Synthesis of Some Novel Potent and Selective Catechol O-Methyltransferase Inhibitors

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A series of disubstituted catechol derivatives was synthesized and tested as potential COMT inhibitors. The most active compounds were more than 1000 times more potent (IC_{50} = 3–6 nM) in vitro than the known COMT inhibitor, 3′,4′-dihydroxy-2-methylpropiophenone (U 0521, IC_{50} = 6000 nM). The new compounds were also highly selective COMT inhibitors with no activity against other essential enzymes involved in the synthesis and metabolism of catecholamines.

Catechol O-methyltransferase (COMT) catalyzes the transfer of the methyl group from S-adenosyl-L-methionine to phenolic hydroxyl groups of a number of compounds with catechol structures.[1] This enzyme is important in the extraneuronal inactivation of catecholamines and drugs with catechol structures.[2] It is present in most tissues, both in the periphery and in the central nervous system. The highest activities have been found in the liver, kidney, and intestine. COMT is probably present in soluble and membrane-bound forms, but the exact character of the two forms has not been established.[2,4,5]

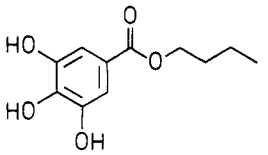
At present there is no effective and selective COMT inhibitor that could be used as a tool in biochemical and pharmaceutical research.[2] Even therapeutic use, alone or in combination with other drugs, for the treatment of diseases like Parkinson's disease, depression, heart failure, or hypertension by inhibiting selectively the metabolism of L-Dopa and endogenous amines (dopamine, adrenaline, noradrenaline) would be worth testing.

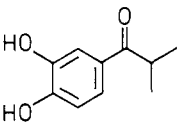
In the treatment of Parkinson's disease the drug of choice is still L-Dopa, the precursor of dopamine.[6] Unfortunately, L-Dopa is a good substrate for various enzymes and hence its oral bioavailability is low.

The greatest losses occur in the gastrointestinal tract through decarboxylation and 3-O-methylation.[7] The decarboxylation route can be blocked by two peripherally acting Dopa decarboxylase inhibitors, carbidopa and benserazide, which reduce peripheral dopamine formation and allow more L-Dopa to reach the brain.[8] When Dopa decarboxylase is inhibited, the 3-O-methylation route becomes dominant, and 3-O-methyldopa (3-OMD) formation is increased.[7,8] This leads to high plasma and brain 3-OMD/L-Dopa ratios, which have been suggested to correlate with the poor clinical potency or side effects of L-Dopa.[9] By combining a COMT inhibitor with L-Dopa and a decarboxylase inhibitor, the losses of L-Dopa through 3-O-methylation should decrease and the bioavailability of L-Dopa should improve.

A preliminary clinical trial was conducted on a known weak COMT inhibitor, butyl gallate, but because of the toxicity of the compound, no further studies were performed.[12] Another known COMT inhibitor, 3′,4′-dihydroxy-2-methylpropiophenone[11] (U 0521, 29), was also tested in one patient.[13] This compound has, however, the disadvantage of inhibiting also tyrosine hydroxylase, which is important for the biosynthesis of catecholamines.[10] The potency of compound **29** is also very low, probably because it is a good substrate for COMT.

The present investigation is based on the hypothesis that a substituted catechol derivative may act as a COMT inhibitor. Therefore, a series of new catechol derivatives





substituted with various chemical groups was synthesized and tested for in vitro activity as COMT inhibitors. The compounds and the in vitro COMT inhibitory activities in brain tissue are presented in Table I.

Chemistry

Of the simple model compounds **1–11**, which were used in quantitative structure–activity relationship studies,[14] the compounds **2**, **3**, **5**, and **6** have been described in literature.[15–18] 4-Chloro-6-nitrocatechol (**1**) was prepared from 4-chloroguaiacol[19] by nitration and subsequent demethylation. 3,4-Dihydroxy-5-nitrobenzonitrile (**4**) was obtained from compound **5** according to the method described by Olah and Keumi.[20] Compound **7** was prepared from 3-bromo-4,5-dimethoxybenzaldehyde[21] by the reac-

† University of Helsinki.

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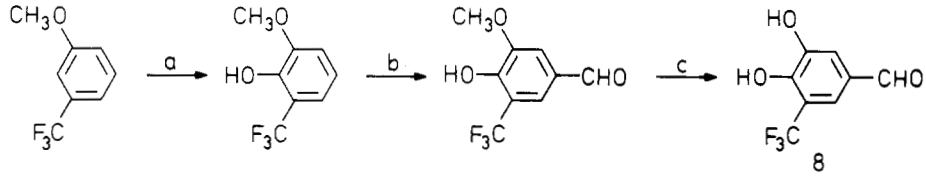
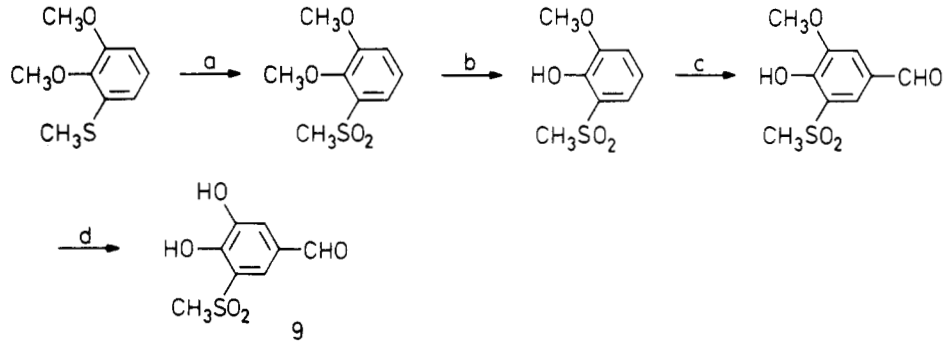
Scheme I[a]



