D. R.; Miller, D. D. Synthesis and β-Adrenoceptor Activity of the Erythro and Threo Isomers of Substituted α-Hydroxytrimetoquinol. *J. Med. Chem.* 1978, *21*, 1173–1178.

Scheme IX

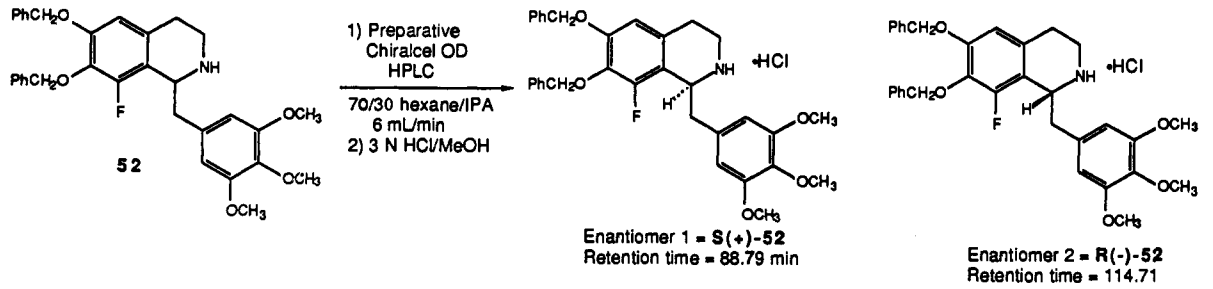


Table I. Comparative Activities of TMQ (1) and Iodinated Analogues on β-Systems (Guinea Pig Trachea and Atria)

	trachea (β_2)[a]			atria (β_1)[a]			selectivity
treatment	pEC_{50}[b]	IA[c]	potency ratio[d]	pEC_{50}[b]	IA[c]	potency ratio[d]	ratio[e] β_2/β_1
TMQ (1)	7.58 ± 0.05	0.99 ± 0.01	1.00	6.76 ± 0.15	0.97 ± 0.02	1.00	1.00
5-ITMQ (5)	5.65 ± 0.16	0.58 ± 0.04	0.0093	>4.5	0.39 ± 0.60	>0.0055	1.68
8-ITMQ (6)	5.93 ± 0.10	0.89 ± 0.02	0.02	5.53 ± 0.17	0.81 ± 0.09	0.055	0.36

[a] Values are the mean ± SEM of n = 3–6. [b] pEC_{50} = −log EC_{50} (M). [c] IA = intrinsic activity = maximum response of drug relative to the response of 10^{-5} M isoproterenol. [d] Potency ratio = EC_{50} (trimetoquinol)/EC_{50} (drug). [e] Selectivity ratio = potency ratio (β_2)/potency ratio (β_1) for each drug.

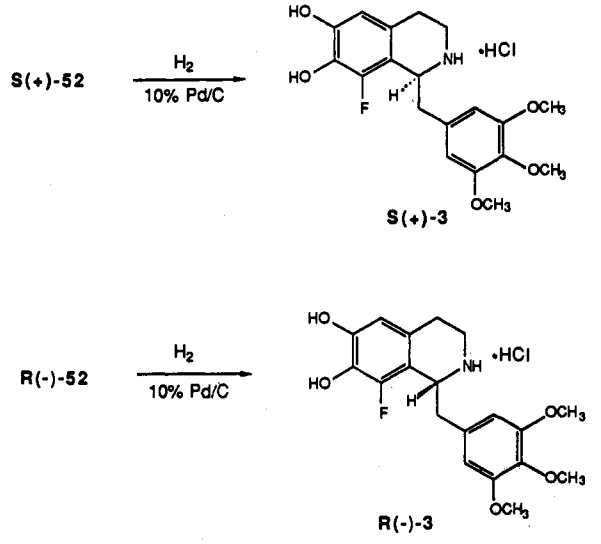
Table II. Comparative Inhibitory Activities of TMQ (1) and Iodinated Analogues against U46619-Induced Responses in Human Platelets and Rat Thoracic Aorta[a]

	platelets (aggregation)		aorta (contraction)		selectivity ratio
drug	pIC_{50}[b]	potency ratio	pK_B[c]	potency ratio	aorta/platelets (τ/α)
TMQ (1)	6.55 ± 0.03	1.00	5.46 ± 0.17	1.00	1.00
5-ITMQ (5)	3.92 ± 0.35	0.002	4.31 ± 0.19[f]	0.07	35.00
8-ITMQ (6)	5.51 ± 0.35[d,e]	0.09	4.84 ± 0.21	0.24	2.67

[a] Values are the mean ± SEM of n = 3–4. [b] pIC_{50} = −log IC_{50} (M). [c] pK_B = −log [[A]/(CR − 1)] where [A] = molar concentration of antagonist and CR = concentration ratio = EC_{50} (plus antagonist)/EC_{50} (control). [d] $p < 0.001$ compared to TMQ (1) in unpaired t test. [e] $p < 0.001$ compared to 5 in unpaired t test. [f] $p < 0.01$ compared to TMQ (1) in unpaired t test.

OD column was chosen because similar tetrahydroisoquinolines, such as laudanosine, have been resolved on this column.[29] Using a mobile phase of 70/30 hexane/2-propanol, the first enantiomer of **52** eluted at 88.79 min and the second enantiomer eluted at 114.71 min. The optical purity of each enantiomer was >99% as determined on an analytical Chiralcel OD HPLC column. The bis(benzyloxy) enantiomers were crystallized as their hydrochloride salts. The bis(benzyloxy)-protected *S* and *R* enantiomers of **52** were deprotected via catalytic hydrogenation with palladium on carbon to afford (*S*)-(+)-**3** and (*R*)-(−)-**3**, respectively (Scheme X). The configuration was determined from the CD spectra of (*S*)-(+)-**3** and (*R*)-(−)-**3** by analogy to the CD spectrum of authentic (*R*)-(+)-TMQ.

Scheme X



Biological Results and Discussion

The concentration-dependent effects of TMQ (1) and iodo analogues **5** and **6** were evaluated in guinea pig right atria and tracheal strips as representative β_1- and β_2-adrenergic receptor systems, respectively (Table I). Both **5** and **6** were less active than TMQ as agonists in both atria and trachea. Whereas TMQ gave a maximal response nearly equal to that of 10^{-5} M isoproterenol in these tissues, both **5** and **6** were only partial agonists on these systems. In particular, analogue **5** gave a maximal effect equal to only 39% and 58% of isoproterenol stimulation in guinea pig atria and trachea, respectively. Analogues **5** and **6** were active in a similar concentration range in both guinea pig atria and trachea which converts to β_2/β_1 selectivity ratios similar to that of TMQ (Table I).

The iodo analogues and TMQ were also evaluated for their comparative inhibitory activity against U46619-induced human platelet aggregation (TXA_2 α receptors) and against U46619-mediated contraction of rat thoracic aorta (TXA_2 τ receptors) as shown in Table II. Both analogues were inhibitors of U46619-induced aggregation with pIC_{50} values of 3.92 and 5.51 for **5** and **6** respectively as compared to TMQ (1) which had a pIC_{50} value of 6.55 (Table II). Each iodinated compound blocked U46619-induced contractions of rat thoracic aorta in a competitive manner with pK_B values of 5.46, 4.31, and 4.84 for TMQ, **5**, and **6**, respectively (Table II). Accordingly, iodine substitution at either the 5- or 8-positions of TMQ caused a reduction

(29) Okamoto, Y.; Kawashima, M.; Hatada, K. XI. Controlled Chiral Recognition of Cellulose Triphenylcarbamate Derivatives Supported on Silica Gel. *J. Chromatogr.* **1986**, *363*, 173–186.

Table III. Comparative Activities of Racemic TMQ (1) and 5,8-Difluoro-TMQ (4) on β-Systems (Guinea Pig Trachea and Atria)

	trachea (β_2)[a]			atria (β_1)[a]			selectivity
treatment	pEC_{50}[b]	IA[c]	potency ratio[d]	pEC_{50}[b]	IA[c]	potency ratio[d]	ratio[e] β_2/β_1
TMQ (1)	7.11 ± 0.09	0.95 ± 0.01	1.00	6.76 ± 0.15	0.97 ± 0.02	1.00	1.00
5,8-DiFTMQ (4)	6.62 ± 0.12	0.94 ± 0.02	0.32	6.30 ± 0.37	0.78 ± 0.06	0.35	0.91

[a] Values are the mean ± SEM of n = 4–9. [b] pEC_{50} = −log EC_{50} (M). [c] IA = intrinsic activity = maximum response of drug relative to the response of 10^{-5} M isoproterenol. [d] Potency ratio = EC_{50} (trimetoquinol)/EC_{50} (drug). [e] Selectivity ratio = potency ratio (β_2)/potency ratio (β_1) for each drug.

Table IV. Comparative Activities of TMQ (1) and 5-CF_3TMQ (7) on β-Systems (Guinea Pig Trachea and Atria)

	trachea (β_2)[a]			atria (β_1)[a]		
treatment	pEC_{50}[b]	IA[c]	pK_B[d]	pEC_{50}[b]	IA[c]	pK_B[d]
TMQ (1) alone	7.09 ± 0.11	0.94 ± 0.01	–	7.91 ± 0.09	0.86 ± 0.02	–
TMQ plus 10^{-4} M 5-CF_3TMQ (7)	6.89 ± 0.14	0.91 ± 0.01	–	5.55 ± 0.08	0.80 ± 0.08	5.32 ± 0.06

[a] Values are the mean ± SEM of n = 3–5. [b] pEC_{50} = −log EC_{50} (M). [c] IA = intrinsic activity = maximum response of drug relative to the response of 10^{-5} M isoproterenol. [d] pK_B = −log [[A]/(CR − 1)] where [A] = molar concentration of antagonist and CR = concentration ratio = EC_{50} (plus antagonist)/EC_{50} (control).

Table V. Comparative Inhibitory Activities of TMQ (1), 5,8-Difluoro-TMQ (4), and 5-CF_3TMQ (7) against U46619-Induced Responses in Human Platelets and Rat Thoracic Aorta[a]

	platelets (aggregation)		aorta (contraction)		selectivity ratio
drug	pIC_{50}[b]	potency ratio	pK_B[d]	potency ratio	aorta/platelets (τ/α)
TMQ (1)	6.36 ± 0.13	1.00	6.13 ± 0.20	1.00	1.00
5,8-DiFTMQ (4)	3.80 ± 0.18	2.77×10^{-3}	3.98 ± 0.46	7.08×10^{-3}	2.58
5-CF_3TMQ (7)	3.08 ± 0.10	5.25×10^{-4}	3.70 ± 0.31	3.72×10^{-3}	7.08

[a] Values are the mean ± SEM of n = 3–10. [b] pIC_{50} = −log IC_{50} (M). [c] pK_B = −log [[A]/(CR − 1)] where [A] = molar concentration of antagonist and CR = concentration ratio = EC_{50} (plus antagonist)/EC_{50} (control).

in TXA_2 receptor antagonist properties. Of interest is that 4 and 5 were about 35- and 3-fold more selective than 1 as antagonists of TXA_2 responses in rat thoracic aorta (TXA_2 τ receptor) than in human platelets (TXA_2 α receptors) (Table II). This similar trend was shown in our previous work[14] with fluorine substitution at the 5- and 8-positions of TMQ (1). Most interesting in this study, however, is the large difference in the reduction in activity between the substitution at the 5- and 8-positions. The TXA_2 antagonist activity of 1 appears to be very sensitive to iodine substitution at the 5-position whereas it is more tolerant to the presence of this substituent at the 8-position.

