temperature for 36 h. The reaction was then cooled to 0 °C and 30 mL of 6 N HCl was slowly added. The ice bath was removed and the mixture was stirred at room temperature for 4 h. THF was removed in vacuo and the resulting aqueous layer was basified to pH 14 with solid KOH. The solution, which appeared to be saturated with KOH, was extracted with 5 × 30 mL of chloroform, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 143 mg of yellow oil. This oil was dissolved in 5 mL of water, and 6 N HCl was added to pH 6. This solution was applied to a Dowex 50W-X8 (H^+ form) resin. Step gradient elution with distilled H_2O, 1 N HCl, 2.3 N HCl, 3.3 N HCl, and finally 4.3 N HCl resulted in product eluting at 4.3 N HCl to give 172 mg (28%) of 4 as a yellow solid after lyophilization: mp 180 °C br, dec; ^1H NMR (D_2O) δ 3.32–3.28 (m, 2 H), 3.02–2.94 (m, 8 H), 1.98–1.89 (m, 2 H), 1.84–1.75 (m, 2 H), 1.61 (br s, 4 H), 1.16–1.15 (d, 6 H); ^{13}C NMR (D_2O) δ 47.40, 45.86, 44.30, 30.76, 23.10, 17.70; HRMS (CI, NH_3) calcd for $C_{12}H_{30}N_4$ (M^+) 231.2549, found 231.2541.

Method B. N^1,N^4-Bis(3-azidobutyryl)-1,4-diaminobutane (**16**, 239 mg, 0.765 mmol) was added to a 25-mL round-bottom flask followed by attachment of a reflux condenser. THF (5 mL) was added via syringe, dissolving all the solid. BH_3·THF solution (1.0 M, 9.18 mL, 9.18 mmol) was slowly added and the resulting mixture was brought to reflux temperature for 24 h. After this time, the mixture was stirred at room temperature for 6 h. An ice bath was used to cool the reaction mixture to 0 °C, followed by slow addition of 1.5 mL of 6 N HCl. This mixture was then heated to reflux for 6 h followed by room temperature stirring overnight. THF was removed in vacuo followed by dilution of the resulting aqueous layer to 9 mL of total volume. This mixture was then applied to a Dowex 50W-X8 (H^+ form) cation-exchange column (100 mL volume of resin). Step gradient elution with distilled H_2O, 1 N HCl, 2.3 N HCl, 3.3 N HCl, and finally 4.3 N HCl resulted in product elution at 4.3 N HCl. The fractions containing the desired product were pooled and excess HCl was removed in vacuo followed by lyophilization to give 85 mg of yellow solid. This material proved impure by ^1H NMR analysis, so it was dissolved in 1 mL of water and was made basic (pH 14) with solid NaOH. Extraction with 3 × 5 mL of $CHCl_3$ and evaporation gave 73 mg of free base. This oil was then taken up in 1 N HCl and lyophilized to give 109 mg (38%) of the 4HCl salt (**4**) as a white solid: mp 250 °C dec; ^1H NMR (D_2O) δ 3.54–3.48 (m, 2 H), 3.23–3.15 (m, 8 H), 2.2–2.09 (m, 2 H), 2.06–1.96 (m, 2 H), 1.82–1.79 (m, 4 H), 1.37–1.35 (d, 6 H); ^{13}C NMR (D_2O) δ 47.10, 45.45, 44.02, 30.49, 22.85, 17.34; HRMS (DCI, NH_3) calcd for $C_{12}H_{30}N_4$ (M^+) 231.2549, found 231.2549. Anal. ($C_{12}H_{30}N_4$·4HCl) C, H, N.

Enzyme Assays and Cell Culture. Methods for the preparation and assay of spermine synthase and for the determination of growth and polyamine content of SV-3T3 cells are described by Pegg et al.[29] Preparation and assay of human SSAT[30] and cell culture experiments using L1210 cells[5] or HT29 cells[31] were carried out as previously described. Metabolism and uptake of compounds **2**–**4** were studied using HPLC analysis of cellular extracts[29] prepared at various times from cells exposed to these compounds as described in the text and legends. The retention time (t_R, min) during a typical experiment were as follows: spermidine (33.9), MeSpd (34.5), spermine (40.0), MeSpm (40.5), and Me_2Spm (40.9).

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Registry No. *dl*-**2**, 137946-02-2; *dl*-**2**·3HCl, 137945-92-7; *dl*-**3**, 137946-01-1; *dl*-**3**·HCl, 137945-93-8; **4**, 137946-03-3; **4**·4 HCl, 137945-94-9; *dl*-**5**, 2835-82-7; *dl*-**6**, 138051-81-7; **7**, 62146-62-7; **8**·HCl, 35517-18-1; *dl*-**9**, 137945-95-0; *dl*-**10**, 137945-96-1; *dl*-**11**, 137945-97-2; *dl*-**12**, 137964-65-9; **13**, 18523-47-2; *dl*-**14**, 137945-98-3; **15**, 137945-99-4; **16**, 137946-00-0; SSAT, EC2.3, 64885-84-3; spermidine, 124-20-9; spermine, 71-44-3; putrescine, 110-60-1; crotonic acid, 3724-65-0; *N*-[4-[(carbobenzyloxy)amino]butyl]-phthalimide, 66917-07-5; *N*-(4-azidobutyl)phthalimide, 66917-06-4; *N*-(4-bromobutyl)phthalimide, 5394-18-3; acrylic acid, 79-10-7.