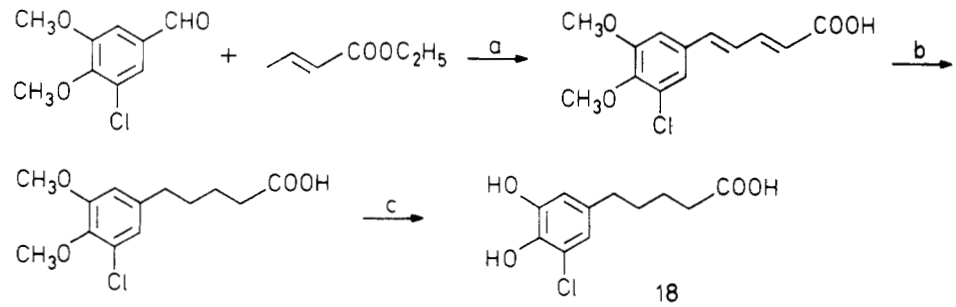
[a] (a) *n*-BuLi, $(CH_3O)_3B$, H_2O_2. (b) Hexamethylenetetramine, TFA. (c) BBr_3.

Scheme II[a]



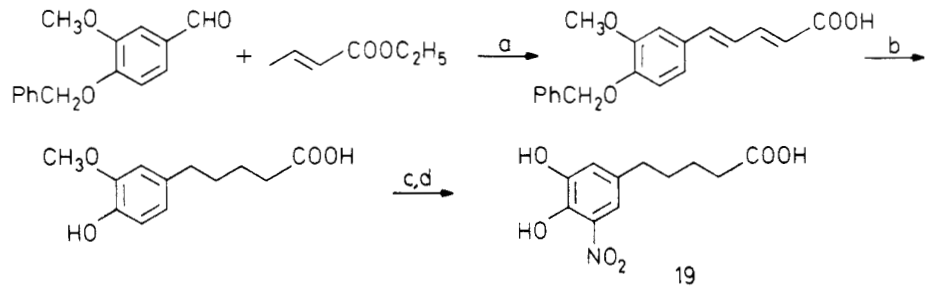
[a] (a) 3-Cl-$C_6H_4CO_3H$. (b) NaCN, DMSO. (c) Hexamethylenetetramine, TFA. (d) HBr.

Scheme III[a]



[a] (a) KOBu-*t*. (b) H_2, Pd/C. (c) HBr.

Scheme IV[a]

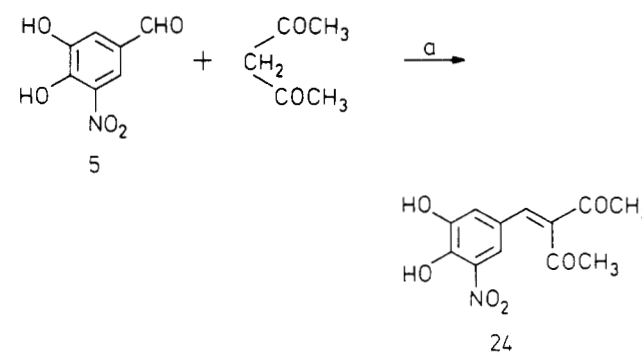


[a] (a) KOBu-*t*. (b) H_2, Pd/C. (c) HNO_3. (d) HBr.

tion with copper(I) cyanide in DMA and subsequent demethylation with boron tribromide. 3,4-Dihydroxy-5-(trifluoromethyl)benzaldehyde (**8**) was prepared from 3-(trifluoromethyl)anisole by a three-step synthesis described in Scheme I. 3,4-Dihydroxy-5-(methylsulfonyl)benzaldehyde (**9**) was synthesized from 2,3-dimethoxythioanisole in a series of reactions shown in Scheme II.

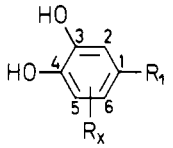
3′,4′-Dihydroxy-5′-nitroacetophenone (**10**) was prepared from 4′-hydroxy-3′-methoxyacetophenone by nitration and subsequent demethylation with concentrated hydrobromic acid in acetic acid. 3,4-Dihydroxy-5-nitrobenzyl alcohol (**11**) was obtained from compound **5** by reduction with sodium borohydride. The amides **12**–**17** were prepared from the corresponding 3,4-diacetoxy-(2, 5, or 6)-chloro- or (5 or 6)-nitrobenzoyl chlorides and 1-aminoadamantane

Scheme V[a]



[a] (a) H^+, THF.

Table I. Synthetic Data, Physical Constants, and COMT Inhibitory Activity of the Compounds Synthesized