The 5,8-difluoro (4) and 5-trifluoromethyl (7) analogues were examined for their β-adrenergic activity in guinea pig trachea and atria. The pEC_{50} values are 7.11 and 6.62 in trachea (β_2) and 6.76 and 6.30 in atria (β_1) for TMQ and the 5,8-difluoro analogue, respectively (Table III). The potency ratio for analogue 4 is 0.32 and 0.35 in trachea and atria respectively and the β_2/β_1 selectivity ratio is 0.91 (Table III). Thus, fluorine substitution at both the 5- and 8-positions of TMQ decreases activity on both trachea and atria relative to TMQ and is virtually nonselective on β-adrenoceptors. The 5-trifluoromethyl analogue 7 has neither agonist nor antagonist activity on β_2-adrenoceptors (guinea pig trachea). In atria (β_1), however, the 5-trifluoromethyl derivative (7) has weak antagonist activity with a pK_B of 5.32 (Table IV). Therefore, substitution of a trifluoromethyl group at the 5-position of TMQ imparts weak antagonist activity for the compound at β_1-receptors.

The comparative ability of TMQ, 5,8-difluoro-TMQ (4), and 5-(trifluoromethyl)-TMQ (7) to inhibit U46619-induced responses in human platelets and rat thoracic aorta is shown in Table V. Both analogues are weak inhibitors of U46619 on human platelets with pIC_{50} values of 3.80 and 3.08 for 4 and 7, respectively, as compared to TMQ (1) which had a pIC_{50} value of 6.36 (Table V). Accordingly, fluorine substitution at both 5- and 8-positions or trifluoromethyl substitution at the 5-position of TMQ causes a significant reduction in TXA_2 antagonist properties in human platelets. With respect to inhibitory action against U46619-induced contraction on rat thoracic aorta, 4 and 7 have pK_B values of 3.98 and 3.70, respectively (Table V). This converts to a potency ratio of 7.08×10^{-3} and 3.72×10^{-3} in comparison to the inhibitory activity of TMQ (1). The τ/α selectivity ratio for difluoro-TMQ is 2.58. Thus, difluoro substitution at the 5- and 8-positions of TMQ possesses not only drastically decreased inhibitory activity but also dramatically decreases aorta/platelet selectivity when compared to monofluoro substituted analogues 2 and 3. The τ/α selectivity ratio for 5-(trifluoromethyl)-TMQ is 7.08. Thus, although the introduction of the trifluoromethyl group at the 5-position of TMQ causes a drastic decrease in inhibitory activity, the selectivity ratio for 7 is comparable to that of the 5-fluoro-TMQ analogue (4) which had a τ/α selectivity ratio of 7.67.[14]

Additional experiments were conducted with the 5,8-difluoro (4) and the 8-iodo (6) derivatives in the presence of propranolol, a nonselective β-antagonist, to determine if the β-adrenergic stimulatory effects were mediated through the activation of β-adrenoceptors in atria and trachea. Due to the reduced potency and intrinsic activity of 5-iodo-TMQ (5) in atria, experiments were completed in the presence of propranolol and 5 in guinea pig trachea only. The concentration–response curves of analogues 4, 5, and 6 were shifted to the right, indicating agonist/antagonist interaction with the β-adrenergic receptors (data not presented). The experimentally determined pK_B values of propranolol against each compound were similar in atria and trachea (data not presented) and comparable to those previously reported for the antagonism of TMQ (1) responses by propranolol in β-adrenergic systems.[30]

The enantiomers of 8-fluoro-TMQ [(S)-(+)-3 and (R)-(−)-3] have been evaluated for their β-adrenergic activity

(30) Mukhopadhyay, A.; Sober, D. J.; Chang, J.; Slenn, R. T.; Amin, H. M.; Miller, D. D.; Feller, D. R. Bronchoselective Actions of a New Series of Trimetoquinol Analogs. *Eur. J. Pharmacol.* 1982, 77, 209–219.

Table VI. Comparative Activities of Racemic TMQ (1), (*S*)-(+)-3, and (*R*)-(−)-3 on β-Systems (Guinea Pig Trachea and Atria)

treatment	trachea (β_2)[a]			atria (β_1)[a]			selectivity ratio[e] β_2/β_1
	pEC_{50}[b]	IA[c]	potency ratio[d]	pEC_{50}[b]	IA[c]	potency ratio[d]	
TMQ (1)	7.41 ± 0.06	0.98 ± 0.01	1.00	7.52 ± 0.21	1.00 ± 0.00	1.00	1.00
(*S*)-(+)-8-FTMQ (*S*)-(+)-3	6.53 ± 0.10	0.90 ± 0.03	0.13	6.62 ± 0.19	0.76 ± 0.04	0.13	1.00
(*R*)-(−)-8-FTMQ (*R*)-(−)-3	5.45 ± 0.13	0.90 ± 0.04	0.01	5.22 ± 0.33	0.64 ± 0.08	0.005	2.00

[a] Values are the mean ± SEM of n = 4–10. [b] pEC_{50} = −log EC_{50} (M). [c] IA = intrinsic activity = maximum response of drug relative to the response of 10^{-5} M isoproterenol. [d] Potency ratio = EC_{50} (trimetoquinol)/EC_{50} (drug). [e] Selectivity ratio = potency ratio (β_2)/potency ratio (β_1) for each drug.

Table VII. Receptor Binding Affinities of Racemic TMQ (1), Racemic 8-FTMQ (3), (*S*)-(+)-3, and (*R*)-(−)-3 in Guinea Pig Left Ventricle (β_1-Adrenoceptor) and Lung Membranes (β_2-Adrenoceptor)

compounds	pK_i values[a]	
	lung	left ventricle
(±)-TMQ (1)	7.31 ± 0.24 (4)	7.06 ± 0.01 (3)
(±)-8-FTMQ (3)	7.24 ± 0.08 (8)	7.01 ± 0.01 (3)
(*S*)-(+)-8-FTMQ (*S*)-(+)-3	7.35 ± 0.03 (4)	7.12 ± 0.06 (3)
(*R*)-(−)-8-FTMQ (*R*)-(−)-3	6.43 ± 0.04 (4)	5.94 ± 0.10 (3)

[a] Values are the mean ± SEM (n).

in guinea pig trachea and atria relative to TMQ. The pEC_{50} values were determined to be 7.41, 6.53, and 5.45 on trachea (β_2) and 7.52, 6.62, and 5.22 on atria (β_1) for TMQ (1), (*S*)-(+)-3 and (*R*)-(−)-3, respectively (Table VI). Thus, (*S*)-(+)-3 is 13-fold and 26-fold more potent than (*R*)-(−)-3 on β_2- and β_1-adrenoceptors, respectively. This follows the same trend as found for TMQ on β-adrenergic systems, that is (*S*)-(−)-TMQ is more potent than (*R*)-(+)-TMQ on β-systems.[4] The potency ratios for (*S*)-(+)-3 were the same (0.13) in trachea and atria indicating no β_2/β_1 selectivity. The potency ratios for (*R*)-(−)-3 are 0.01 on trachea and 0.005 on atria which corresponds to a β_2/β_1 selectivity ratio of 2.00. This differs from the activity of the racemic mixture of 8-fluoro-TMQ (3) in which the β_2/β_1 selectivity ratio was 8.[12] It is of interest to note that both (*S*)-(+)-3 and (*R*)-(−)-3 are partial agonists on β_1-adrenoceptors.

Binding assays were also performed to characterize the interaction of TMQ (1), racemic 8-FTMQ (3), (*S*)-(+)-3, and (*R*)-(−)-3 with β_1- and β_2-adrenergic receptors in isolated guinea pig left ventricular and lung membranes, respectively (Table VII).[31] 8-FTMQ (3), (*S*)-(+)-3, and racemic TMQ (1) possessed similar affinities for β_1- and β_2-adrenergic receptors, whereas (*R*)-(−)-3 demonstrated lower affinity for β_1- and β_2-adrenergic receptors. The 8-fluoro compounds also exhibited β_2/β_1 selectivity ratios similar to that of racemic TMQ (1). Furthermore, the data obtained in functional and receptor binding assays confirm the stereoselectivity of the 8F isomers for interaction with β_1- and β_2-adrenergic receptors (Tables VI and VII). Moreover, the potency of racemic 8-FTMQ (3) was significantly greater than the potencies of either (*R*)- or (*S*)-8-FTMQ, whereas no receptor binding potency differences were noted for these compounds. At this time, however, the reason for the differences in receptor binding and biological potencies is unclear. One possible explanation is that the isomers of 8-FTMQ possess additional postreceptor sites of action which lead to the observed differences in functional activity.

The enantiomers of 8-fluoro-TMQ were also examined for their inhibitory activity against U46619-induced human platelet aggregation. Both enantiomers were concentration-dependent inhibitors of U46619 on human platelets with pIC_{50} values of 5.61 and 4.40 for (*R*)-(−)-3 and (*S*)-(+)-3, respectively, and were about 5- and 100-fold less potent than TMQ (1) [pIC_{50} = 6.36] (Table VIII). In this TXA_2 system, (*R*)-(−)-3 is more potent than (*S*)-(+)-3, which is in contrast to the stereoselectivity found on β-adrenergic receptors. The same trend was found for TMQ (1) on TXA_2 systems, in which (*R*)-(+)-TMQ was more potent than (*S*)-(−)-TMQ.[8] In contrast to the *R*-(−) isomer which possessed TXA_2 antagonist activity in rat aorta, (*S*)-(+)-3 exhibited contractile responses equal to 65% of the maximal response to U46619 (Table VIII). The maximal contractile response (*S*)-(+)-3 was blocked completely by SQ 29,548 (3×10^{-4} M), a TXA_2 receptor antagonist.[32] These studies do not rule out the possibility that (*S*)-(+)-3 activates other prostaglandin receptor sites in vascular smooth muscle. Thus, the 8-fluoroTMQ isomers exhibit differential responses on PGH_2/TXA_2 receptors in rat aorta (τ) and human platelets (α).

In summary, substitution at the 5- or 8-positions of TMQ with iodine, 5,8-difluoro substitution, or 5-trifluoromethyl substitution yields compounds which are less potent but which possess selective TXA_2 antagonism for τ receptors over α receptors. This is known most notably with the 5-iodo derivative **5** which had a τ/α selectivity ratio of 35. In contrast to previous reports with fluorine;[12] however, 5-iodo- and 8-iodo-TMQ were considerably less active than TMQ as β_1- and β_2-adrenergic agonists and exhibited little β_2/β_1 selectivity. It would appear that either increased size or enhanced lipophilicity of the iodine addition to the 5- or 8-positions of TMQ contribute to the reduction in β-adrenergic stimulatory activity and thromboxane A_2 antagonist activity. The decrease in potency seen with the 5,8-difluoro substitution of TMQ on β-adrenergic systems and TXA_2 systems as well as the nonselective action among the subtypes in these systems may be attributed to a steric effect. The elimination of β-agonist activity and the introduction of selective, although weak, β_1-antagonist activity by inserting a trifluoromethyl group at the 5-position of TMQ would also appear to be the result of either increased steric bulk or the greatly enhanced lipophilicity. Similarly, the TXA_2 antagonist activity was greatly reduced with the introduction of the 5-trifluoromethyl group, most notably in human platelets (TXA_2 α receptor).

On β-adrenergic systems, (*S*)-(+)-3 was at least 10-fold more potent than (*R*)-(−)-3. Conversely, (*R*)-(−)-3 was about 14-fold more potent as an antagonist of TXA_2-mediated aggregation in human platelets than (*S*)-(+)-3. In contrast to platelets, (*S*)-(+)-3 was an agonist in rat aorta whereas (*R*)-(−)-3 was an antagonist. These results demonstrate that optimal activity for β-adrenoceptor agonist

(31) Engel, G.; Hoyer, D.; Berthold, R.; Wagner, H. (±)[^{125}Iodo]-cyanopindolol, a New Ligand for β-Adrenoceptors: Identification and Quantitation of Subclasses of β-Adrenoceptors in Guinea Pig. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1981, *317*, 277–285.