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Binding of Phenylalkylamine Derivatives at 5-HT1C and 5-HT2 Serotonin Receptors: Evidence for a Lack of Selectivity

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Certain phenylalkylamine derivatives have been considered to bind selectively at 5-HT2 serotonin receptors. It is now recognized that the most widely used derivatives, i.e., 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropanes where X = Me (DOM), Br (DOB), and I (DOI) (**1**–**3**, respectively) also bind at the more recently identified population of serotonin 5-HT1C receptors. The purpose of the present investigation was to determine whether simple phenylalkylamines bind selectively at one population of receptors over the other. An examination of 34 derivatives reveals (i) similar structure–affinity relationships and (ii) a significant correlation (r = >0.9, n = 25) between 5-HT1C and 5-HT2 affinity. None of the compounds included in the present study displayed more than a 10-fold selectivity for one population of these receptors over the other; the results suggest that these compounds (including the widely used 5-HT2 agonists DOB and DOI) are 5-HT1C/5-HT2 agents.

The 5-HT2 population of serotonin (5-hydroxytryptamine) receptors has been implicated in cardiovascular function, muscle contraction, depression, anxiety, psychoses, and hallucinogenic activity (see refs 1–3 for recent reviews). Much of the impetus for clinical research in this area is directly related to the discovery of the "selective"

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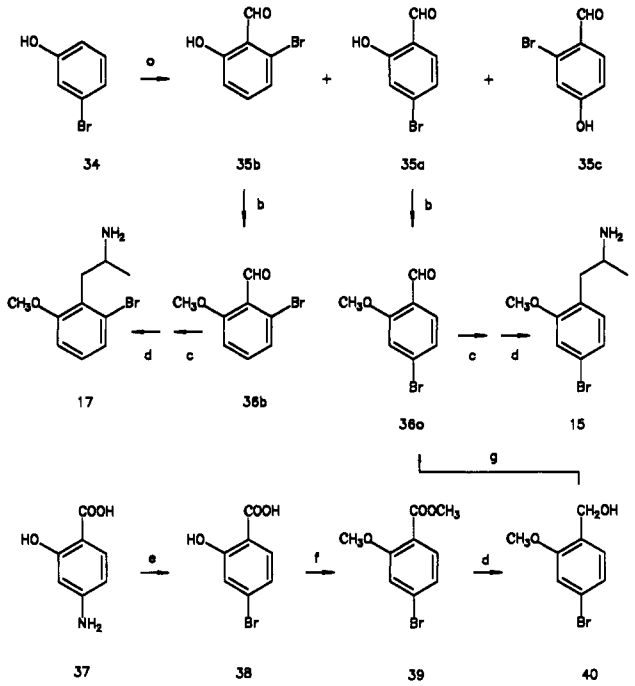
5-HT2 antagonist ketanserin.[4] Initially, it was thought that 5-HT2 receptors might constitute antagonist binding sites because serotonin (5-HT) itself displays low affinity (K_i ca. 500 nM) for these receptors.[5] In 1983, we proposed, on the basis of drug discrimination studies, that appropriately substituted phenylalkylamines constitute the first class of 5-HT2 serotonin agonists.[6] Agents such as, for example, 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropane where X = CH_3 (DOM; 1), Br (DOB; 2), and I (DOI; 3) were subsequently shown to bind at 5-HT2 receptors with high affinity[7] and are routinely employed now as 5-HT2 agonists.[8] Soon after our original studies were published,[6,7] Pazos et al.[9] reported the discovery of a new population of 5-HT receptors referred to as 5-HT1C receptors. We subsequently found that the supposedly selective 5-HT2 agonists 1–3 also bind at 5-HT1C receptors with high affinity,[10,11] and Burris and Sanders-Bush[12] have reported that DOM (1) is a 5-HT1C agonist. Thus, these three phenylalkylamines are not 5-HT2-selective agents but may constitute a class of 5-HT1C/5-HT2 agonists. On the basis of rather limited structure–affinity relationship (SAFIR) data, we speculated that 5-HT1C and 5-HT2 receptors might be more closely related to one another than to other 5-HT receptors.[10,11] That is, there appeared to be some striking structural similarities for the few high-affinity compounds examined; to date, however, a systematic structure–affinity investigation of the 5-HT1C binding of phenylalkylamines has not been reported.

With the recent cloning of 5-HT1C and 5-HT2 receptors, e.g. ref 13, it has now been shown that there is nearly an 80% homology between the transmembrane portions of the two receptors.[14] In fact, some investigations have referred to 5-HT2 and 5-HT1C receptors as 5-HT2A and 5-HT2B,[15] or as 5-HT2α and 5-HT2β receptors.[14] 5-HT2 antagonists, such as ketanserin, also show little selectivity for 5-HT2 versus 5-HT1C receptors.[16] As a consequence, many of the actions originally attributed to 5-HT2 receptor mechanisms may in fact involve a 5-HT1C mechanism or a combination of 5-HT1C and 5-HT2 mechanisms. For example, we have recently reported that the human hallucinogenic potency, drug discrimination potency, and hyperthermic potency of phenylalkylamine derivatives (previously shown to correlate significantly with 5-HT2 affinity)[1,11,17] also correlate with 5-HT1C affinity.[18] Due to the potential clinical applications, it is important to develop agonists and antagonists that can discriminate between these two populations of receptors.