compd	R_x	R_1	yield, %	mp, °C	recrys solv	formula	anal.	COMT inhibitory activity in brain tissue IC_{50}, nM
1	5-NO_2	1-Cl	63	108–111	MeOH–H_2O	$C_6H_4ClNO_4$	CHClN	25
2[a]	5-NO_2	1-NO_2	45	168–169	MeOH–H_2O	$C_6H_4N_2O_6$		12
3[b]	5-NO_2	1-COOH	58	220–225	MeOH–H_2O	$C_7H_5NO_6$		620
4	5-NO_2	1-CN	33	195–197	MeOEt–$PhCH_3$	$C_7B_4N_2O_4$	CHN	30
5[c]	5-NO_2	1-CHO	60	145–148	2-PrOH–H_2O	$C_7H_5NO_5$		24
6[d]	5-CHO	1-CHO	51	192–195	MeOH–H_2O	$C_8H_6O_4$		160
7	5-CN	1-CHO	74	218–220	EtOH	$C_8H_5NO_3$	CHN	160
8	5-CF_3	1-CHO	68	188–192	PhMe	$C_8H_5F_3O_3$		2300
9	5-CH_3SO_2	1-CHO	53	169–171	MeCOEt	$C_8H_8O_5S$	CHS	20000
10	5-NO_2	1-$COCH_3$	35	161–169	2-PrOH	$C_8H_7NO_5$	CHN	16
11	5-NO_2	1-CH_2OH	65	100 dec	Et_2O	$C_7H_7NO_5$	CHN	180
12	2-Cl	1-CONH-(1-adamantyl)	63	245–250	MeOH–H_2O	$C_{17}H_{20}ClNO_3$	CHClN	12000
13	5-Cl	1-CONH-(1-adamantyl)	71	244–247	MeOH–H_2O	$C_{17}H_{20}ClNO_3$	CHClN	400
14	6-Cl	1-CONH-(1-adamantyl)	68	225–230	EtOH–H_2O	$C_{17}H_{20}ClNO_3$	CHClN	2700
15	5-NO_2	1-CONH-(1-adamantyl)	85	207–209	MeOH–H_2O	$C_{17}H_{20}N_2O_5$	CHN	19
16	6-NO_2	1-CONH-(1-adamantyl)	75	250–255	EtOH–H_2O	$C_{17}H_{20}N_2O_5$	CHN	1100
17	5-CN	1-CONH-(1-adamantyl)	45	253–255	2-PrOH	$C_{18}H_{20}N_2O_3$	CHN	90
18	5-Cl	1-$(CH_2)_4$-COOH	71	99–101	PhMe	$C_{11}H_{13}ClO_4$	CHCl	8000
19	5-NO_2	1-$(CH_2)_4$-COOH	16	135–138	EtOAc–hexane	$C_{11}H_{13}NO_6$	CHN	90
20	5-NO_2	1-CH=$C(CN)_2$	50	208–210	MeOH–H_2O	$C_{10}H_5N_3O_4$	CHN	20
21	5-NO_2	1-CH=C(CN)$COOC_2H_5$	56	205–210	MeOH–H_2O	$C_{12}H_{10}N_2O_6$	CHN	50
22	5-NO_2	1-CH=C(CN)$CON(CH_3)_2$	23	183–185	EtOH	$C_{12}H_{11}N_3O_5$	CHN	18
23	5-NO_2	1-CH=$C(COCH_3)_2$	45	179–181	MeOH	$C_{12}H_{11}NO_6$	CHN	18
24	5-CF_3	1-CH=$C(COCH_3)_2$	45	200–205 dec	$CHCl_3$	$C_{13}H_{11}F_3O_4$		1500
25	5-NO_2	1-CH=$C(CH_3)COCH_3$	30	139–141	2-PrOH	$C_{11}H_{11}NO_2$	CHN	12
26	5-NO_2	1-CH=$CHCOC_6H_5$	68	198–200	2-PrOH	$C_{15}H_{11}NO_5$	CHN	6
27	5-NO_2	1-CH=$CHCOC_6H_2$-3,4,5-$(CH_3O)_3$	44	213–214	2-PrOH	$C_{18}H_{17}NO_8$	CHN	5
28	5-NO_2	1-CH=$CCH_2CH_2C(=O)CH=CHC_6H_2$-3,4-$(OH)_2$-5-$NO_2$	78	>350	MeCOEt	$C_{19}H_{14}N_2O_9$	CHN	3
29[e]	5-H	1-$COCH(CH_3)_2$	35	94–96	PhMe	$C_{10}H_{12}O_3$		6000

[a] Reference 15. [b] Reference 16. [c] Reference 17. [d] Reference 18. [e] Reference 11.

followed by deacetylation. The syntheses of compounds **18** and **19** were described in Schemes III and IV. Compounds **20–28** were obtained by condensation of compound **5** or **8** with compounds with an active methyl or methylene group in acidic conditions as illustrated in Scheme V for compound **23**.

Biological Results and Discussion

The in vitro COMT inhibitory activities of the compounds synthesized were determined in an enzyme preparation isolated from the brains of female Han: WISTAR rats with 3,4-dihydroxybenzoic acid as the substrate. The enzyme activity was measured by determining the concentration of 4-hydroxy-3-methoxybenzoic acid formed by HPLC with an electrochemical detector. The results are summarized in Table I.

Preliminary quantitative structure–activity relationship studies, including among others compounds **1–11**, indicated that the electronic effects of the 1- and 5-substituents on the catechol ring, lowering the pK_a of the hydroxyl groups and LUMO energy of the molecules, explained most of the biological variation.[14] However, compounds **8** and **9**, bearing trifluoromethyl or methylsulfonyl groups, respectively, at the ortho position, were both almost inactive (IC_{50} = 2300 and 20000 nM). The most active compound in this series (**1–11**) was compound **2** (IC_{50} = 12 nM), bearing two nitro groups at positions ortho and para to the same hydroxy group. This compound (**2**) was thus about 500 times more potent than **29**. If the nitro group at the para position in compound **2** was replaced by chlorine (**1**), cyano (**4**), formyl (**5**), or acetyl (**10**), the activity was decreased by about half compared to that of compound **2**.

Because some of these simple model substances (**1–11**) would be expected to have limited value as true drugs because of their toxicity or low oral absorption,[22] attempts have been made to improve the pharmacological properties and, if possible, the activities of these compounds by substituting the catechol moiety by other means. Therefore, a new series of catechol derivatives (**12–28**), substituted with various groupings, was synthesized.