(32) Ogletree, M. L.; Harris, D. N.; Greenberg, R.; Haslanger, M. F.; Nakane, M. Pharmacological Actions of SQ 29,548 a Novel Selective Thromboxane Antagonist. *J. Pharmacol. Exp. Ther.* 1985, *234*, 435–441.

Table VIII. Comparative Inhibitory Activities of TMQ (1), (S)-(+)-3, and (R)-(−)-3 against U46619-Induced Responses in Human Platelets and Rat Thoracic Aorta[a]

drug	platelets (aggregation)		aorta (contraction)		selectivity ratio aorta/platelets (τ/α)
	pIC_{50}[b]	potency ratio	pK_B[c] or pEC_{50}[d]	potency ratio	
TMQ (1)	6.36 ± 0.13	1.00	5.50 ± 0.14[c]	1.00	1.00
(S)-(+)-8-FTMQ (S)-(+)-3	4.40 ± 0.08	0.01	5.71 ± 0.27[d]	–	–
(R)-(−)-8-FTMQ (R)-(−)-3	5.61 ± 0.19	0.18	5.63 ± 0.13[c]	1.35	7.50

[a] Values are the mean ± SEM of n = 3–5. [b] pIC_{50} = −log IC_{50} (M). [c] pK_B = −log [[A]/(CR − 1)] where [A] = molar concentration of antagonist and CR = concentration ratio = EC_{50} (plus antagonist)/EC_{50} (control). [d] pEC_{50} = −log EC_{50}. Maximum effect of (S)-(+)-8-FTMQ was 65% relative to U46619 (100%).

and TXA_2 antagonist properties of 8-fluoro-TMQ (3) isomers is associated with the S and R configuration, respectively.

Experimental Section

Melting points (uncorrected) were determined on a Thomas-Hoover capillary melting point apparatus. Infrared data were collected on an Analect RFX-40 FTIR spectrophotometer or a Beckman 4230 spectrophotometer. The NMR spectra were obtained on either an IBM AF-250 FTNMR spectrometer (250 Hz) or an IBM AF-279 FTNMR spectrometer (270 Hz) and are reported in parts per million. Mass spectra were obtained at the College of Pharmacy by use of a Kratos MS25 RFA double-focusing mass spectrometer or at the Ohio State University Chemical Instrumentation Center by use of a VG 70-250S (or Kratos MS-30) mass spectrometer. Optical rotations were taken on a JASCO J-500A spectropolarimeter. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, and were within ±0.4% of the theoretical values for the elements indicated. All solvents were dried prior to use.

3-Hydroxy-2-iodo-4-methoxybenzaldehyde (10). To a stirred solution of isovanillin 9 (12.76 g, 83.95 mmol) in pyridine (48 mL) at 0 °C was added a solution of iodine monochloride (13.61 g, 84.01 mmol) in dioxane (82 mL). The reaction mixture was stirred at room temperature for 6 days. The solvents were evaporated under reduced pressure to give an oil which was added to water (200 mL) and acidified with 6 N HCl. The solution was extracted with ethyl acetate (2 × 150 mL), and the ethyl acetate extracts were washed successively with 5% sodium bisulfite solution, water (2 × 200 mL), and brine (200 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a solid. The solid was crystallized from ethyl acetate/diethyl ether to give 17.10 g (73%) of 10 as light yellow needles: mp 169–171.5 °C; IR (KBr, cm^{-1}) 3300, 1655; ^1H NMR ($CDCl_3$) δ 10.03 (s, 1 H, ArCHO), 7.56 (d, J = 8.4 Hz, 1 H, ArH), 6.93 (d, J = 8.4 Hz, 1 H, ArH), 6.31 (s, 1 H, ArOH), 3.99 (s, 3 H, $ArOCH_3$); MS m/z 278 (M^+, base). Anal. ($C_8H_7IO_3$) C, H.

2-Iodo-3,4-dimethoxybenzaldehyde (11). To a solution of 10 (25.12 g, 90.36 mmol) in acetone (250 mL) was added potassium carbonate (25.00 g) and dimethyl sulfate (22.77 g, 180.72 mmol). The mixture was refluxed for 6 h, cooled, and concentration under reduced pressure. Water (100 mL) was added, and the solution was extracted with diethyl ether (2 × 100 mL). The organic extracts were combined, washed with water (100 mL), dried ($MgSO_4$), and concentrated to give an oil which was crystallized from dichloromethane/petroleum ether to give 22.30 g (85%) of 11 as a white crystal: mp 77–78 °C; IR (KBr, cm^{-1}) 1660; ^1H NMR ($CDCl_3$) δ 10.00 (s, 1 H, CHO), 7.70 (d, J = 8.5 Hz, 1 H, ArH), 6.90 (d, J = 8.5 Hz, 1 H, ArH), 3.94 (s, 3 H, $ArOCH_3$), 3.84 (s, 3 H, $ArOCH_3$); MS m/z 292 (M^+, base). Anal. ($C_9H_9IO_3$) C, H.

2-Iodo-3,4-dimethoxybenzyl Alcohol (13). To a solution of 11 (6.55 g, 22.77 mmol) in dry THF (50 mL) was added sodium borohydride (2.58 g, 68.25 mmol). The mixture was stirred at 40 °C for 3 h. The excess sodium borohydride was destroyed by the addition of water. Tetrahydrofuran and water were evaporated under reduced pressure, and to the residue was added water (100 mL) followed by extraction with diethyl ether (3 × 100 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure to give an oil. The oil was crystallized from diethyl ether/hexane to give 5.5 g (83%) of 13 as a white solid: mp 90–92 °C; IR (KBr, cm^{-1}) 3300; ^1H NMR ($CDCl_3$) δ 7.13 (d, J = 8.4 Hz, 1 H, ArH), 6.89 (d, J = 8.4 Hz, 1 H, ArH), 4.66 (s, 2 H, CH_2OH), 3.87 (s, 3 H, $ArOCH_3$), 3.84 (s, 3 H, $ArOCH_3$); MS m/z 294 (M^+, base). Anal. ($C_9H_{11}IO_3$) C, H.

2-Iodo-3,4-dimethoxyphenylacetonitrile (15). To a solution of 13 (15.90 g, 54.08 mmol) in chloroform (30 mL) at 0 °C was added phosphorus tribromide (14.63 g, 54.03 mmol) dropwise. The solution was stirred for 2 h at 0 °C, at which time water was added to the reaction mixture and the organic layer was separated. The aqueous layer was extracted with chloroform (2 × 50 mL). The organic layers were combined, washed with water (2 × 50 mL), dried ($MgSO_4$), and concentrated to give an oil. The oil was crystallized from ethanol to give 14.92 g (77%) of 2-iodo-3,4-dimethoxybenzyl bromide as white crystals: mp 47–49 °C; ^1H NMR ($CDCl_3$) δ 7.23 (d, J = 8.4 Hz, 1 H, ArH), 6.83 (d, J = 8.4 Hz, 1 H, ArH), 4.65 (s, 2 H, CH_2Br), 3.86 (s, 3 H, $ArOCH_3$), 3.83 (s, 3 H, $ArOCH_3$); MS m/z 358 (M^+ + 2), 356 (M^+), 277 (base).

The benzyl bromide (12.33 g, 34.54 mmol) was dissolved in dimethylformamide (50 mL). Sodium cyanide (8.46 g, 172.65 mmol) was added, and the mixture was stirred at room temperature for 30 min. The reaction mixture was poured into water (50 mL) and extracted with dichloromethane (3 × 50 mL). The organic extracts were combined, washed with water (100 mL), dried ($MgSO_4$), and concentrated to give an oil. The oil was crystallized from methanol/acetone to give 7.61 g (73%) of 15 as white crystals: mp 77–78 °C; IR (KBr, cm^{-1}) 2250; ^1H NMR ($CDCl_3$) δ 7.24 (d, J = 8.4 Hz, 1 H, ArH), 6.92 (d, J = 8.4 Hz, 1 H, ArH), 3.88 (s, 3 H, $ArOCH_3$), 3.84 (s, 3 H, $ArOCH_3$), 3.80 (s, 2 H, CH_2CN); MS m/z 303 (M^+, base). Anal. ($C_{10}H_{10}INO_2$) C, H, N.

3,4-Dihydroxy-2-iodophenylacetonitrile (18). To a solution of 15 (3.03 g, 10.00 mmol) in dichloromethane (10 mL) at −78 °C was added 1 M boron tribromide in dichloromethane (30 mL, 30 mmol) dropwise. The reaction mixture was allowed to attain room temperature and stirred at that temperature overnight. The mixture was concentrated under reduced pressure to give a solid. The solid was dissolved in ethyl acetate (50 mL), washed with brine (3 × 50 mL), and water (3 × 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a solid which was crystallized from ethyl acetate/diethyl ether to give 1.88 g (68%) of 18 as white crystals: mp 135–137 °C; IR (KBr, cm^{-1}) 3480, 3200, 2230; ^1H NMR ($CDCl_3$) δ 6.91 (d, J = 8.3 Hz, 1 H, ArH), 6.88 (d, J = 8.3 Hz, 1 H, ArH), 5.57 (s, 2 H, 2 ArOH), 3.72 (s, 2 H, CH_2CN); MS m/z 275 (M^+), 273 (base). Anal. ($C_8H_6INO_2$) C, H, N.

4,5-Dihydroxy-3-iodophenylacetonitrile (19). Prepared by the procedure as described for compound 18; from 17 (3.03 g, 10.00 mmol) with 1 M boron tribromide in dichloromethane (30 mL, 30 mmol) was obtained 1.89 g (69%) of 19 as white crystals: mp 153–154 °C; IR (KBr, cm^{-1}) 2250; ^1H NMR (CD_3OD) δ 7.11 (d, J = 1 Hz, 1 H, ArH), 6.76 (d, J = 1 Hz, 1 H, ArH), 3.30 (s, 2 H, CH_2CN); MS m/z 275 (M^+), 127 (base). Anal. ($C_8H_6INO_2$) C, H, N.

3,4-Bis(benzyloxy)-2-iodophenylacetonitrile (21). To a solution of 18 (1.50 g, 5.45 mmol) in acetone (50 mL) were added potassium carbonate (1.5 g, 10.85 mmol), potassium iodide (1.00 g, 6.02 mmol), and benzyl bromide (1.86 g, 10.90 mmol). The mixture was refluxed overnight, cooled, and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (100 mL) and washed with water (3 × 50 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a solid which was crystallized from ethyl acetate/diethyl ether to give 2.27 g (92%) of 21 as white crystals: mp 105.5–106.5 °C; IR (KBr, cm^{-1}) 3060, 3025, 2230; ^1H NMR ($CDCl_3$) δ 7.50–7.32 (m, 10 H, ArH), 7.23 (d, J = 8.4 Hz, 1 H, ArH), 7.01 (d, J = 8.4 Hz, 1 H, ArH), 5.15 (s, 2 H, $ArCH_2O$), 5.04 (s, 2 H, $ArCH_2O$), 3.81 (s, 2 H, CH_2CN); MS m/z 455 (M^+), 91 (base). Anal. ($C_{22}H_{18}INO_2$) C, H, N.