We have recently proposed, on the basis of molecular modeling studies, that helix III of the transmembrane segments of 5-HT1C and 5-HT2 receptors constitutes a key binding region for phenylalkylamines such as 1–3.[19] Indeed, this region of the receptor is nearly identical for both, and this could account for the observed similarities in affinity. On the basis of this information, it may not be possible to achieve selectivity by making simple changes to the aromatic moiety of derivatives of 1. To obtain

Scheme I[a]



[a] (a) $CHCl_3$/NaOH; (b) MeI/KF-Al_2O_3; (c) $EtNO_2$; (d) AlH_3; (e) t-Bu nitrite/copper(II) bromide; (f) MeI/K_2CO_3; (g) pyridinium dichromate.

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empirical support for this hypothesis, we examined the 5-HT1C and 5-HT2 binding of a series of simple phenylalkylamine derivatives to determine whether or not there was any difference in affinity at the two populations of receptors. A secondary goal of this work, particularly if differences were found between 5-HT1C and 5-HT2 binding, was to establish some preliminary structure–affinity relationships for 5-HT1C binding of phenylalkylamines.

Chemistry

All of the required compounds were obtained from the appropriately substituted benzaldehyde derivatives via condensation with nitromethane or nitroethane to give the corresponding nitrostyrene, followed by reduction of the nitrostyrene with $LiAlH_4$ or AlH_3 to the desired amine. For example, amine **11** was prepared in this manner from 2-methoxy-4-methylbenzaldehyde (**32**). The corresponding *N*-monomethyl derivatives (e.g. **7** and **13**) were prepared by acylation of the primary amine (i.e., **6** and **14**) with ethyl chloroformate, followed by reduction of the carbamate with $LiAlH_4$. Some compounds were available from previous studies conducted in our laboratories.

We had previously reported the synthesis of what we believed to be compound **15**.[20] The assignment of its structure was based on that of 4-bromo-2-hydroxybenzaldehyde (**35a**) which was prepared by Reimer–Tiemann formylation of 3-bromophenol (**34**) following a literature procedure.[21] It was later shown by Kobayashi et al.,[22] however, that this formylation affords 2-bromo-4-hydroxybenzaldehyde (**35c**) as the major product. We reexamined this formylation reaction and obtained three bromohydroxybenzaldehyde derivatives (Scheme I). The major product (15%) was 2-bromo-6-hydroxybenzaldehyde (**35b**; mp 50–52 °C) (incorrectly assigned the structure of **35a** in the original literature);[21] the desired 4-bromo-2-hydroxybenzaldehyde (**35a**; mp 49–51 °C) was obtained in 4.8% yield, whereas the third product, 2-bromo-4-hydroxybenzaldehyde (**35c**), was obtained in 1.2% yield. It might be noted that under the conditions used by Kobayashi (calcium hydroxide/sodium carbonate mixture) **35a** was obtained in 0.8% yield,[22] whereas we found that the use of sodium hydroxide solution results in a 6-fold higher yield of the desired aldehyde. Both **35a** and **35b** were O-methylated to their corresponding ethers **36a** and **36b**, respectively, converted to their nitropropene derivatives, and reduced with AlH_3 to afford **15** and **17**, respectively (Scheme I). Interestingly, and causing further confusion, the HCl salts of these amines have nearly identical melting points. To verify the structure of **36a**, it was prepared by a less equivocal method (Scheme I). 4-Aminosalicylic acid (**37**) was converted via its diazonium salt to the corresponding bromo acid **38** which was methylated to the *O*-methyl methyl ester **39**; attempts to reduce **39** directly to aldehyde **36a** were unsuccessful. Compound **39** was reduced with AlH_3 to alcohol **40**, and pyridinium dichromate oxidation of this alcohol afforded **36a**, identical to that obtained above. On the basis of these studies, we conclude that the "**15**" reported earlier by us[20] is actually **17**. The corresponding iodo analogue **18** was prepared from 4-iodo-2-methoxybenzaldehyde which, in turn, was also prepared from 4-aminosalicylic acid (**37**) using a method similar to that described above.

Results and Discussion

(a) 5-HT1C Structure–Affinity Relationships. Binding data are provided in Table I. The unsubstituted parent compound **4** binds with little affinity at 5-HT1C receptors. Incorporation of a methoxy or ethoxy group at the 4-position (comparing **4** with **9**, or **21** with **23** and **24**) has relatively little effect on affinity. The more lipophilic 4-benzyloxy group (i.e., **10**) enhances affinity by about 1 order of magnitude. Likewise, the presence of lipophilic alkyl or halogen substituents at the 4-position also enhances affinity (e.g. compare **4** with **8**, **4** with **7**, **21** with **2**, **3**, **26**, and **30**). A 2-methoxy substituent appears beneficial for binding, and its presence increases affinity by 1 order of magnitude (compare **5** with **11**, and **8** with **15**). The 2,5-dimethoxy-substituted compound **21** binds at 5-HT1C receptors with about four times its affinity at 5-HT2 receptors; further substitution at the 4-position with alkyl or halogen substituents (e.g. **2**, **3**, **26**, **30**) results in compounds with the highest affinity. Interestingly, removal of the 5-methoxy group results only in a 2–3-fold decrease in affinity at 5-HT1C receptors (compare **11** with **1**, **16** with **2**, **18** with **3**, and **14** with **23**), whereas this same modification reduces 5-HT2 affinity by 15–30-fold. Indeed, it was the preliminary results with these compounds that prompted the synthesis of most of the new compounds reported in this study.