It appeared soon that the compounds bearing a nitro group at the position ortho to a hydroxyl group were superior in potency to other groups. Other electron-withdrawing substituents (R_x), such as halogen (**12, 13**) or cyano (**17**), at the position ortho, and halogen (**14**) or nitro (**16**) at the position para to another hydroxyl group gave considerably lower activities. It was, however, possible to vary the substituent (R_1) at the para position within considerably wider limits without loss of activity (**15, 19–23, 25–27**). Substituents (R_1) containing a carbonyl group, especially those conjugated with the benzene ring directly or through a carbon–carbon double bond, increase the activity markedly (**21–23, 25–27**). Compounds **26–28**, which have a multiple conjugated system throughout the whole molecule, were the most active (IC_{50} = 6, 5, and 3

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Table II. Specificity of the New COMT Inhibitors to Some Essential Enzymes Involved in the Metabolism of Catecholamines (IC_{50}, nM)

compd	COMT	TH[a]	DBH[b]	DDC[c]	MAO-A[d]	MAO-B[e]
26	12	50 000	50 000	50 000	50 000	50 000
27	6	21 000	50 000	50 000	50 000	50 000
29	6000	24 000	50 000	50 000	50 000	50 000

[a] TH = tyrosine hydroxylase.[25] [b] DBH = dopamine-β-hydroxylase.[26] [c] DDC = dopamine decarboxylase.[27] [d] MAO-A. [e] MAO-B = monoamine oxidase A[28] and B[29].

nM, respectively) in this series. These compounds were about 1000–2000 times more potent than **29**. Increasing the lipophilic properties of the substituent R_1 also had a positive effect on the activity (cf. **15**). It is interesting to note that these new potent COMT inhibitors were very poor substrates for COMT, although they contain a catechol moiety. For example no O-methylated products (**30** or **31**) were detected by gas chromatography after incubation of compound **23** with the enzyme preparation.

In pharmacological tests, these compounds, e.g. **23**, at an oral dose of 30 mg/kg, administered in combination with L-Dopa and carbidopa inhibited the formation of 3OMD in rat serum by 80%.[23] The same dose of compound **29** had no effect.

The new compounds were extremely specific COMT inhibitors and not inhibitors of other essential enzymes involved in the metabolism of catecholamines, as shown in Table II. In addition, these compounds are rather weak inhibitors of the conjugating enzyme phenol sulfotransferase[24] (IC^{50} values on high micromolar level, e.g. IC_{50} values for compounds **2**, **10**, and **23** were 150, 125, and 210 μM, respectively). They are not readily sulfoconjugated by this enzyme, since only 1–5% of compounds are excreted as sulfoconjugates. The inhibition mechanism seems to be tight-binding competitive with respect to DBA and uncompetitive with respect to SAM. A more detailed study will be reported elsewhere.[30] The compounds were in general very atoxic; for example, the acute oral toxicity in Wistar rats was about 2500 mg/kg for compound **23**.

Experimental Section

Melting points were determined on a Kofler block and they are uncorrected. Elemental analyses were performed at the Mikroanalytisches Laboratorium by Dr. Ilse Beetz. The compounds were analzyed within a range of ±0.4% of the theoretical values for C, H, Cl, N, and S. The ^{1}H NMR spectra of all new compounds were measured on a JEOL FX90Q spectrometer with TMS as internal standard and were in agreement with the assigned structures. The mass spectra were recorded on a JEOL HX100 mass spectrometer. The purity of all the compounds synthesized was checked by TLC on a silica gel plate (Merck) using the solvent system toluene–dioxane–acetic acid (8:1:1). Gas chromatographic determinations were made on Micromat HRGC 412 gas chromatograph (FID detector) equipped with fused silica capillary column (25 m; i.d. 0.32 mm) coated with OV-1. The temperature was programmed from 150 °C to 240 °C at 25 °C min.

Biological Test Procedures. The enzyme preparation was obtained by homogenizing the tissues from the brains of female Han: WISTAR rats (average weight 100 g) in 10 mM phosphate buffer (pH 7.4) (1:10 weight g/mL) which contained 0.5 mM dithiothreitol. The homogenate was centrifuged at 15000*g* for 20 min. The supernatant was recentrifuged at 100000*g* for 60 min. All procedures were carried out at +4 °C.

The determination of IC_{50} values was performed by measuring the COMT activity in several drug concentrations of the reaction mixture (total volume 250 μL) containing the enzyme preparation (0.5 mg of protein in 25 μL), 0.4 mM 3,4-dihydroxybenzoic acid, as substrate, 5 mM $MgCl_2$ solution, 0.2 mM *S*-adenosyl-L-methione and COMT inhibitor in 0.1 M phosphate buffer (pH 7.4). To the control mixtures no COMT inhibitor was added. The mixture was incubated for 30 min at 37 °C; thereafter the reaction was stopped by perchloric acid, and the proteins precipitated were removed by centrifugation (at 4000*g* for 10 min).

The activity of the enzyme was measured by determining the concentration of 4-hydroxy-3-methoxybenzoic acid formed from the substrate by HPLC with an electrochemical detector. Chromatography was performed by injecting 20 μL of the sample in a 4.6 mm × 150 mm Spherisorb ODS column (particle size 5 μm). The reaction products were eluted from the column with 20% MeOH containing 0.1 M sodium dihydroen phosphate, 20 mM citric acid, and 0.15 mM EDTA, pH = 3.2, at a flow rate of 1.5 mL/min. The electrochemical detector was set at 0.9 V against an Ag/AgCl electrode. The concentration of 4-hydroxy-3-methoxybenzoic acid was compared with the control samples and those containing COMT inhibitor. The IC_{50} values of triplicate measurements were reliable to about ±10%.

4-Chloro-2-methoxy-6-nitrophenol. To a solution of 4-chloroguaiacol[19] (31.7 g, 0.2 mol) in dichloromethane (100 mL), was gradually added a 1 M solution of nitric acid in dichloromethane (200 mL) with stirring at 0–5 °C. The mixture was stirred for 10 min at room temperature and washed with water and the solvent was evaporated in vacuo. The residue was triturated with ether and the crystalline product was filtered: yield 19.0 g (47%); mp 101–104 °C; ^{1}H NMR (DMSO-d_6) δ 3.95 (s, 3 H), 7.36 (d, 1 H, *J* = 2 Hz), 7.53 (d, 1 H, *J* = 2 Hz), 10.7 (br s, 1 H).