4,5-Bis(benzyloxy)-3-iodophenylacetonitrile (22). Prepared by the same procedure as described for compound 21; from 19

(3.11 g, 11.30) with potassium carbonate (3.12 g, 22.60 mmol), potassium iodide (1.00 g, 6.02 mmol), and benzyl bromide (3.87 g, 22.61 mmol) was obtained 3.74 g (73%) of **22** as white crystals: mp 99.5–101 °C; IR (KBr, cm^{-1}) 2250; ^1H NMR ($CDCl_3$) δ 7.46–7.29 (m, 11 H, ArH), 6.92 (d, J = 1 Hz, 1 H, ArH), 5.10 (s, 2 H, $ArCH_2O$), 4.99 (s, 2 H, $ArCH_2O$), 3.63 (s, 2 H, CH_2CN); MS m/z 455 (M^+), 91 (base). Anal. ($C_{22}H_{18}INO_2$) C, H, N.

***N*-[2-[3,4-Bis(benzyloxy)-2-iodophenyl]ethyl]-3′,4′,5′-trimethoxyphenylacetamide (25).** To a cool solution (0 °C) of **21** (3.72 g, 8.20 mmol) in dry THF (20 mL) was added dropwise a 1 M BH_3·THF solution (24.5 mL, 24.5 mmol). The mixture was refluxed under an argon atmosphere for 18 h and cooled to 0 °C, and methanol was added cautiously to quench the reaction. The mixture was concentrated under reduced pressure to an oil. The oil was dissolved in methanol (50 mL) and reconcentrated under reduced pressure (this was repeated two more times) to give the oily phenethylamine. The oil was dissolved in toluene (100 mL), and commercially available (Aldrich) 3,4,5-trimethoxyphenylacetic acid (**24**, 1.85 g, 8.20 mmol) was added. The mixture was refluxed for 72 h with removal of water via a Dean–Stark trap. The mixture was cooled and concentrated under reduced pressure to give a solid. The solid was dissolved in dichloromethane (150 mL), washed with water (100 mL), 10% HCl (2 × 100 mL), water (2 × 100 mL), 10% sodium bicarbonate solution (2 × 100 mL), and water (2 × 100 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give a white solid. The solid was crystallized from ethyl acetate to give 3.05 g (56%) of **25** as a white solid: mp 126–127 °C; IR (KBr, cm^{-1}) 3270, 1640; ^1H NMR ($CDCl_3$) δ 7.50–7.31 (m, 10 H, ArH), 6.83 (d, J = 8.4 Hz, 1 H, ArH), 6.68 (d, J = 8.4 Hz, 1 H, ArH), 6.40 (s, 2 H, 2 ArH), 5.46 (br, 1 H, NH), 5.11 (s, 2 H, $ArCH_2O$), 4.99 (s, 2 H, $ArCH_2O$), 3.84 (s, 3 H, $ArOCH_3$), 3.80 (s, 6 H, 2 $ArOCH_3$), 3.47 (s, 2 H, $COCH_2Ar$), 3.45 (t, 2 H, $ArCH_2C$), 2.90 (t, 2 H, CH_2N); MS m/z 667 (M^+). Anal. ($C_{33}H_{34}INO_6$) C, H, N.

***N*-[2-[4,5-Bis(benzyloxy)-3-iodophenyl]ethyl]-3′,4′,5′-trimethoxyphenylacetamide (26).** Phenylacetamide **26** was synthesized by the procedure previously described for phenylacetamide **25**; from phenylacetonitrile **22** (2.74 g, 6.02 mmol), with 1 M BH_3·THF solution (18.06 mL, 18.06 mmol) and 3,4,5-trimethoxyphenylacetic acid (**24**, 1.36 g, 6.02 mmol) was obtained 2.66 g (66%) of **26** as a white solid: mp 123–124 °C; IR (KBr, cm^{-1}) 3310, 1640; ^1H NMR ($CDCl_3$) δ 7.50–7.31 (m, 10 H, ArH), 7.17 (d, J = 1.8 Hz, 1 H, ArH), 6.77 (d, J = 1.8 Hz, 1 H, ArH), 6.40 (s, 2 H, 2 ArH), 5.50 (br, 1 H, NH), 5.07 (s, 2 H, $ArCH_2O$), 4.99 (s, 2 H, $ArCH_2O$), 3.83 (s, 3 H, $ArOCH_3$), 3.82 (s, 6 H, 2 $ArOCH_3$), 3.47 (s, 2 H, $COCH_2Ar$), 3.42 (t, 2 H, $ArCH_2C$), 2.69 (t, 2 H, CH_2N); MS m/z 667 (M^+). Anal. ($C_{33}H_{34}INO_6$) C, H, N.

6,7-Bis(benzyloxy)-5-iodo-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline Oxalate (28). To a solution of phenylacetamide **25** (1.00 g, 1.50 mmol) in acetonitrile was added phosphorus oxychloride (1.64 g, 10.75 mmol). The mixture was refluxed for 5 h under an argon atmosphere, cooled, and concentrated under reduced pressure to afford an oil. The oil was dissolved in methanol (25 mL) and reconcentrated under reduced pressure (this was repeated two more times). The oil was dissolved in methanol (50 mL) and cooled to 0 °C, whereupon excess sodium borohydride (2.30 g, 60.81 mmol) was added. The mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was dissolved in water (25 mL), and 10% sodium hydroxide solution (25 mL) was added. The mixture was extracted with diethyl ether (2 × 50 mL). The organic extracts were washed with water (2 × 50 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated to give an oil. The oil was dissolved in methanol, and a solution of oxalic acid dihydrate (189 mg, 1.5 mmol) in methanol was added. The solvent was evaporated, and the residue was crystallized from methanol/diethyl ether to give 750 mg (68%) of **28** as a white solid: mp 199.5–200.5 °C; ^1H NMR (DMSO-d_6) δ 7.44–7.32 (m, 10 H, ArH), 6.90 (s, 1 H, ArH), 6.65 (s, 2 H, 2 ArH), 5.03 (d, J = 11.6 Hz, 1 H, $ArCH_2O$), 4.95 (d, J = 11.6 Hz, 1 H, $ArCH_2O$), 4.91 (s, 2 H, $ArOCH_3$), 4.65 (br, 1 H, ArCHNH), 3.76 (s, 6 H, 2 $ArOCH_3$), 3.41 (s, 3 H, $ArOCH_3$), 3.3–2.7 (m, 6 H, 3 CH_2); MS FAB 652 ($MH^+ - C_2H_2O_4$), 91 (base). Anal. ($C_{33}H_{34}INO_5 \cdot C_2H_2O_4$) C, H, N.

6,7-Bis(benzyloxy)-8-iodo-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (29). Prepared by the same procedure as described for compound **28**; from phenylacetamide **26** (1.20 g, 1.80 mmol) with phosphorus oxychloride (2.47 g, 16.14 mmol) and sodium borohydride (2.50 g, 66.14 mmol) was obtained 790 mg (67%) of **29** as a white solid: mp 140.5–142 °C dec; ^1H NMR ($CDCl_3$) δ 7.53–7.31 (m, 10 H, ArH), 6.78 (s, 1 H, ArH), 6.63 (s, 2 H, 2 ArH), 5.11 (s, 2 H, $ArCH_2O$), 5.05 (d, J = 10.2 Hz, 1 H, $ArCH_2O$), 5.00 (d, J = 10.2 Hz, 1 H, $ArCH_2O$), 4.24 (dd, J = 2.7 and 10.9 Hz, 1 H, ArCHNH), 3.88 (s, 6 H, 2 $ArOCH_3$), 3.85 (s, 3 H, $ArOCH_3$), 3.34–2.60 (m, 6 H, 3 CH_2); MS m/z 470 (M^+ – trimethoxybenzyl), 91 (base). Anal. ($C_{33}H_{34}INO_5 \cdot 0.5H_2O$) C, H, N.

6,7-Dihydroxy-5-iodo-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (5). An equivolume mixture of methanol and concentrated hydrochloric acid (6 mL) was added to **28** (200 mg, 0.27 mmol). The mixture was refluxed for 10 min under an argon atmosphere. The solvent and excess HCl were evaporated. The residue was dissolved in ethanol, and the solution was evaporated to dryness (this was repeated three times). The residue was crystallized form methanol to give 62 mg (46%) of **5** as white needles: mp 217–218 °C dec; ^1H NMR (CD_3OD) δ 6.59 (s, 1 H, ArH), 6.53 (s, 2 H, 2 ArH), 4.64 (dd, J = 6.1 and 8.2 Hz, 1 H, ArCHNH), 3.79 (s, 6 H, 2 $ArOCH_3$), 3.70 (s, 3 H, $ArOCH_3$), 3.55–3.30 and 3.00–2.80 (m, 6 H, 3 CH_2); MS FAB 472 (MH^+ – HCl), 85 (base). Anal. ($C_{19}H_{22}INO_5 \cdot HCl$) C, H, N.

6,7-Dihydroxy-8-iodo-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (6). According to the same procedure as described for compound **5**; from **29** (361 mg, 0.55 mmol) with an equivolume mixture of methanol and concentrated hydrochloric acid (6 mL) was obtained 188 mg (67%) of **6** as white needles: mp 180–181.5 °C dec; ^1H NMR (pyridine-d_5) δ 7.17 (s, 2 H, 2 ArH), 7.07 (s, 1 H, ArH), 5.39 (dd, J = 2.9 and 9.6 Hz, 1 H, ArCHNH), 3.92–3.77 (m, 2 H, CH_2), 3.80 (s, 3 H, $ArOCH_3$), 3.77 (s, 6 H, 2 $ArOCH_3$), 3.55–3.41 (m, 2 H, CH_2), 3.81–3.16 (m, 2 H, CH_2NH); MS FAB 472 (MH^+ – HCl), 290 (base). Anal. ($C_{19}H_{22}INO_5 \cdot HCl \cdot 0.5H_2O$) C, H, N.

3,4-Dimethoxy-2-(trifluoromethyl)benzaldehyde (12). To a solution of 2-iodo-3,4-dimethoxybenzaldehyde **11** (4.12 g, 14.10 mmol) in *N*-methyl-2-pyrrolidinone (80 mL) in an anhydrous environment under argon was added Cu^{II} (5.37 g, 28.20 mmol) and sodium trifluoroacetate (7.67 g, 56.40 mmol). The solution was heated under argon. At 165 °C, the solution began to liberate carbon dioxide and was allowed to stir at 175 °C for 4 h. The solution was cooled, diluted with diethyl ether (150 mL), and washed with water (1 × 200 mL). The aqueous layer was extracted once with diethyl ether (200 mL), and the combined ether layers were washed with water (2 × 400 mL) and brine (1 × 400 mL), dried ($MgSO_4$), and concentrated to a brown oil. Flash column chromatography using a gradient solvent system of dichloromethane/hexane, 20:80 to 50:50 (R_f = 0.60 silica on glase using dichloromethane as solvent), afforded **12** as a yellow oil, 1.31 g (40%): IR (neat, cm^{-1}) 1687; ^1H NMR (acetone-d_6) δ 10.21–10.20 (br q, 1 H, CHO), 7.72 (d, J_m = 8.7 Hz, 1 H, ArH), 7.42 (d, J_m = 8.6 Hz, 1 H, ArH), 4.02 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3); ^{13}C NMR BB (acetone-d_6) δ 189.13 (q, $J_{C-C-C-CF_3}$ = 5.4 Hz, CHO), 158.93 (s, aromatic C bonded to OCH_3), 149.20 (s, aromatic C bonded to OCH_3), 129.28 (s, aromatic C bonded to aldehyde), 126.72 (s, aromatic C), 125.33 (q, J_{CF_3} = 275.6 Hz, CF_3), 124.26 (q, J_{C-CF_3} = 30.5 Hz, aromatic C bonded to CF_3), 116.42 (s, aromatic C), 61.91 (s, OCH_3), 56.96 (s, OCH_3); ^{19}F NMR ($CFCl_3$ = 0 ppm external ref) δ −52.50; MS m/z 234 (M^+, base). Anal. ($C_{10}H_9F_3O_3$) C, H.