We have previously found that stereochemistry of the α-methyl substituent has little influence on affinity;[5] although only limited data are provided, this is supported by the present results. Removal of the α-methyl group (compare **11** with **12**, **15** with **16**, and **2** with **29**) and N-monomethylation (compare **2** with **27**, **6** with **7**, and **11** with **13**) also have little effect on affinity; however, the presence of an *N*-*n*-propyl substituent essentially abolishes the affinity of **28**.

(b) 5-HT1C versus 5-HT2 Selectivity. The above structure–affinity relationships closely parallel those that we have previously reported for the binding of phenylalkylamines at 5-HT2 receptors.[5,7,23] Those structural features important for 5-HT1C binding are the same features important for 5-HT2 binding. Consequently, one might not expect to see much selectivity. This appears to be the case (Table I). Most of the compounds in Table I bind at 5-HT1C receptors with several (range = 2–8) times the affinity they possess for 5-HT2 receptors; the 2,5-dimethoxy-substituted analogues, however, typically bind at 5-HT1C receptors with about half the affinity they display for 5-HT2 receptors. This distinction may or may not be significant except that it is the latter compounds (e.g. **1–3**) that are routinely used as 5-HT2 agonists. Linear regression analysis reveals that there is a significant correlation between the 5-HT1C and 5-HT2 affinities of the compounds in Table I ($r = 0.911$, $n = 25$).[24] (+)-LSD was

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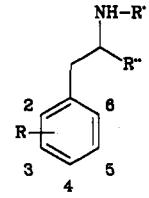
Table I. 5-HT1C and 5-HT2 Binding Data for Phenylalkylamine Derivatives[a]



	R'	R''	R2	R3	R4	R5	R6	K_i value (nM)[b] 5-HT1C	K_i value (nM)[b] 5-HT2	ratio[c]
4	H	Me	H	H	H	H	H	>10000	>10000	–
5[d]	H	Me	H	H	Me	H	H	>10000	>10000	–
6	H	Me	H	H	Pr	H	H	1250 (300)	2345 (700)	2
7	Me	Me	H	H	Pr	H	H	1935 (450)	2525 (230)	1
8	H	Me	H	H	Br	H	H	1485 (350)	ND	–
9[d]	H	Me	H	H	OEt	H	H	>10000	>10000	–
10[d]	H	Me	H	H	OBz	H	H	1210 (125)	ND	–
11	H	Me	OMe	H	Me	H	H	610 (50)	1435 (80)	2
12	H	H	OMe	H	Me	H	H	110 (25)	825 (100)	7
13	Me	Me	OMe	H	Me	H	H	1480 (120)	ND	–
14	H	Me	OMe	H	OMe	H	H	4475 (130)	>10000	>2
15	H	Me	OMe	H	Br	H	H	100 (20)	830 (30)	8
16	H	H	OMe	H	Br	H	H	220 (5)	1030 (90)	5
17	H	Me	OMe	H	H	H	Br	1560 (495)	>10000	>6
18	H	Me	OMe	H	I	H	H	130 (40)	580 (130)	5
19[e]	H	Me	H	OMe	OMe	OMe	H	5710	16500	3
20	H	Me	H	H	$-OCH_2O-$		H	2290 (320)	2190 (110)	1
20 (–)	H	Me	H	H	$-OCH_2O-$		H	2100 (125)	3425[e]	1
21[e]	H	Me	OMe	H	H	OMe	H	1217	5200	4
22	H	Me	OMe	H	COOPr	OMe	H	>10000	2460[e]	<1
23[e]	H	Me	OMe	H	OMe	OMe	H	2666	1250	0.5
24	H	Me	OMe	H	OEt	OMe	H	2300 (240)	2200 (120)	1
1[e]	H	Me	OMe	H	Me	OMe	H	193	100	0.5
1 (–)	H	Me	OMe	H	Me	OMe	H	90 (10)	65 (5)	1.5
25[e]	H	Me	OMe	H	Et	OMe	H	101	100	1
26[e]	H	Me	OMe	H	Pr	OMe	H	14	69	5
2 (–)	H	Me	OMe	H	Br	OMe	H	50 (6)	24[e]	0.5
2	H	Me	OMe	H	Br	OMe	H	70 (10)	41[e]	0.5
2 (+)	H	Me	OMe	H	Br	OMe	H	84 (4)	145[e]	2
27[d]	Me	Me	OMe	H	Br	OMe	H	98 (21)	80 (10)	1
28[d]	Pr	Me	OMe	H	Br	OMe	H	>10000	2460[e]	<1
29	H	H	OMe	H	Br	OMe	H	36 (6)	34 (4)	1
3[e]	H	Me	OMe	H	I	OMe	H	30	19	0.6
30[e]	H	Me	OMe	H	Bu	OMe	H	26	58	2
31[e]	(+)-LSD							3.8	2.5	1.5

[a] Synthesis, 5-HT2 binding data, or both 5-HT2 and 5-HT1C binding data have been reported earlier for some of these compounds (see footnotes *d* and *e*); where binding data have been previously reported, SEM are not provided here. [b] K_i values are followed by SEM; SEM was not determined for compounds with K_i values >10000 nM. [c] 5-HT1C selectivity; ratio = K_i(5-HT2)/K_i(5-HT1C). [d] The synthesis of compounds **5** (ref 25), **9** and **10** (ref 26), **27** (ref 20), and **28** (27) have been previously reported. [e] K_i value previously reported (refs 3, 7, 11, 20, 28).

included for comparative purposes. It is concluded that these compounds can not be considered selective for one of these two populations of receptors over the other.