4-Chloro-6-nitrocatechol (1). A mixture of 4-chloro-2-methoxy-6-nitrophenol (10.2 g, 0.05 mol) in concentrated hydrobromic acid (200 mL) was refluxed for 2 h. The solution was cooled to 0 °C and filtered, and the product was washed with cold water: yield 6.0 g (63%); mp 108–111 °C; ^{1}H NMR (DMSO-d_6) δ 7.06 (d, 1 H, *J* = 2 Hz), 7.38 (d, 1 H, *J* = 2 Hz), 10.5 (br s, 2 H).

3,4-Dihydroxy-5-nitrobenzonitrile (4). A solution of 3,4-dihydroxy-5-nitrobenzaldehyde (**5**[17]) (9.2 g, 0.05 mol) and hydroxylammonium chloride (4.9 g, 0.07 mol) in formic acid (50 mL) was refluxed for 1 h. The solution was cooled to 0 °C and filtered, and the product was washed with cold formic acid. Recrystallization from butanone–toluene yielded 3.0 g (33%) of the desired product: mp 193–195 °C; ^{1}H NMR (DMSO-d_6) δ 7.31 (J, 1 H, *J* = 2 Hz), 7.87 (J, 1 H, *J* = 2 Hz), 9.9 (br s, 2 H).

3-Cyano-4,5-dimethoxybenzaldehyde. A mixture of 3-bromo-4,5-dimethoxybenzaldehyde[21] (6.8 g, 0.028 mol) and copper(I) cyanide (2.7 g, 0.03 mol) in DMA (40 mL) was refluxed for 4 h with stirring. The solvent was evaporated in vacuo, and the residue was treated with water and filtered. The crude product was recrystallized from 2-propanol: yield 2.1 g (39%); mp 109–112 °C.

3-Cyano-4,5-dihydroxybenzaldehyde (7). To a solution of 3-cyano-4,5-dimethoxybenzaldehyde (1.9 g, 0.01 mol) in dichloromethane (40 mL) was added a 1 M solution of boron tribromide in dichloromethane (30 mL) at 0 °C with stirring. The solution was stirred overnight at 20 °C. The solvent was evaporated and the residue treated with dilute hydrochloric acid. After filtration the product was crystallized from EtOH: yield 1.2 g (74%); mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 7.43 (d, 1 H, *J* = 2 Hz), 7.70 (d, 1 H, *J* = 2 Hz), 9.74 (s, 1 H), 10.7 (br s, 2 H).

2-Methoxy-6-(trifluoromethyl)phenol. A solution containing 1.6 M butyllithium in hexane (160 mL), THF (300 mL), and *N,N,N',N'*-tetramethylethylenediamine (40 mL) was cooled to -78 °C, and 3-(trifluoromethyl)anisole (43.3 g, 0.25 mol) was added with stirring under a nitrogen atmosphere. The solution was warmed to room temperature and then again cooled to -78 °C after which trimethyl borate (35 mL) was added. The solution was warmed to 20 °C, and concnetrated ammonia solution (50 mL) was added. The solvents were evaporated in vacuo, and to the

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residue were added 98–100% formic acid (60 mL) and 35% hydrogen peroxide (25 mL). The solution was extracted with ether–petroleum ether (1:1). The organic phase was separated, and the product was extracted with 2.5 N sodium hydroxide solution. The aqueous phase was acidified with hydrochloric acid and extracted with dichloromethane. Most of the solvent was removed in vacuo after which petroleum ether was added. The crystalline product was filtered: yield 8.5 g (18%); mp 51–53 °C; ^{1}H NMR ($CDCl_3$) δ 3.9 (s, 3 H), 6.10 (s, 1 H), 6.7–7.1 (m, 3 H); HRMS m/z (M^+) calcd 192.0398, found 192.0396.

4-Hydroxy-3-methoxy-5-(trifluoromethyl)benzaldehyde. A solution of 2-methoxy-6-(trifluoromethyl)phenol (7.6 g, 0.04 mol) and hexamethylenetetramine (5.6 g, 0.04 mol) in trifluoroacetic acid (80 mL) was refluxed for 1 h. The solvent was removed in vacuo, 1 N hydrochloric acid (50 mL) was added to the residue, and the solution was extracted with dichloromethane. Most of the solvent was evaporated in vacuo, and petroleum ether was added, which the product was crystallized: yield 2.8 g (32%); mp 151–152 °C; ^{1}H NMR ($CDCl_3$) δ 4.02 (s, 3 H), 6.76 (s, 1 H), 7.56 (d, 1 H, J = 2 Hz), 7.68 (d, 1 H, J = 2 Hz), 9.87 (s, 1 H); HRMS m/z (M^+) calcd 220.0347, found 220.0344.

3,4-Dihydroxy-5-(trifluoromethyl)benzaldehyde (8). A solution of 4-hydroxy-3-methoxy-5-(trifluoromethyl)benzaldehyde (2.2 g, 0.01 mol) in 1 M boron tribromide in dichloromethane (65 mL) was stirred for 2 h at room temperature. Hydrochloric acid was added and the organic phase was separated. The solvent was evaporated in vacuo, and the residue was crystallized from toluene: yield 1.4 g (68%); mp 188–192 °C; ^{1}H NMR (DMSO-d_6) δ 7.46 (d, 1 H, J = 2 Hz), 7.60 (d, 1 H, J = 2 Hz), 9.78 (s, 1 H), 10.7 (br s, 2 H); MS m/z (M^+) 206 (100), 186 (79), 177 (4), 158 (19), 130 (15), 10 (43); HRMS m/z (M^+) calcd 206.0191, found 206.0186.