3,4-Dimethoxy-2-(trifluoromethyl)benzyl Alcohol (14). To a solution of benzaldehyde **12** (4.33 g, 18.49 mmol) in THF–H_2O (9:1 v/v) (100 mL) cooled to 0 °C was added sodium borohydride (1.40 g, 36.98 mmol). The solution was heated to 40 °C for 2.5 h, cooled, and quenched with water and NaH_2PO_4. The solution was concentrated and taken up into diethyl ether (100 mL) and water (100 mL), the layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 100 mL). The combined organic layers were dried ($MgSO_4$) and concentrated to a yellow oil. Gravity column chromatography was performed using a gradient solvent system of ethyl acetate/hexane, 20:80 to 50:50, to yield **14** as a yellow oil, 3.81 g (87%): IR (neat, cm^{-1}) 3600–3200, absence of C=O; ^1H NMR ($CDCl_3$/TMS) δ 7.29 (d, J_m = 8.7 Hz, 1 H, ArH), 7.06 (d, J_m = 8.6 Hz, 1 H, ArH), 4.76 (d, J = 1.2 Hz,

2 H, CH_2), 3.89 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 1.97 (br s, 1 H, OH); MS m/z 236 (M^+, base). Anal. ($C_{10}H_{11}F_3O_3$ C, H.

3,4-Dimethoxy-2-(trifluoromethyl)benzyl Cyanide (16). To a solution of benzyl alcohol 14 (463 mg, 1.96 mmol) in dichloromethane (10 mL) cooled to 0 °C was added phosphorus tribromide (0.20 mL, 2.16 mmol). The solution was allowed to stir for 2 h at 0 °C and quenched with ice water (10 mL), at which time saturated sodium bicarbonate solution (10 mL) and dichloromethane (10 mL) were added. The organic layer was separated, washed with water (2 × 20 mL) and brine (1 × 20 mL), dried ($MgSO_4$), and concentrated to afford the benzyl bromide as a light yellow oil, 431 mg (73.5%): IR (neat, cm^{-1}) absence of OH; ^1H NMR ($CDCl_3$/TMS) δ 7.16 (d, J_o = 8.6 Hz, 1 H, ArH), 7.02 (d, J_o = 8.6 Hz, 1 H, ArH), 4.62 (q, J = 1.4 Hz, 2 H, CH_2Br), 3.90 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3); MS m/z 219 (M^+ – Br, base).

To a solution of the above 3,4-dimethoxy-2-(trifluoromethyl)benzyl bromide (2.21 g, 7.39 mmol) in dimethylformamide (50 mL) was added sodium cyanide (1.81 g, 36.94 mmol), and the solution was allowed to stir at room temperature for 30 min. Water (100 mL) was added, and the solution was extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with water (2 × 300 mL) and brine (1 × 100 mL), dried ($MgSO_4$), and concentrated to an orange oil. Gravity column chromatography was performed using a gradient solvent system of ethyl acetate/hexane, 20:80 to 50:50. The resultant light yellow oil was crystallized from ethyl acetate/hexane to afford **16** as white needles, 1.67 g (92%): mp 41–43 °C; IR (KBr, cm^{-1}) 2253; ^1H NMR ($CDCl_3$/TMS) δ 7.27 (d, J_o = 8.6 Hz, 1 H, ArH), 7.07 (d, J_o = 8.6 Hz, 1 H, ArH), 3.91 (s, 3 H, OCH_3), 3.89 (s, 3 H, OCH_3), 3.85 (q, J = 1.9 Hz, 2 H, CH_2CN); MS m/z 245 (M^+, base). Anal. ($C_{11}H_{10}F_3NO_2$) C, H, N.

3,4-Dihydroxy-2-(trifluoromethyl)benzyl Cyanide (20). To a solution of benzyl cyanide **16** (1.66 g, 6.77 mmol) in dichloromethane (25 mL) cooled to 0 °C under argon was added 1 M boron tribromide in dichloromethane (20.30 mL). The solution was allowed to stir overnight at room temperature, concentrated under reduced pressure, and taken up into ethyl acetate (100 mL) and water (100 mL). The organic layer was separated, washed with water (3 × 100 mL) and brine (1 × 100 mL), dried ($MgSO_4$), treated with charcoal, and concentrated to a brown powder. Recrystallization from ethyl acetate/hexane afforded crystalline **20**, 1.21 g (82%): mp 149–151 °C; IR (KBr, cm^{-1}) 3400–3100, 2280; ^1H NMR (acetone-d_6) δ 7.08 (d, J_o = 8.2 Hz, 1 H, ArH), 6.93 (d, J_o = 8.2 Hz, 1 H, ArH), 3.94 (q, J = 2.0 Hz, 2 H, CH_2CN); MS m/z 217 (M^+, base). Anal. ($C_9H_6F_3NO_2$) C, H, N.

3,4-Bis(benzyloxy)-2-(trifluoromethyl)benzyl Cyanide (23). To a solution of benzyl cyanide **20** (1.15 g, 5.30 mmol) in acetone (50 mL) was added potassium carbonate (1.65 g, 11.93 mmol), potassium iodide (133 mg, 0.80 mmol), and 97% benzyl chloride (1.40 mL, 11.93 mmol). The solution was stirred overnight at reflux, cooled, concentrated under reduced pressure, and taken up into ethyl acetate (50 mL) and water (50 mL). The ethyl acetate layer was washed with water (3 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated. The residue was passed through a gravity silica column using 20% ethyl acetate/80% hexane (R_f = 0.38 silica on glass using 20% ethyl acetate/80% hexane as solvent) to yield **23** as an orange oil, 1.81 g (86%): IR (neat, cm^{-1}) 2254; ^1H NMR ($CDCl_3$/TMS) δ 7.44–7.30 (m, 11 H, ArH), 7.17 (d, J_o = 8.6 Hz, 1 H, ArH), 5.17 (s, 2 H, $ArCH_2O$), 5.06 (s, 2 H, $ArCH_2O$), 3.88 (br q, 2 H, CH_2CN); MS m/z 306 (M^+ – benzyl), 91 (base). Anal. ($C_{23}H_{18}F_3NO_2$) C, H, N.

***N*-[2-[3,4-Bis(benzyloxy)-2-(trifluoromethyl)phenyl]ethyl]-3′,4′,5′-trimethoxyphenylacetamide (27).** To a solution of benzyl cyanide **23** (1.61 g, 4.05 mmol) in dry THF (50 mL) cooled to 0 °C under argon was added 1 M diborane in THF (16.20 mL). The solution was refluxed overnight under argon, cooled to 0 °C, quenched with methanol (50 mL), and concentrated. The residue was taken up into methanol (50 mL) and concentrated (repeated two more times). The resultant oil was taken up into toluene (50 mL), and to the solution was added commercially available (Aldrich) 3,4,5-trimethoxyphenylacetic acid (**24**, 0.92 g, 4.05 mmol). The solution was refluxed for 72 h with azeotropic removal of water via a Dean–Stark trap, cooled, and concentrated under reduced pressure to an oil. The oil was taken up into dichloromethane (50 mL), washed with water (1 × 50 mL), 10% HCl solution (2 × 50 mL), saturated sodium bicarbonate solution (2 × 50 mL), and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated to a beige solid. Recrystallization from toluene/hexane gave **27** a white solid, 2.10 g (85%): mp 88–90 °C; IR (KBr, cm^{-1}) 3302, 1641; ^1H NMR ($CDCl_3$/TMS) δ 7.47–7.29 (m, 10 H, ArH), 7.00 (d, J_o = 8.5 Hz, 1 H, ArH), 6.72 (d, J_o = 8.5 Hz, 1 H, ArH), 6.41 (s, 2 H, ArH), 5.48 (br t, 1 H, NH), 5.14 (s, 2 H, $ArCH_2O$), 5.02 (s, 2 H, $ArCH_2O$), 3.85 (s, 3 H, OCH_3), 3.82 (s, 6 H, 2 OCH_3), 3.47–3.40 (m, 4 H, 2 $ArCH_2$), 2.92–2.87 (m, 2 H, CH_2N); MS m/z 609 (M^+), 91 (base). Anal. ($C_{34}H_{34}F_3NO_6$) C, H, N.

6,7-Bis(benzyloxy)-1-(3′,4′,5′-trimethoxybenzyl)-5-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (30). To a solution of amide **27** (816 mg, 1.34 mmol) in dry acetonitrile (50 mL) under argon was added phosphorus oxychloride (0.87 mL, 9.37 mmol). The solution was stirred at reflux for 5 h, cooled, and concentrated under reduced pressure. The residue was taken up into methanol (50 mL) and concentrated (repeated two more times). The residue was taken up into methanol (50 mL) and cooled to 0 °C, and to the solution was added sodium borohydride (253 mg, 6.70 mmol). The solution was allowed to stir overnight at room temperature under argon and concentrated under reduced pressure. The residue was dissolved into diethyl ether (100 mL), water (50 mL), and 10% sodium hydroxide solution (50 mL). The water layer was extracted with diethyl ether (2 × 50 mL) and brine (1 × 300 mL), dried ($MgSO_4$), and concentrated to a yellow oil. The oil was passed through a gravity silica column using 1% methanol/99% chloroform as the solvent. The resultant oil was converted to the hydrochloride salt using 3 N methanolic HCl. Recrystallization from dichloromethane/hexane gave **30** as white crystals, 338 mg (40%): mp 146–149 °C; ^1H NMR ($CDCl_3$/TMS) δ 7.38–7.30 (m, 10 H, ArH), 6.48 (s, 1 H, ArH), 6.42 (s, 2 H, ArH), 5.04 (d, 1 H, H_A of H_{AB}, J = 9.9 Hz, $ArCH_2O$), 4.96 (d, 1 H, H_B of H_{AB}, J = 9.9 Hz, $ArCH_2O$), 4.86 (d, 1 H, H_A of H_{AB}, J = 11.7 Hz, $ArCH_2O$), 4.77 (d, 1 H, H_B of H_{AB}, J = 11.7 Hz, $ArCH_2O$), 3.81 (s, 3 H, OCH_3), 3.79 (s, 6 H, 2 OCH_3), 3.57–3.50 (m, 1 H, ArCHN), 3.22–3.10 (m, 6 H, $ArCH_2CH_2N$ and $ArCH_2$); MS m/z 593 (M^+ – HCl), 412 (M^+ – trimethoxybenzyl – HCl), 322, 181, 91 (base). Anal. ($C_{34}H_{34}F_3NO_5$·HCl) C, H, N.

6,7-Dihydroxy-1-(3′,4′,5′-trimethoxybenzyl)-5-(trifluoromethyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (7). A solution of tetrahydroisoquinoline **30** (150 mg, 0.24 mmol) in an equivolume mixture of methanol/concentrated hydrochloric acid (20 mL) was refluxed for 2 h, cooled, and concentrated under reduced pressure. The residue was taken up into methanol (25 mL) and concentrated (repeated two more times). The resultant solid was recrystallized from methanol/diethyl ether to afford **7** as a white powder, 81 mg (75%): mp 260–263 °C dec; IR (KBr, cm^{-1}) 3400–3200; ^1H NMR (CD_3OD) δ 6.81 (s, 1 H, ArH), 6.63 (s, 2 H, ArH), 4.74 (dd, J = 5.8 and 8.5 Hz, 1 H, ArCHN), 3.83 (s, 6 H, 2 OCH_3), 3.76 (s, 3 H, OCH_3), 3.49–3.02 (m, 6 H, $ArCH_2CH_2N$ and $ArCH_2$); MS FAB 414 (M^+ – HCl), 232 (base). Anal. ($C_{20}H_{22}F_3NO_5$·HCl) C, H, N.