(c) Summary. The present investigation compared the 5-HT1C and 5-HT2 binding affinities of a series of phenylalkylamine derivatives in order to provide empirical support for our previous hypothesis[19] that simple phenalkylamines may not distinguish between these two receptors. Indeed, there is a significant correlation between 5-HT1C and 5-HT2 binding affinities. Structure–affinity relationships formulated for 5-HT1C binding essentially echo those previously reported for 5-HT2 binding.[5,7,23] One of the more unexpected findings of the present study is that removal of the 5-methoxy group of 2,5-dimethoxy-substituted derivatives seems to have a greater effect on 5-HT2 binding than on 5-HT1C binding and compounds lacking this methoxy group (e.g **12**, **15**, **16**) are among the more selective compounds examined; nevertheless, none of these phenylalkylamine derivatives displays more than a 10-fold selectivity for 5-HT1C or 5-HT2 receptors. The present results do, however, provide experimental binding data that support previous observations of structural similarity between the two receptors.

Experimental Section

Synthesis. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 5ZDX FT-IR spectrophotometer, and proton magnetic resonance spectra were obtained using a JEOL FX90Q FT-NMR spectrometer at 89.55 MHz. Chemical shift values are reported in parts per million (δ) relative

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to tetramethylsilane as an internal standard. Elemental analysis was performed by Atlantic Microlab (Norcross, GA) and are within 0.4% of theory. With the exception of the compounds described below, all compounds were previously synthesized in our laboratory and were available from earlier studies.

(±)-1-(4-*n*-Propylphenyl)-2-aminopropane Hydrochloride (6). Compound 6 was prepared from 4-*n*-propylbenzaldehyde via the same procedure employed for the preparation of 11. The aldehyde was obtained in 94% yield from its commercially available diethyl acetal by heating at reflux a THF (80-mL) solution of the acetal (5 g) with 10% HCl for 6.5 h; the crude aldehyde was purified by convertion to its bisulfite adduct (mp 137 °C), and the free aldehyde (yellow oil) was liberated by hydrolysis with 4% NaOH (to pH 9). The crude nitropropene intermediate, prepared as described for 11 and obtained as an oil in 91% yield, was reduced with $LiAlH_4$ by heating at reflux for 22 h in THF. An ethereal solution of the crude amine was saturated with HCl gas to provide 1.1 g (57%) of 6 as a white precipitate, mp 173 °C. A small portion of the free base was converted to its maleate salt for purpose of characterization, mp 172–173 °C (lit.[29] mp 172–173 °C).

(±)-*N*-Methyl-1-(4-*n*-propylphenyl)-2-aminopropane Fumarate (7). A solution of ethyl chloroformate (0.55 g, 5 mmol) in anhydrous Et_2O (5 mL) was added in a dropwise manner to a stirred solution of 6 (free base; 0.17 g, 0.96 mmol) and NEt_3 (0.15 g, 1.5 mmol) in Et_2O (5 mL) at room temperature. The reaction mixture was allowed to stir for 3 h; additional ethyl chloroformate (0.2 g, 1.8 mmol) was added, and stirring was continued for an additional 2 h. The solids were removed by filtration, and the filtrate was washed with H_2O (25 mL), dried ($MgSO_4$), and evaporated to dryness under reduced pressure to yield 0.22 g of crude carbamate as a yellow oil. A solution of this oil (0.22 g, 0.92 mmol) in anhydrous Et_2O (10 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ (0.17 g, 4.5 mmol) in Et_2O (15 mL) at 0 °C. The mixture was heated at reflux for 4 h and allowed to stir at room temperature for 15 h. Excess $LiAlH_4$ was decomposed by the dropwise addition of wet Et_2O. The solids were removed by filtration and washed with Et_2O (2 × 25 mL). The combined washings and filtrate were further washed with H_2O (3 × 25 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to yield 0.11 g of material. Dropwise addition of a solution of the crude material in EtOAc (5 mL) to a stirred solution of fumaric acid (0.13 g, 1.1 mmol) in EtOAc (20 mL) afforded 0.12 g (38% overall) of 7 as white crystals after recrystallization from an absolute EtOH/anhydrous Et_2O mixture, mp 120–121 °C (lit.[29] mp 115–116 °C).