1,2-Dimethoxy-3-(methylsulfonyl)benzene. To a solution of 2,3-dimethoxythioanisole (3.68 g, 0.02 mol) in dichloromethane (50 mL) was added 3-chloroperoxybenzoic acid (3.6 g, 0.02 mol) with stirring. Stirring was continued for a further 18 h at room temperature. Sodium hydroxide (1 N, 30 mL) was added, the dichloromethane phase was separated, and the solvent was evaporated in vacuo: yield 3.7 g (86%); mp 80–83 °C (petroleum ether); ^{1}H NMR ($CDCl_3$) δ 3.22 (s, 3 H), 3.93 (s, 3 H), 4.03 (s, 3 H), 7.08–7.30 (m, 3 H); HRMS m/z (M^+) calcd 216.0456, found 216.0451.

2-Methoxy-6-(methylsulfonyl)phenol. A mixture of 1,2-dimethoxy-3-(methylsulfonyl)benzene (5.0 g, 0.023 mol) and sodium cyanide (5.7 g, 0.116 mol) in DMSO (20 mL) was heated for 2 h at 100 °C. The excess sodium cyanide was removed by filtration, and the solution was acidified with hydrochloric acid and extracted with ethyl acetate. The organic phase was washed with water, and the solvent was evaporated in vacuo: yield 3.5 g (75%); mp 80–82 °C (petroleum ether); ^{1}H NMR (DMSO-d_6) δ 3.20 (s, 3 H), 3.67 (s, 3 H), 6.44 (dd, 1 H, J = 8 Hz, 8 Hz), 6.98 (dd, 1 H, J = 8 Hz, 2 Hz), 7.14 (dd, 1 H, J = 8 Hz, 2 Hz); HRMS m/z (M^+) calcd 202.0300, found 202.0299.

4-Hydroxy-3-methoxy-5-(methylsulfonyl)benzaldehyde. A solution of 2-methoxy-6-(methylsulfonyl)phenol (3.0 g, 0.015 mol) and hexamethylenetetramine (4.2 g, 0.03 mol) in trifluoroacetic acid (60 mL) was refluxed overnight. The solvent was removed in vacuo, water was added to the residue, and the solution was extracted with ethyl acetate. The solvent was evaporated and dichloromethane was added, after which the product was crystallized: yield 1.5 g (43%); mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ 3.25 (s, 3 H), 3.99 (s, 3 H), 7.65 (d, 1 H, J = 2 Hz) 7.99 (d, 1 H, J = 2 Hz), 9.95 (s, 1 H).

3,4-Dihydroxy-5-(methylsulfonyl)benzaldehyde (9). A mixture of 4-hydroxy-3-methoxy-5-(methylsulfonyl)benzaldehyde (1.4 g, 0.006 mol) in concentrated hydrobromic acid (28 mL) was refluxed overnight. After cooling, the solution was extracted with ethyl acetate. The extract was washed with water, concentrated in vacuo, and filtered: yield 0.7 g (53%); mp 169–171 °C; ^{1}H NMR (DMSO-d_6) δ 3.24 (s, 3 H), 7.50 (d, 1 H, J = 2 Hz), 7.81 (d, 1 H, J = 2 Hz), 9.82 (s, 1 H).

3,4-Dihydroxy-5-nitrobenzyl Alcohol (11). To a solution of sodium borohydride (6.0 g, 0.16 mol) in water (50 mL) was gradually added 3,4-dihydroxy-5-nitrobenzaldehyde (5) (9.15 g, 0.05 mol) with stirring at room temperature. The mixture was stirred for 1 h after which it was acidified with hydrochloric acid. The solution was filtered to remove tarry impurities and extracted four times with ether. The ether extract was dried and concentrated to a volume of about 100 mL, and the crystalline solid was filtered: yield 6.0 g (65%); mp 100 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.43 (s, 2 H), 4.60 (br s, 1 H), 7.10 (d, 1 H, J = 2 Hz), 7.34 (d, 1 H, J = 2 Hz) 10.0 (br s, 2 H).

***N*-(1-Adamantyl)-3,4-dihydroxy-5-nitrobenzamide (15).** A solution of 3,4-diacetoxy-5-nitrobenzoic acid (0.85 g, 0.003 mol), a catalytic amount of DMF, and thionyl chloride (0.5 mL) in toluene (10 mL) was heated for 1 h at 80 °C. The solvent was evaporated in vacuo, and the residue was dissolved in dichloromethane (5 mL) and added to a mixture of 1-aminoadamantane hydrochloride (0.56 g, 0.0003 mol) and triethylamine (1 mL) in dichloromethane (10 mL). The mixture was stirred for 15 min at 0 °C and then for 15 min at 20 °C. Water was added to the reaction mixture, dichloromethane phase was separated, and the solvent was evaporated in vacuo, yielding a yellow viscous oil of 1.2 g (100%). The intermediate product was dissolved in MeOH (10 mL), a catalytic amount of sulfuric acid was added, and the solution was refluxed for 3 h. Water (20 mL) was added, and on cooling the product was crystallized: yield 0.85 g (90%); mp 207–208 °C; ^{1}H NMR (DMSO-d_6) δ 1.65 (s, 6 H), 2.05 (s, 9 H), 7.50 (d, 1 H, J = 2 Hz), 7.64 (s, 1 H), 7.85 (d, 1 H, J = 2 Hz), 10.45 (br s, 2 H).

5-(3-Chloro-4,5-dimethoxyphenyl)-2,4-pentadienoic Acid. To a solution of 3-chloro-4,5-dimethoxybenzaldehyde (10.0 g, 0.05 mol) and ethyl crotonate (7.6 g, 0.067 mol) in N-methylpyrrolidone (65 mL) was added potassium *tert*-butoxide (6.7 g, 0.06 mol) with stirring. The solution was stirred for 0.5 h and then poured onto a mixture of ice and hydrochloric acid. The solution was extracted with ether. The ether solution was washed with water and then extracted with $NaHCO_3$ solution. The aqueous phase was acidified with hydrochloric acid, and the semisolid product was separated and crystallized from ethyl acetate: yield 7.3 g (55%); mp 166–170 °C; ^{1}H NMR ($CDCl_3$) δ 3.89 and 3.92 (d, 6 H), 5.75 (d, 1 H, J = 12 Hz), 6.6–7.2 (m, 4 H), 7.96 (dd, 1 H, J = 12 Hz, J = 16 Hz), 10.0 (br s, 1 H); HRMS m/z (M^+) calcd 268.0502, found 268.0508.