***N*,*N*-Dimethyl-2,5-difluoro-4-hydroxy-3-methoxybenzylamine (36).** A mixture of 3,6-difluoro-2-hydroxyanisole[25] (**35**, 5.00 g, 31.09 mmol), 37% formaldehyde (6 mL), and 40% dimethylamine (8 mL) in ethanol (30 mL) was heated at reflux for 3 h. The resultant solution was evaporated to give a residue which was purified via flash chromatography using 100% diethyl ether as the solvent. Recrystallization from dichloromethane/diethyl ether gave 6.20 g (91%) of **36** as colorless crystals: mp 116.5 °C; IR (KBr, cm^{-1}) 2970, 2920, 1595, 1465, 1370, 1355, 1070; ^1H NMR ($CDCl_3$) δ 6.77 (dd, J = 6.4 and 10.8 Hz, 1 H, ArH), 3.96 (d, J = 1.6 Hz, 3 H, OCH_3), 3.47 (d, J = 1.6 Hz, 2 H, CH_2), 2.29 (s, 6 H, 2 CH_3). Anal. ($C_{10}H_{13}F_2NO_2$) C, H, N.

2,5-Difluoro-4-hydroxy-3-methoxybenzyl Cyanide (38). To a solution of benzylamine **36** (12.0 g, 55.0 mmol) in methanol (50 mL) was added iodomethane (15.0 g, 106.0 mmol), and the resultant mixture was stirred at room temperature for 4 h. The solvent was evaporated to give a residue which was washed with diethyl ether and recrystallized with methanol to afford 18.0 g (90%) of the ammonium salt: dec point > 280 °C. The ammonium salt (15.0 g, 42.0 mmol) was dissolved in dimethyl sulfoxide (100 mL), sodium cyanide (4.0 g, 82.0 mmol) was added, and the mixture was stirred for 2 h at 0 °C and then for 15 h at room temperature. The solution was poured into water (200 mL),

acidified with concentrated hydrochloric acid, and extracted with ethyl acetate (3 × 100 mL). The combined extracts were dried ($MgSO_4$) and evaporated to give a solid residue. Purification by flash chromatography using 25% hexane in diethyl ether and recrystallization from dichloromethane/hexane gave nitrile **38**, 7.3 g (88%): mp 97 °C; IR (KBr, cm^{-1}) 3200, 2270, 1513, 1475, 1065; ^1H NMR ($CDCl_3$) δ 6.90 (dd, J = 9.8 and 6.36 Hz, 1 H, ArH), 5.64 (br s, 1 H, OH), 4.05 (d, J = 1.9 Hz, 3 H, OCH_3), 3.67 (s, 2 H, CH_2). Anal. ($C_9H_7F_2NO_2$) C, H, N.

2,5-Difluoro-3,4-dihydroxyphenylacetonitrile (40). To a solution of **38** (6.0 g, 30.1 mmol) in dichloromethane (60 mL) cooled in an ice bath under a nitrogen atmosphere was added 1 M boron tribromide in dichloromethane (60 mL) dropwise over 5 min. The solution was stirred for 1 h at 0 °C and then for 20 h at room temperature. The resultant mixture was poured into 10% cold ammonium hydroxide solution (150 mL), and the organic layer was extracted with water (2 × 50 mL). The combined extracts were acidified with concentrated hydrochloric acid, extracted with ethyl acetate (5 × 75 mL), and dried (Na_2SO_4). The product was purified via short flash chromatography using 100% diethyl ether as the solvent. Recrystallization from diethyl ether/cyclohexane gave 4.0 g (72%) of **40**: mp 135 °C; IR (KBr, cm^{-1}) 3370, 3180, 2290, 1650, 1620, 1560, 1490; ^1H NMR (CD_3OD) δ 6.62 (dd, J = 6.7 and 10.82 Hz, 1 H, ArH), 3.77 (d, J = 1.3 Hz, 2 H, CH_2). Anal. ($C_8H_5F_2NO_2$) C, H, N.

3,4-Bis(benzyloxy)-2,5-difluorophenylacetonitrile (42). A mixture of catechol **40** (8.5 g, 46.0 mmol), benzyl chloride (12.0 g, 94.0 mmol), potassium carbonate (13.0 g, 94.0 mmol), and sodium iodide (0.7 g, 4.7 mmol) in acetone (1 liter) was refluxed for 24 h, followed by filtration and removal of solvent. The residue obtained was taken up into ethyl acetate and washed with water and dried ($MgSO_4$). Flash column chromatography (10% ethyl acetate in hexane) followed by recrystallization from diethyl ether/hexane gave 14.6 g (87%) of **42**: mp 27–28 °C; IR (KBr, cm^{-1}) 2250, 1495, 1470, 1105, 1065; ^1H NMR ($CDCl_3$) δ 7.36 (m, 10 H, ArH), 6.91 (dd, J = 10.5 and 6.4 Hz, 1 H, ArH), 5.12 (s, 2 H, $ArCH_2O$), 5.11 (s, 2 H, $ArCH_2O$), 3.68 (s, 2 H, CH_2). Anal. ($C_{22}H_{17}F_2NO_2$) C, H, N.

2-[3,4-Bis(benzyloxy)-2,5-difluorophenyl]ethylamine Hydrochloride (44). To a solution of nitrile **42** (6.4 g, 17.5 mmol) in dry THF (40 mL) was added 1 M diborane in THF (95 mL) over 20 min with ice-bath cooling. The resultant mixture was stirred at room temperature for 1 h and then refluxed for 15 h followed by addition of methanol (20 mL) at 0 °C and refluxing for 1 h. The solution was concentrated, and the resultant oily residue was taken up into dichloromethane, washed with saturated sodium bicarbonate solution, dried (Na_2SO_4), and concentrated to the oily amine, 6.1 g (94%). The free amine in chloroform was converted to the hydrochloride salt by bubbling HCl gas into the solution. Recrystallization from dichloromethane/cyclohexane gave **44** as colorless crystals: mp 107–108 °C; IR (KBr, cm^{-1}) 3030, 1590, 1570, 1500, 1355, 1105; ^1H NMR ($CDCl_3$) δ 7.33 (m, 10 H, ArH), 6.80 (dd, J = 10.49 and 6.68 Hz, 1 H, ArH), 5.06 (s, 4 H, 2 $ArCH_2O$), 3.09 (m, 4 H, 2 CH_2). Anal. ($C_{22}H_{21}F_2NO_2$·HCl) C, H, N.

***N*-[2-[3,4-Bis(benzyloxy)-2,5-difluorophenyl]ethyl]-3′,4′,5′-trimethoxyphenylacetamide (46).** A mixture of free amine **44** (6.1 g, 17.7 mmol), 3,4,5-trimethoxyphenylacetic acid (4.0 g, 17.7 mmol), and toluene (150 mL) was refluxed for 3 days with azeotropic removal of water using a Dean–Stark trap. The solvent was removed to give a residue which was purified by flash column chromatography using 60% ethyl acetate in hexane as the solvent. Recrystallization from dichloromethane/cyclohexane gave 8.9 g (94%) of amide **46**: mp 78–79 °C; IR (KBr, cm^{-1}) 3290, 1645, 1500, 1475, 1460, 1255, 1140; ^1H NMR ($CDCl_3$) δ 7.34 (m, 10 H, ArH), 6.57 (dd, J = 10.8 and 6.4 Hz, 1 H, ArH), 6.41 (s, 2 H, ArH), 5.47 (br t, 1 H, NH), 5.08 (s, 2 H, $ArCH_2O$), 5.07 (s, 2 H, $ArCH_2O$), 3.83 (s, 3 H, OCH_3), 3.82 (s, 6 H, 2 OCH_3), 3.47 (s, 2 H, CH_2), 3.42 (q, 2 H, CH_2), 2.73 (t, 2 H, CH_2). Anal. ($C_{33}H_{33}F_2NO_6$) C, H, N.

6,7-Bis(benzyloxy)-1-(3′,4′,5′-trimethoxybenzyl)-5,8-difluoro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (48). A mixture of amide **46** (0.80 g, 1.38 mmol) and phosphorus oxychloride (0.80 g, 5.23 mmol) in dry benzene (30 mL) was refluxed for 2 h under a nitrogen atmosphere. The resulting solution was washed with saturated sodium bicarbonate solution, dried (Na_2SO_4), and purified via flash column chromatography (25% diethyl ether in dichloromethane) to give the oily dihydroisoquinoline, 0.55 g (71%): IR (KBr, cm^{-1}) 2920, 2840, 1585, 1565, 1450, 1123; ^1H NMR ($CDCl_3$) δ 7.32 (m, 10 H, ArH), 6.47 (s, 2 H, ArH), 5.13 (s, 2 H, $ArCH_2O$), 4.98 (s, 2 H, $ArCH_2O$), 4.06 (d, J = 2.4 Hz, 2 H, CH_2), 3.79 (s, 3 H, OCH_3), 3.78 (s, 6 H, 2 OCH_3), 3.65 (br t, J = 7.2 Hz, 2 H, CH_2), 2.60 (br t, J = 7.5 Hz, 2 H, CH_2). To a solution of the dihydroisoquinoline (1.60 g, 2.86 mmol) in ethanol (50 mL) was added sodium borohydride (2.30 g, 60.00 mmol) with ice-bath cooling. The resulting mixture was stirred at room temperature for 30 min and then refluxed for 15 h. The solution was concentrated, taken up into chloroform, washed with saturated sodium bicarbonate solution, and concentrated to an oily residue. Purification via flash column chromatography using 100% diethyl ether as the solvent followed by treatment with 4 N ethanolic HCl (5 mL) and evaporation to dryness in vacuo gave tetrahydroisoquinoline **48** as the hydrochloride salt, 0.95 g (56%): IR (KBr, cm^{-1}) 2940, 1595, 1465, 1130; ^1H NMR ($CDCl_3$) δ 7.37 (m, 10 H, ArH), 6.39 (s, 2 H, ArH), 5.12 (s, 2 H, $ArCH_2O$), 5.10 (s, 2 H, $ArCH_2O$), 4.97 (br t, 1 H, CH), 3.80 (s, 3 H, OCH_3), 3.72 (s, 6 H, 2 OCH_3), 3.34 (dd, 1 H, CH_2), 3.29 (dd, 2 H, CH_2), 3.03 (m, 1 H, CH_2), 2.80 (br d, 2 H, CH_2). Anal. ($C_{33}H_{33}F_2NO_5$·HCl) C, H, N.

5,8-Difluoro-6,7-dihydroxy-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (4). A mixture of tetrahydroisoquinoline **48** (0.85 g, 1.42 mmol) and 10% palladium on carbon (0.50 g) in ethanol (20 mL) was hydrogenated using a Parr apparatus at 50 psi for 2 h. The resulting mixture was filtered and evaporated to give a residue which was recrystallized twice from ethanol to afford 0.36 g (60%) of **4** as colorless crystals: mp 154–155 °C; IR (KBr, cm^{-1}) 3400, 1600, 1510, 1490, 1245, 1125; ^1H NMR (DMSO-d_6) δ 9.85 (br s, 1 H, OH), 9.75 (br s, 1 H, OH), 6.60 (s, 2 H, ArH), 4.84 (br t, 1 H, CH), 3.73 (s, 6 H, 2 OCH_3), 3.63 (s, 3 H, OCH_3), 3.34–3.12 (m, 4 H, 2 CH_2), 2.85 (m, 2 H, CH_2). Anal. ($C_{19}H_{21}F_2NO_5$·HCl·H_2O) C, H, N.