(±)-1-(2-Methoxy-4-methylphenyl)-2-aminopropane Hydrochloride (11). Ammonium acetate (1.3 g, 17.3 mmol) was added to a solution of aldehyde **32** (1.3 g, 8.7 mmol) in nitroethane (25 mL), and the reaction mixture was heated at reflux for 8 h and stirred at room temperature for 60 h. The nitroethane was evaporated under reduced pressure, and a solution of the resultant orange oil in $CHCl_3$ (35 mL) was washed with H_2O (25 mL). The aqueous portion was extracted with $CHCl_3$ (15 mL), and the organic fractions were dried ($MgSO_4$) and evaporated to yield a crude oil. Distillation of the oil (Kugelrohr bath temperature 72 °C; 0.075 mmHg) (lit.[30] bp 132 °C; 0.4 mmHg) gave 1.6 g (91%) of 1-(2-methoxy-4-methylphenyl)-2-nitropropene. A solution of this material (1.6 g, 7.9 mmol) in anhydrous Et_2O (30 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ (1.6 g, 47.4 mmol) in Et_2O (45 mL) at 0 °C. The reaction mixture was heated at reflux for 6 h, following which it was allowed to stir at room temperature for 16 h. Excess $LiAlH_4$ was decomposed by the dropwise addition of 15% NaOH solution. The solids were removed by filtration and washed with Et_2O (2 × 25 mL). The combined washings and filtrate were dried ($MgSO_4$), and the solvent was evaporated under reduced pressure. Kugelrohr distillation (bath temperature 35 °C; 0.14 mmHg) of the crude product afforded 1.0 g (74%) of 11 as its free base. A portion of this material was converted to its HCl salt and recrystallized from a MeOH/Et_2O mixture: mp (softening at 111 °C) 127 °C (lit.[30] mp 162–162.5 °C); NMR (DMSO-d_6) δ 0.87–0.94 (d, 3 H, CH_3), 2.27 (s, 3 H, Ar-CH_3), 2.42–2.50 (d, 2 H, CH_2), 2.80–3.20 (m, 1 H, CH), 3.73 (s, 3 H, OCH_3), 6.60–7.20 (m, 3 H, Ar-H); IR (KBr) 3451.6 cm^{-1} (NH). Due to the discrepancy with the previously reported melting point, 11 was submitted for microanalysis. Anal. ($C_{11}H_{17}NO\cdot HCl\cdot 0.2H_2O$) C, H, N.

2-(2-Methoxy-4-methylphenyl)aminoethane Maleate (12). Compound 12 was prepared from 32 via its nitroethene intermediate (98% yield after Kugelrohr distillation at 105 °C; 0.04 mmHg to afford a yellow oil) followed by $LiAlH_4$ reduction as described for the synthesis of 11. The crude amine was distilled (Kugelrohr bath temperature 33 °C; 0.09 mmHg) to yield 0.23 g (45%) of 12 as its free base. A small portion was converted to the maleate salt, mp 101–103 °C after recrystallization from absolute EtOH/anhydrous Et_2O. Anal. ($C_{10}H_{15}NO\cdot C_4H_4O_4\cdot 0.5H_2O$) C, H, N.

(±)-*N*-Methyl-1-(2-methoxy-4-methylphenyl)-2-aminopropane Maleate (13). Compound 13 was prepared from 11 using the same acylation/reduction procedure employed for the synthesis of 7. The crude amine was distilled (Kugelrohr bath temperature 42 °C; 0.23 mmHg) to afford 0.1 g (91%) of the free base of 13. A small portion was converted to the maleate salt by the addition of an ethereal solution to a cold ethereal solution of maleic acid; the precipitated material was washed with hot Et_2O to afford 13: mp 100–101 °C; NMR (DMSO-d_6) δ 1.02–1.10 (d, 3 H, CH_3), 2.29 (s, 3 H, Ar-CH_3), 2.59 (s, 3 H, N-CH_3), 2.88–3.55 (m, 3 H, CH_2, CH), 3.78 (s, 3 H, OCH_3), 6.01 (d, 2 H, maleate CH), 6.68–7.08 (m, 3 H, Ar-H), 8.29 (br s, D_2O-exchangeable, NH^+); IR (KBr) 3437.5 cm^{-1} (NH). Anal. ($C_{12}H_{19}NO\cdot C_4H_4O_4$) C, H, N.

(±)-1-(4-Bromo-2-methoxyphenyl)-2-aminopropane (15). 4-Bromo-2-methoxybenzaldehyde (**36a**) (0.4 g, 2 mmol) was converted to its nitropropene intermediate in 91% yield (mp 80–82 °C after recrystallization from Et_2O) employing the procedure described for 11. A solution of this nitropropene (0.5 g, 2 mmol) in THF (10 mL) was added in a dropwise fashion under a N_2 atmosphere to a stirred solution of AlH_3 prepared by addition of 100% H_2SO_4 and THF (10 mL) to a suspension of $LiAlH_4$ (0.23 g, 5.9 mmol) in THF (10 mL) at 0 °C under N_2. The reaction mixture was allowed to stir at room temperature overnight, and excess AlH_3 was decomposed by the successive dropwise addition of wet THF (10 mL), H_2O (0.3 mL), 15% NaOH (0.3 mL), and H_2O (0.6 mL). The mixture was filtered, and the filtrate was dried ($MgSO_4$) and evaporated to dryness under reduced pressure to yield 0.5 g of a yellow oil. The maleate salt 15 was prepared in 52% yield (0.4 g), mp 119–122 °C after recrystallization from absolute EtOH: NMR ($CDCl_3$) δ 1.33 (d, 3 H, CH_3), 2.90 (d, 2 H, CH_2), 3.69 (m, 1 H, CH), 3.80 (s, 3 H, OCH_3), 6.20 (s, 2 H, CH=CH), 6.90–7.15 (m, 3 H, Ar-H). A sample of the hydrochloride salt (mp 171–173 °C after recrystallization from 2-PrOH/anhydrous Et_2O) was prepared for comparison with an authentic reference sample of the salt previously reported,[20] mp 173–175 °C; mixture mp 129–134 °C. Anal. ($C_{10}H_{14}BrNO\cdot C_4H_4O_4$) C, H, N, Br.