5-(3-Chloro-4,5-dimethoxyphenyl)pentanoic Acid. A solution of 5-(3-chloro-4,5-dimethoxyphenyl)-2,4-pentadienoic acid (3.7 g), 10% palladium on charcoal (1.0 g), and concentrated hydrochloric acid (3 mL) in acetic acid (30 mL) was hydrogenated at normal pressure and room temperature. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo: yield 3.2 g (85%) of a viscous oil; ^{1}H NMR ($CDCl_3$) δ 1.5–1.8 (m, 4 H), 2.2–2.7 (m, 4 H), 3.84 and 3.86 (d, 6 H), 7.61 (d, 1 H, J = 2 Hz), 7.76 (d, 1 H, J = 2 Hz), 10.6 (br s, 1 H); HRMS m/z (M^+) calcd 272.0815, found 272.0819.

5-(3-Chloro-4,5-dihydroxyphenyl)pentanoic Acid (18). A solution of 5-(3-chloro-4,5-dimethoxyphenyl)pentanoic acid (3.2 g) and acetic acid (8 mL) in concentrated hydrobromic acid (10 mL) was refluxed for 3 h. A saturated solution of sodium sulfate was added to the reaction mixture. The crystalline product was filtered, washed with water, and recrystallized from toluene: yield 2.0 g (71%); mp 99–101 °C; ^{1}H NMR (DMSO-d_6) δ 1.4–1.7 (m, 4 H), 2.1–2.6 (m, 4 H), 6.54 (s, 2 H), 8–11 (br s, 3 H).

5-(3,4-Dihydroxy-5-nitrophenyl)pentanoic Acid (19). 4-(Benzyloxy)-3-methoxybenzaldehyde (242.0 g, 1 mol) was condensed with ethyl crotonate (186.0 g, 1.6 mol) as described above to form 5-[4-(benzyloxy)-3-methoxyphenyl]-2,4-pentadienoic acid as a semisolid product: ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3 H), 5.16 (s, 2 H), 5.67 (d, 1 H, J = 12 Hz), 6.6–7.1 (m, 5 H), 7.2–7.5 (m, 5 H), 7.93 (dd, 1 H, J = 12 Hz, J = 15 Hz), 11.5 (br s, 1 H).

The product (210 g) was dissolved in DMF (500 mL), and 10% palladium on charcoal catalyst (22 g) was added. The mixture was hydrogenated at 60 °C and normal pressure. After filtration, the solvent was evaporated in vacuo and the residue was dissolved in dichloromethane (1 L) and washed with water (2 L). The product was extracted with saturated $NaHCO_3$ solution. After acidification of the aqueous phase with hydrochloric acid, the product was extracted with dichloromethane. The solvent was evaporated in vacuo, leaving 5-(4-hydroxy-3-methoxyphenyl)-pentanoic acid as an oil (180 g): ^{1}H NMR ($CDCl_3$) δ 1.5–1.8 (m, 4 H), 2.0–2.7 (m, 4 H), 3.87 (s, 3 H), 7.5–7.9 (m, 3 H), 6–10 (2-H). This product was dissolved in dichloromethane (1 L), and 1 M HNO_3–dichloromethane solution (820 mL) was gradually added with stirring and cooling (0–5 °C). The solution was stirred for 10 min at 0 °C. The organic phase was washed with water and

the solvent was evaporated in vacuo to give 5-(4-hydroxy-3-methoxy-5-nitrophenyl)pentanoic acid as a semisolid mass. After crystallization from acetic acid–water, the yield was 45 g; mp 102–104 °C; ^{1}H NMR (DMSO-d_6) δ 1.59–1.85 (m, 4 H), 2.25–2.75 (m, 4 H), 3.96 (s, 3 H), 6.95 (d, 1 H, J = 2 Hz), 7.48 (d, 1 H, J = 2 Hz), 10.4 (br s, 2 H).

The above product was dissolved in a mixture of acetic acid (500 mL) and concentrated hydrobromic acid (500 mL) and refluxed for 4 h. A saturated Na_2SO_4 solution (1 L) was added to the reaction mixture, and the solution was allowed to stand overnight at 5 °C. The crystals were filtered and washed with 50% acetic acid and then recrystallized from ethyl acetate–hexane: yield 32.0 g (16%); mp 135–138 °C; ^{1}H NMR (DMSO-d_6) δ 1.46–1.6 (m, 4 H), 2.05–2.6 (m, 4 H), 6.94 (J, 1 H, J = 2 Hz), 7.17 (J, 1 H, J = 2 Hz), 9.5–11.4 (br s, 3 H).

3,4-Dihydroxy-5-nitro-ω,ω-dicyanostyrene (20). A solution of **5** (3.66 g, 0.02 mol), malononitrile (3.3 g, 0.05 mol), and ammonium acetate (1 g) in MeOH (10 mL) was refluxed for 4 h. Water was added, and the product was filtered: yield 2.3 g (50%); mp 205–209 °C; ^{1}H NMR (DMSO-d_6) δ 7.76 (d, 1 H, J = 2 Hz), 7.98 (d, 1 H, J = 2 Hz), 8.34 (s, 1 H), 9.2 (br s, 2 H).