2-Hydroxy-3-(trifluoromethyl)anisole (34). To a solution of 2.15 M *n*-butyllithium in hexane (63.6 mL) in dry THF (200 mL) cooled to −78 °C under argon was added dropwise a solution of commercially available (Aldrich) 3-(trifluoromethyl)anisole (**33**, 20.00 g, 113.50 mmol) in dry THF (20 mL). After complete addition, the mixture was warmed to 0 °C and allowed to stir for 4 h. Cu^IBr (16.28 g, 113.50 mmol) was then charged into the reaction vessel, and the solution was allowed to stir for an additional 2 h at 0 °C. The flask was equipped with a Pasteur pipette with an attached drying tube. Dry air was drawn into the reaction mixture at 0 °C for 45 min by applying a vacuum aspirator to the flask. Concentrated hydrochloric acid (22.40 mL) was added, and the solution was stirred at room temperature for 20 h. 10% Hydrochloric acid solution (75 mL) was added, and the solution was extracted with diethyl ether (2 × 200 mL). The combined ether layers were washed with saturated sodium bicarbonate solution (1 × 100 mL), dried ($MgSO_4$), and concentrated under reduced pressure to a brown oil. Gravity column chromatography was performed using a gradient solvent system of dichloromethane/hexane, 20:80 to 40:60 (R_f = 0.67 silica on glass using dichloromethane as solvent). The resultant light yellow solid was recrystallized form ethanol/water to yield **34** as white needles, 12.77 g (58%): mp 51–53 °C; $FeCl_3$ positive; IR (KBr, cm^{-1}) 3410, ^1H NMR (acetone-d_6) δ 9.65 (br s, 1 H, OH), 7.21 (d, J = 8 Hz, 1 H, ArH), 7.06 (dd, J_m = 1.1 Hz, J_o = 8 Hz, 1 H, ArH), 6.88 (apparent t, J = 8 Hz, ArH), 3.84 (s, 3 H, OCH_3); MS m/z 192 (M^+). Anal. ($C_9H_7O_4F_3$) C, H.

***N*,*N*-Dimethyl-4-hydroxy-3-methoxy-5-(trifluoromethyl)benzylamine Oxalate (37).** To a solution of 37% formaldehyde (6.34 mL, 78.06 mmol) and 40% dimethylamine (8.78 mL, 78.06 mmol) in ethanol (100 mL) was added phenol **34** (10.00 g, 52.04 mmol). The solution was heated to reflux for 2 h, cooled, and concentrated under reduced pressure. The resultant solid was dissolved into methanol (50 mL) to which oxalic acid dihydrate (6.56 g, 52.04 mL) in methanol (20 mL) was added. The white crystalline precipitate was collected to yield **37**, 13.23 g (75%): mp 201–202 °C; IR (KBr, cm^{-1}) 3026, 3227; ^1H NMR (DMSO-d_6) δ 7.36 (s, 1 H, ArH), 7.22 (s, 1 H, ArH), 4.11 (s, 2 H, CH_2), 3.85 (s, 3 H, OCH_3), 2.26 (s, 6 H, 2 CH_3); MS m/z 249 ($M^+ - C_2H_2O_4$), 205 ($M^+ - C_2H_2O_4 - NMe_2$), 185, 58 (base). Anal. ($C_{11}H_{14}F_3NO_2$·$C_2H_2O_4$) C, H, N.

4-Hydroxy-3-methoxy-5-(trifluoromethyl)benzyl Cyanide (39). To a solution of the free base of benzylamine **37** (7.32 g, 29.37 mmol) in dichloromethane (100 mL) was added iodomethane (14.00 mL), and the solution was allowed to stir overnight at room temperature. The solution was concentrated under reduced pressure, the residue was dissolved in dimethyl sulfoxide (100 mL) to which sodium cyanide (2.16 g, 44.05 mmol) was added, and the solution was allowed to stir at room temperature for 7 h. The reaction mixture was diluted with water (100 mL), acidified to pH 1 (litmus) with 6 N hydrochloric acid solution, extracted with ethyl acetate (2 × 600 mL) and brine (1 × 600 mL), dried ($MgSO_4$), and concentrated under reduced pressure to a yellow solid. The solid was recrystallized from ethyl acetate/hexane to yield **39** as yellow needles, 4.90 g (72%): mp 110–111 °C; IR (KBr, cm^{-1}) 3364, 2264; ^1H NMR ($CDCl_3$/TMS) δ 7.06 (d, J = 1.2 Hz, 1 H, ArH), 6.99 (d, J = 1.7 Hz, 1 H, ArH), 6.15 (s, 1 H, OH), 3.97 (s, 3 H, OCH_3), 3.72 (s, 2 H, CH_2); MS m/z 231 (M^+, base). Anal. ($C_{10}H_6F_3NO_2$) C, H, N.

3,4-Dihydroxy-5-(trifluoromethyl)benzyl Cyanide (41). To a solution of benzyl cyanide **39** (3.63 g, 15.70 mmol) in dichloromethane (25 mL) cooled to 0 °C under argon was added 1 M boron tribromide in dichloromethane (31.40 mL). The solution was warmed to room temperature and allowed to stir overnight at which time the reaction mixture was concentrated under reduced pressure and taken up into ethyl acetate (250 mL). The organic solution was washed with water (2 × 250 mL) and brine (1 × 250 mL), dried ($MgSO_4$), and concentrated to a white solid which was recrystallized from ethyl acetate/hexane to afford **41** as a white solid, 3.27 g (96%): mp 151–152 °C; IR (KBr, cm^{-1}) 3230, 2267; ^1H NMR (acetone-d_6) δ 8.85 (br s, 2 H, 2 OH), 7.14 (d, J_m = 1.5 Hz, 1 H, ArH), 7.07 (d, J_m = 1.5 Hz, 1 H, ArH), 3.88 (s, 2 H, CH_2); MS m/z 217 (M^+), 197 (base). Anal. ($C_9H_6F_3NO_2$) C, H, N.

3,4-Bis(benzyloxy)-5-(trifluoromethyl)benzyl Cyanide (43). To a solution of catechol **41** (3.83 g, 17.64 mmol), potassium carbonate (5.48 g, 36.69 mmol), and potassium iodide (0.44 g, 2.65 mmol) in acetone (100 mL) was added 97% benzyl chloride (4.7 mL, 39.7 mmol). The solution was stirred at reflux for 5 h, cooled, and concentrated under reduced pressure. The residue was dissolved into ethyl acetate (100 mL) and water (100 mL). The organic layer was separated, washed with water (2 × 100 mL) and brine (1 × 100 mL), dried ($MgSO_4$), and concentrated to an orange oil. The oil was passed through a gravity silica column using 20% ethyl acetate/80% hexane as the solvent to yield a yellow solid which was recrystallized form ethyl acetate/hexane to afford **43** as white cubic crystals, 5.79 g (82%): mp 68–70 °C; IR (KBr, cm^{-1}) 2254; ^1H NMR ($CDCl_3$/TMS) δ 7.44–7.30 (m, 10 H, ArH), 7.17 (s, 1 H, ArH), 7.14 (s, 1 H, ArH), 5.17 (s, 2 H, $ArCH_2O$), 5.09 (s, 2 H, $ArCH_2O$), 3.73 (s, 2 H, CH_2CN); MS m/z 306 (M^+ – benzyl), 91 (base). Anal. ($C_{23}H_{16}F_3NO_2$) C, H, N.

2-[3,4-Bis(benzyloxy)-5-(trifluoromethyl)phenyl]ethylamine Hydrochloride (45). To a solution of benzyl cyanide **43** (5.10 g, 12.83 mmol) in dry THF (100 mL) cooled to 0 °C under argon was added 1 M diborane in THF (51.3 mL). After refluxing for 18 h under argon, the solution was cooled to 0 °C and cautiously quenched with methanol (50 mL). The solution was concentrated under reduced pressure, taken up into methanol (50 mL), and concentrated again (repeated once again). The resultant colorless oil was taken up into methanol (20 mL), and with ice-bath cooling 6 N methanolic HCl (2.25 mL) was added. The solution was concentrated, taken up into methanol (100 mL), and concentrated (repeated once again). Recrystallization from methanol/diethyl ether gave **45** as a white solid, 5.16 g (90%): mp 147–150 °C; IR (KBr, cm^{-1}) 3400; ^1H NMR (CD_3OD) δ 7.52–7.26 (m, 11 H, ArH), 7.15 (d, J_m = 1.8 Hz, 1 H, ArH), 5.23 (s, 2 H, $ArCH_2O$), 5.04 (s, 2 H, $ArCH_2O$), 3.23–3.17 (m, 2 H, CH_2N), 3.01–2.95 (m, 2 H, $ArCH_2$); MS m/z 401 (M^+ – HCl), 91 (base). Anal. ($C_{23}H_{22}F_3NO_2$·HCl·0.5H_2O) C, H, N.

***N*-[2-[3,4-Bis(benzyloxy)-5-(trifluoromethyl)phenyl]ethyl]-3′,4′,5′-trimethoxyphenylacetamide (47).** Phenylethylamine hydrochloride **45** (4.76 g, 10.65 mmol) dissolved into dichloromethane (100 mL) was washed with saturated sodium bicarbonate solution (3 × 20 mL) and water (3 × 100 mL), dried ($MgSO_4$), and concentrated to an oil. The oil was dissolved into toluene (100 mL), and to the solution was added commercially available (Aldrich) 3,4,5-trimethoxyphenylacetic acid (2.41 g, 10.65 mmol). The mixture was heated to reflux for 72 h with removal of water via a Dean–Stark trap, cooled, and concentrated to a yellow solid. The solid was taken up into dichloromethane (75 mL), washed with water (1 × 75 mL), 10% HCl solution (2 × 75 mL), water (1 × 75 mL), saturated sodium bicarbonate solution (2 × 75 mL), and brine (1 × 75 mL), dried ($MgSO_4$), and concentrated under reduced pressure to a yellow oil which solidified upon standing. Recrystallization from hot toluene/diethyl ether produced **47** as white crystals, 5.15 g (79%): mp 114–115 °C; IR (KBr, cm^{-1}) 3280, 1649; ^1H NMR ($CDCl_3$/TMS) δ 7.46–7.30 (m, 10 H, ArH), 7.01 (s, 1 H, ArH), 6.98 (s, 1 H, ArH), 6.40 (s, 2 H, 2 ArH), 5.51 (br t, 1 H, NH), 5.11 (s, 2 H, $ArCH_2O$), 5.06 (s, 2 H, $ArCH_2O$), 3.83 (s, 3 H, OCH_3), 3.82 (s, 6 H, 2 OCH_3), 3.51–3.43 (m, 4 H, $ArCH_2C$ and $ArCH_2CO$), 2.78 (t, J = 7.1 Hz, 2 H, CH_2N); MS m/z 609 (M^+), 91 (base). Anal. ($C_{34}H_{34}F_3NO_6$) C, H, N.