2-(4-Bromo-2-methoxyphenyl)aminoethane Maleate (16). Compound 16 was prepared in the same manner as its α-methyl counterpart 15 from **36a** using nitromethane in place of nitroethane. The nitroethene intermediate (mp 92–94 °C after recrystallization from Et_2O; 96% yield) was reduced with AlH_3 to afford 1.3 g (87%) of 16 as its free base. A portion was converted to the maleate salt, mp 125–127 °C after recrystallization from an 8:2 EtOAc/2-PrOH mixture: NMR (DMSO-d_6) δ 2.86 (t, 2 H, J = 8.1 Hz, CH_2), 3.02 (t, 2 H, J = 8.1 Hz, CH_2), 3.85 (s, 3 H, OCH_3), 6.12 (s, 2 H, CH=CH), 7.08 (m, 3 H, Ar-H), 7.86 (br s, NH_2), 8.03 (s, 1 H, COOH). Anal. ($C_9H_{12}BrNO\cdot C_4H_4O_4$) C, H, N, Br.

(±)-1-(2-Bromo-6-methoxyphenyl)-2-aminopropane Maleate (17). This compound was prepared from **36b** exactly as described for 15. The nitropropene intermediate was obtained in 86% yield (mp 54–57 °C after recrystallization from Et_2O) and was reduced by AlH_3 and converted to the maleate salt in 62%

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yield (mp 134–136 °C after recrystallization from absolute EtOH): NMR ($CDCl_3$ + DMSO-d_6) δ 1.20 (d, 3 H, CH_3), 3.10 (m, 2 H, CH_2), 3.50 (m, 1 H, CH), 6.15 (s, 2 H, CH=CH), 7.00 (m, 1 H, Ar-(4)H), 7.20 (d, 2 H, Ar-(3,5)H), 8.00 (br s, 4 H, NH_2, 2 COOH). HCl salt: mp 173–175 °C (absolute EtOH). Anal. ($C_{10}H_{14}BrNO \cdot C_4H_4O_4$) C, H, N, Br.

(±)-1-(4-Iodo-2-methoxyphenyl)-2-aminopropane Maleate (18). Compound 18 was prepared from 4-iodo-2-methoxybenzaldehyde, which was in turn prepared in 64% yield from 4-iodosalicylic acid (mp 229 °C; lit.[31] mp 226–227 °C), in the same manner employed for the synthesis of **32** except that AlH_3 was used in place of $LiAlH_4$: aldehyde mp 84–85 °C (lit.[21] mp 85 °C); NMR ($CDCl_3$) δ 3.92 (s, 3 H, OCH_3), 7.25–7.60 (m, 3 H, Ar-H), 10.40 (s, 1 H, CHO). The aldehyde was converted in 52% yield to its nitropropene intermediate (mp 110–111 °C, from aqueous EtOH) via the procedure described for **11**, and the nitropropene was reduced with AlH_3 as described for **15** to yield 0.6 g (97%) of the free base of **18**. A small portion was converted to the maleate salt, mp 117–118 °C. Anal. ($C_{10}H_{14}INO \cdot C_4H_4O_4 \cdot 0.5H_2O$) C, H, N.

2-Methoxy-4-methylbenzaldehyde (32). Pyridinium dichromate (9.3 g, 24.6 mmol) was added to a solution of alcohol **33** (2.5 g, 16.4 mmol) in CH_2Cl_2 (40 mL) under a N_2 atmosphere. The reaction mixture was allowed to stir at room temperature for 51 h; Et_2O (100 mL) was added, and the solids were removed by filtration. The filtrate was evaporated to dryness under reduced pressure, and a saturated $NaHSO_3$ solution (10 g) was added to the resultant brown residue. The bisulfite adduct was collected by filtration, dried (3.2 g), and suspended in Et_2O (100 mL); 4% NaOH solution (80 mL) was added to liberate the aldehyde. The Et_2O solution was separated; the aqueous fraction was washed with Et_2O (2 × 50 mL), and the combined organic fractions were washed with H_2O (50 mL), dried ($MgSO_4$), and evaporated to dryness to yield 1.5 g (59%) of **32** as a white crystalline solid: mp 42.5–43 °C (although **32** has been previously mentioned in the literature,[30] it was not obtained as a solid material); NMR ($CDCl_3$) δ 2.40 (s, 3 H, Ar-CH_3), 3.91 (s, 3 H, OCH_3), 6.78–6.88 (m, 2 H, Ar-H), 7.67–7.76 (d, 1 H, Ar-H), 10.38 (s, 1 H, CHO).

2-Methoxy-4-methylbenzyl Alcohol (33). Iodomethane (40 g) and K_2CO_3 (31 g) were added to a solution of 4-methylsalicylic acid in acetone (50 mL) at room temperature, and the reaction mixture was then heated at reflux for 48 h. An additional portion of MeI (20 g) was added, and refluxing was continued for another 24 h. The reaction mixture was allowed to cool to room temperature and was stirred overnight (16 h). The solids were removed by filtration, and the solvent was evaporated under reduced pressure to afford a crude oil. Distillation (Kugelrohr bath temperature 78 °C; 0.24 mmHg) gave 8.8 g (74%) of methyl 2-methoxy-4-methylbenzoate as a colorless oil: IR (neat) 1728.9 cm^{-1} (C=O). A solution of this ester (5 g, 27.8 mmol) in dry THF (10 mL) was added in a dropwise manner to a stirred suspension of $LiAlH_4$ in THF (25 mL) at 0 °C under a N_2 atmosphere. The reaction mixture was heated at reflux for 25.5 h and allowed to stir at room temperature for an additional 91 h, and excess $LiAlH_4$ was decomposed by the addition of 15% NaOH at 0 °C. The mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in Et_2O (100 mL), the Et_2O solution was washed with H_2O (50 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give a crude oil. Distillation of the oil (Kugelrohr bath temperature 32 °C; 0.45 mmHg) (lit.[32] bp 280–290 °C) gave 2.7 g (63%) of the desired product.