3-(3,4-Dihydroxy-5-nitrobenzylidene)-2,4-pentanedione (23). A solution of **5** (1.83 g, 0.01 mol) and 2,4-pentanedione (1.00 g, 0.01 mol) in THF (10 mL) was saturated with gaseous hydrogen chloride. After standing overnight at 5 °C, the product was filtered and washed with ether: yield 1.2 g (45%); mp 175–178 °C; ^{1}H NMR (DMSO-d_6) δ 2.27 (s, 3 H), 2.41 (s, 3 H), 7.12 (d, 1 H, J = 2 Hz), 7.59–7.62 (t, 2 H); MS m/z (M^+) 265 (10), 250 (6), 222 (7), 208 (12), 43 (100).

3-(3,4-Dihydroxy-5-nitrophenyl)-1-phenylprop-2-en-1-one (26). This compound was prepared in a similar manner as compound **23**, with methanol as solvent: yield 68%; mp 192–195 °C; ^{1}H NMR (DMSO-d_6) δ 7.45–7.80 (m, 7 H), 7.94 (d, 2 H, J = 2 Hz), 8.05–8.20 (m, 2 H), 10.3 (br s, 2 H).

3-(3,4-Dihydroxy-5-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (27). This compound was obtained analogously to **26**: yield 44%; mp 213–216 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 3 H), 3.89 (s, 6 H), 7.40 (s, 2 H), 7.47–7.92 (m, 4 H), 10.5 (br s, 2 H).

2,5-Bis(3,4-dihydroxy-5-nitrobenzylidene)cyclopentanone (28). A solution of **5** (3.66 g, 0.02 mol) and cyclopentanone (1.2 g, 0.014 mol) in MeOH (10 mL) was saturated with gaseous hydrogen chloride. After standing overnight at 5 °C, the product was filtered: yield 3.2 g (77%), mp 300 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.11 (s, 4 H), 7.32 (s, 2 H), 7.43 (d, 2 H, J = 2 Hz), 7.67 (d, 2 H, J = 2 Hz), 10.3 (br s, 4 H).

3-(4-Hydroxy-3-methoxy-5-nitrobenzylidene)-2,4-pentanedione (30) was synthesized in a similar manner as that for compound **23** from 4-hydroxy-3-methoxy-5-nitrobenzaldehyde and 2,4-pentanedione: mp 152–154 °C; HRMS m/z (M^+) calcd 279.0743, found 279.0741.

3-(3-Hydroxy-4-methoxy-5-nitrobenzylidene)-2,4-pentanedione (31). To a suspension of **23** (10.6 g, 0.04 mol) and silver oxide (4.6 g, 0.02 mol) in *N*-methylpyrrolidone (70 mL) was added methyl iodide (5 mL) with stirring at room temperature. The mixture was stirred for 24 h at 20 °C. Water (300 mL) and ethyl ether (200 mL) were added. The organic phase was separated and concentrated in vacuo. The residue was crystallized from MeOH: yield 2.5 g (22%); mp 172–174 °C; HRMS m/z (M^+) calcd 279.0743, found 279.0745.

Registry No. **1**, 116313-87-2; **2**, 7659-29-2; **3**, 84211-30-3; **4**, 116313-86-1; **5**, 116313-85-0; **6**, 116315-07-2; **7**, 28911-18-4; **8**, 116314-64-8; **9**, 116314-63-7; **10**, 116313-84-9; **11**, 116314-71-7; **12**, 118724-86-0; **13**, 116314-29-5; **14**, 116314-31-9; **15**, 116314-18-2; **16**, 116314-24-0; **17**, 116314-33-1; **18**, 116314-10-4; **19**, 116313-81-6; **20**, 116313-73-6; **21**, 116314-52-4; **22**, 116314-66-0; **23**, 116313-94-1; **24**, 116314-70-6; **25**, 116313-74-7; **26**, 116313-76-9; **27**, 116313-75-8; **28**, 116313-82-7; **29**, 5466-89-7; **30**, 118724-87-1; **31**, 118724-88-2; COMT, 9012-25-3; 4-chloroguaiacol, 16766-30-6; 4-chloro-2-methoxy-6-nitrophenol, 118724-89-3; 3-bromo-4,5-dimethoxybenzaldehyde, 6948-30-7; 3-cyano-4,5-dimethoxybenzaldehyde, 116314-61-5; 3-(trifluoromethyl)anisole, 454-90-0; 2-methoxy-6-(trifluoromethyl)phenol, 116314-59-1; 4-hydroxy-3-methoxy-5-(trifluoromethyl)benzaldehyde, 116314-60-4; 2,3-dimethoxythioanisole, 51506-47-9; 1,2-dimethoxy-3-(methylsulfonyl)benzene, 38452-45-8; 2-methoxy-6-(methylsulfonyl)phenol, 118724-90-6; 4-hydroxy-3-methoxy-5-(methylsulfonyl)benzaldehyde, 116315-11-8; 3,4-diacetoxy-5-nitrobenzoic acid, 116314-97-7; 1-aminoadamantane hydrochloride, 665-66-7; 3-chloro-4,5-dimethoxybenzaldehyde, 18268-68-3; ethyl crotonate, 10544-63-5; 5-(3-chloro-4,5-dimethoxyphenyl)-2,4-pentadienoic acid, 116314-08-0; 5-(3-chloro-4,5-dimethoxyphenyl)pentanoic acid, 116314-09-1; 4-(benzyloxy)-3-methoxybenzaldehyde, 2426-87-1; 5-[4-(benzyloxy)-3-methoxyphenyl]-2,4-pentadienoic acid, 102018-94-0; 5-(4-hydroxy-3-methoxyphenyl)pentanoic acid, 6342-85-4; 5-(4-hydroxy-3-methoxy-5-nitrophenyl)pentanoic acid, 116314-03-5; malononitrile, 109-77-3; 2,4-pentanedione, 123-54-6; dimethyl malonate, 108-59-8; cyclopentanone, 120-92-3; 4-hydroxy-3-methoxy-5-nitrobenzaldehyde, 6635-20-7.