2,3-Bis(benzyloxy)-5,6,13,13a-tetrahydro-1-iodo-9,10,11-trimethoxy-8*H*-dibenzo[*a*,*g*]quinolizine Hydrochloride (51). To a solution of hexamethylphosphoramide (20 mL) and copper powder (769 mg, 12.11 mmol) in a steel tube was added trifluoromethyl iodide (2.59 g, 13.22 mmol). The solution was stirred and heated to 120 °C for 2.5 h and cooled. In an Atmosbag (Aldrich) purged with and under argon, the solution was filtered through Celite (to remove excess copper) into a 50-mL round-bottom flask equipped with stir bar and the free base of tetrahydroisoquinoline **29** (400 mg, 0.61 mmol). The resultant green solution was stirred under argon at room temperature overnight. The mixture was heated to 70 °C and monitored by TLC (1% methanol/99% chloroform and several drops of ammonium hydroxide). After 1 week, TLC showed the absence of starting material, and the reaction mixture was cooled and diluted with ice–water (25 mL) and ethyl acetate (25 mL), and the layers were separated. The water layer was extracted with ethyl acetate (25 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (1 × 50 mL), dried ($MgSO_4$), and concentrated. The residue was passed through a silica gravity column using 55% ethyl acetate/45% hexane. The resultant oil was converted to the hydrochloride salt using 3 N methanolic HCl and treated with charcoal in methanol. Recrystallization from methanol/diethyl ether gave **51** as white crystals, 61 mg (14%): mp 202–203 °C dec; ^1H NMR ($CDCl_3$/TMS) δ 7.49–7.30 (m, 10 H, ArH), 6.85 (s, 1 H, ArH), 6.43 (s, 1 H, ArH), 5.11 (s, 2 H, $ArCH_2O$), 5.06 (d, H_A of H_{AB}, J = 10.1 Hz, 1 H, $ArCH_2O$), 4.96 (d, H_B of H_{AB}, J = 10.1 Hz, 1 H, $ArCH_2O$), 4.77–4.70 (m, 1 H, ArCHN), 4.64 (d, H_A of H_{AB}, J = 16.6 Hz, 1 H, C-8-methylene), 4.45 (d, H_B of H_{AB}, J = 16.6 Hz, 1 H, C-8-methylene), 3.94 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 3.94–3.85 (m, obscured under methoxy peaks, 1 H, CH_2), 3.75–3.65 (m, 1 H, CH_2), 3.41–3.38 (br m, 2 H, CH_2), 2.99–2.69 (m, 2 H, CH_2); MS m/z 663 (M^+), 91 (base). Anal. ($C_{34}H_{34}INO_5$·HCl·1H_2O) C, H, N.

Preparative Chiralcel OD HPLC Separation of the Enantiomers of 6,7-Bis(benzyloxy)-8-fluorotrimetoquinol (52). The free base of 6,7-bis(benzyloxy)-8-fluoro-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (**52**)[12] was resolved on a preparative Chiralcel OD (Diacel) HPLC column (2 cm × 50 cm). The mobile phase was 70/30 hexane/2-propanol, and the flow rate was 6 mL/min. The separation was performed using a Beckman System Gold using an UV detector: k'_1 = 2.51; k'_2 = 3.53; α = 1.41; R_s = 0.69. Enantiomer 1: retention time = 88.79 min. Enantiomer s: retention time = 114.71 min. The hydrochloride salts were made using 3 N methanolic HCl and crystallized from diethyl ether.

Enantiomer 1, (*S*)·HCl: mp 207.5–209.5 °C dec; ^1H NMR (CD_3OD) δ 7.47–7.26 (m, 10 H, ArH), 6.86 (s, 1 H, ArH), 6.53 (s, 2 H, ArH), 5.16 (s, 2 H, $ArCH_2O$), 5.02 (s, 2 H, $ArCH_2O$), 4.97–4.92 (m, 2 H, ArCHNH), 3.79 (s, 6 H, OCH_3 × 2), 3.73 (s, 3 H, OCH_3), 3.53–3.44 (m, 1 H, CH_2), 3.35–3.26 (m, obscured by CD_3OD peak, 2 H, CH_2), 3.13–3.05 (m, 3 H, CH_2); $[\alpha]^{25}_D$ = +15.6° (c = 2.5 mg/mL in methanol); CD (reported in molecular ellipticity, in methanol) $[\theta]_{237}$ = +15 030.

Enantiomer 2, (*R*)·HCl: mp 208–209 °C; ^1H NMR (CD_3OD) same as enantiomer 1 described above; $[\alpha]^{25}_D$ = −14.7° (c = 4.6 mg/mL in methanol); CD (reported in molecular ellipticity, in methanol) $[\theta]_{234}$ = −17 403.

(*S*)-(+)-3,4-Dihydroxy-8-fluoro-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline [(*S*)-(+)-3]. To a Parr

bottle containing 10% palladium on carbon (18 mg) purged with argon was added enantiomer 1 of (*S*)-(+)-**52** (180 mg, 0.31 mmol) in methanol (50 mL). The solution was hydrogenated at 40 psi overnight and filtered through Celite, and the filtrate was concentrated to a dark orange oil. Trituration from diethyl ether gave (*S*)-(+)-**3** as a beige solid, 44 mg (35%): dp 115–120 °C; $FeCl_3$ positive; ^1H NMR (CD_3OD) δ 6.56 (s, 2 H, ArH), 6.51 (s, 1 H, ArH), 3.81 (s, 6 H, 2 OCH_3), 3.74 (s, 3 H, OCH_3), 3.49–2.91 (m, 7 H, 3 CH_2, CH); $[\alpha]^{25}_D$ = +4.4° (*c* = 2.5 mg/mL in methanol); CD (reported in molecular ellipticity, in methanol) $[\theta]_{221}$ = −6906, $[\theta]_{235}$ = +6089, $[\theta]_{253}$ = −182, $[\theta]_{278}$ = +1545.

(*R*)-(−)-3,4-Dihydroxy-8-fluoro-1-(3′,4′,5′-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline [(*R*)-(−)-3]. Procedure same as for (*S*)-(+)-**3** using enantiomer 2 of (*R*)-(−)-**52** (100 mg, 0.17 mmol). Trituration from diethyl ether gave (*R*)-(−)-**3** as a beige solid, 60 mg (88%): dp 116–120 °C; $FeCl_3$ positive; ^1H NMR (CD_3OD) same as (*S*)-(+)-**3** described above; $[\alpha]^{25}_D$ = −4.2° (*c* = 2.5 mg/mL in methanol); CD (reported in molecular ellipticity, in methanol) $[\theta]_{223}$ = +1091, $[\theta]_{235}$ = −7815, $[\theta]_{255}$ = −273, $[\theta]_{280}$ = −818.

β-Adrenergic Studies. Male albino Hartley guinea pigs (300–400 g) were used in all experiments. The isolation and procedures for testing of each compound (10^{-9} to 3×10^{-5}) with isolated spontaneously beating guinea pig right atria and tracheal strips were identical to those of Sober et al.[33] Guinea pig tracheal strips were partially contracted in the presence of 3×10^{-7} M carbachol, and cumulative concentration–response curves[34] were obtained for each compound in the tissues. Each successive drug concentration was added only after the effects of the previous concentration reached a maximum and remained constant. In other experiments, propranolol (30 nM) was added 30 min prior to the construction of concentration-response curve to analogues (*S*)-(+)-**3**, (*R*)-(−)-**3**, and **4**–**7**.

Inhibitory Activities in Rat Aorta. Thoracic aorta were isolated from male Sprague–Dawley rats, and spirally cut strips were prepared and mounted in a 10-mL water-jacketed chamber containing physiological salt solution as described previously.[35] U46619-induced isometric contractions of rat aortic strips were recorded on a polygraph. In experiments where TMQ or analogues (100 mM) were tested as inhibitors U46619-induced contractions, aortic strips were equilibrated for 2 h in Krebs-Heinseleit solution containing 3×10^{-6} M indomethacin. Cumulative concentration response curves (CRC's) of U46619 were obtained by the method of van Rossum.[34]

Platelet Antiaggregatory Activities. Blood was collected from normal human volunteers who reported to be free of medication for at least 10 days prior to blood collection. Platelet-rich plasma (PRP) was prepared as described previously[21] and used for all studies. Platelet aggregation studies were performed according to the turbidometric method of Born[36] in a Payton Model 600 dual-channel aggregometer interfaced to an Apple microcomputer for acquisition, quantification, presentation, and management of platelet aggregation data.[37] U46619 was used at the minimum concentration required to stimulate maximal aggregation in the presence of 1 mM aspirin. Inhibitors were added 1 min prior to induction of platelet activation, and inhibitory concentration-50 (IC_{50}) values for each inhibitor were determined from changes in the amplitude of light transmittance for 4 min after U46619 addition.

Data Analyses. Effective concentration-50 (EC_{50}) values of the drugs in atria and trachea, and of U46619 in rat aorta were determined graphically from individual plots of percent response versus log concentration and expressed as a pEC_{50} value. Blockade of drug response by propranolol in atria and trachea, and of U46619 responses in rat aorta by the drugs were also quantified by calculating their K_B values according to the method of Furchgott and Bursztyn.[38] A 5% level of significance was used to determine differences between control and drug-treated groups of data.

Radioligand Binding Analysis. An 150-μL aliquot of isolated membrane preparation containing 20–35 μg of protein, 50 μL of [I^{125}]iodocyanopindolol (ICYP) (20–50 pM), 25 μL of Tris-saline buffer, and 25 μL of competing drug at various concentrations (10^{-9}–10^{-3} M) were incubated for 55 min at 37 °C. Membrane-bound and free radioligand were separated by rapid filtration through Whatman GF/B filters using a Brandel Cell Harvester. The filters were washed with 10 mL of ice-cold buffer to remove any remaining free radioligand. An *n* of 3–8 was performed for each drug. Specific binding of ICYP was defined as the amount of radioligand bound in the absence of competing ligand minus the amount bound in the presence of 10^{-6} M propranolol. The radioactivity of the filter discs were measured in a Beckman Model 8000 gamma counter. Data were analyzed using a PC version of the radioligand binding program LIGAND.[39]

Acknowledgment. We thank the National Institutes of Health for their support in the form of Grant HL-22533.

(33) Sober, D. J.; Chang, J.; Fairchild, E. H.; Fowble, J. W.; Mukhopadhyay, A.; Feller, D. R.; Miller, D. D. Selective and Potent β_2-Adrenoceptor Agents Within the Tetrahydroisoquinoline Class: Effect of Methyl Substitution at the Benzylic Carbon of the 1-(3,4,5-Trimethoxybenzyl) Group of Trimetoquinol. *J. Med. Chem.* **1981**, *24*, 970–974.

(34) van Rossum, J. M. Cumulative Dose-Response Curves. II. Techniques for Making Dose-Response Curves in Isolated Organs and the Evaluation of Drug Parameters. *Arch. Intern. Pharmacodyn.* **1963**, *143*, 299–330.

(35) Fedyna, J. S.; Adejare, A.; Miller, D. D.; Feller, D. R. Pharmacological Evaluation of the β-Adrenergic Agonist and Thromboxane Antagonist Properties of N-Substituted Trimetoquinol Analogs. *Eur. J. Pharmacol.* **1987**, *135*, 161–171.

(36) Born, G. V. R. Aggregation of Blood Platelets by Adenosine Diphosphate and Its Reversal. *Nature (London)* **1962**, *194*, 927–929.

(37) Huzoor-Akbar; Romstedt, K.; Manhire, B. Computerized Aggregation Instruments: A Highly Efficient and Versatile System for Acquisition, Quantitation, Presentation and Management for Platelet Aggregation Data. *Thromb. Res.* **1983**, *32*, 335–341.

(38) Furchgott, R. F.; Bursztyn, P. Comparison of Dissociation Constants and of Relative Efficacies of Selected Agonists Acting on Parasympathetic Receptors. *Ann. N. Y. Acad. Sci.* **1967**, *144*, 882–898.

(39) McPherson, G. A. In *KINETIC, EBDA, LIGAND AND LOWERY: A Collection of Computer Programs for the Analysis of Radioligand Binding Experiments*; Elsevier: London, 1985; p 125.