4-Bromo-2-hydroxybenzaldehyde (35a) and 2-Bromo-6-hydroxybenzaldehyde (35b). Formylation of 3-bromophenol was performed essentially according to the method of Glennon et al.[20] Chloroform (42 g) was added in a dropwise manner over a period of 15 min to a suspension of 3-bromophenol (**34**) (30 g, 173 mmol) in aqueous NaOH (55.4 g in 75 mL of H_2O) with the temperature maintained between 70 and 75 °C. The mixture was cooled to 0 °C, made acidic (pH 3) by the addition of 1 N HCl, and extracted with EtOAc (3 × 100 mL). The combined extracts were dried ($MgSO_4$), and the solvent was removed under reduced pressure to afford 44.2 g of a liquid. An ethereal solution (200 mL) of this liquid was layered over a saturated solution of sodium bisulfite and allowed to stand at room temperature for 5 d. The bisulfite adduct was collected by filtration and washed with Et_2O (50 mL). The solid material (42.5 g) was suspended in 0.5 N HCl (200 mL), and the mixture was stirred with heating at 50 °C for 2 h. The mixture was extracted with EtOAc (3 × 100 mL), and the combined extracts were dried ($MgSO_4$) and evaporated to dryness to yield 10.7 g of a product mixture. Kugelrohr distillation afforded 9.6 g of an oil that was subjected to steam distillation until 2.5 L of distillate had been collected. Upon allowing the distillation flask to cool to room temperature, **35c** precipitated as a colorless solid and was collected by filtration [0.4 g (1.2%), mp 149–153 °C (lit.[22] mp 156.5–158 °C)]. The distillate was extracted with Et_2O (3 × 100 mL). The combined filtrates were dried ($MgSO_4$) and evaporated to dryness to give 8.8 g of an oil. The oil was dissolved in boiling 95% EtOH (10 mL), and after 48 h of standing at 0–5 °C, 4.4 g of **35b** was obtained as a white solid, mp 50–52 °C (lit.[22] mp 51–52 °C). The ethanolic filtrate was evaporated to dryness, and NMR spectrometric analysis revealed a 3:1 mixture of **35a** and **35b**. The mixture was subjected to column chromatography using silica gel (Davisil 62, 60 g) as the stationary phase and petroleum ether as eluent. An additional 0.9 g of **35b** was obtained (total yield 5.3 g; 15%). Later eluate contained the desired **35a**; recrystallization of crude **35a** from petroleum ether gave 1.7 g (4.8%) of **35a**, mp 49–51 °C (lit.[22] mp 50–51.5 °C).

4-Bromo-2-methoxybenzaldehyde (36a). Method A. Hydroxyaldehyde **35a** (1.2 g, 6 mmol) and MeI (1.3 g, 9 mmol) were added to a suspension of 5 g of KF/Al_2O_3[33] (35% anhydrous KF, 28 mmol) in DMSO (15 mL) and dioxane (15 mL). The reaction mixture was stirred overnight (ca. 16 h), the solids were removed by filtration, and the dioxane was removed by evaporation under reduced pressure. Ice–water (100 mL) was added to the residue, and after a period of 3 h the aldehyde was collected by filtration to yield 1.1 g (87%) of **36a**: mp 63–65 °C (lit.[22] mp 67–69 °C); NMR ($CDCl_3$) δ 3.90 (s, 3 H, OCH_3), 7.05–7.30 (m, 2 H, Ar(5,6)-H), 7.70 (m, 1 H, Ar(3)-H), 10.40 (s, 1 H, CHO).

Method B. Pyridinium dichromate (1.3 g, 3.4 mmol) was added to a solution of alcohol **40** (0.45 g, 2.1 mmol) in CH_2Cl_2 (10 mL) under a nitrogen atmosphere, and the mixture was allowed to stir at room temperature for 65 h. The remainder of the procedure was the same as that used for the preparation of **32** to yield 0.16 g (35%) of **36a**, mp 63–65 °C. The products obtained by the two methods were identical by thin-layer chromatography and NMR.

2-Bromo-6-methoxybenzaldehyde (36b). Hydroxy aldehyde **35b** was methylated using the same procedure used for the methylation of **35a** to yield 0.9 g (67%) of **36b**: mp 50–52 °C (lit.[22] mp 57.5–58 °C); NMR ($CDCl_3$) δ 3.90 (s, 1 H, OCH_3), 6.85–7.05 (m, 1 H, Ar(4)-H), 5.15–7.45 (m, 2 H, Ar(3,5)-H), 10.40 (s, 1 H, CHO).

4-Bromo-2-methoxybenzyl Alcohol (40). MeCN (40 mL) was added to anhydrous copper(II) bromide (8.8 g, 39.3 mmol) under a N_2 atmosphere, followed by *tert*-butyl nitrite (5.1 g, 49 mmol) and an additional volume of MeCN (10 mL). The stirred mixture was cooled on an ice bath, and 4-aminosalicylic acid (**37**) (5 g, 32.7 mmol) was added in small portions. Additional MeCN (20 mL) was added, and the reaction mixture was allowed to stir for 2 h at ice-bath temperature. The mixture was poured into 20% HCl (200 mL) and extracted with Et_2O (2 × 200 mL), the combined ethereal extracts were washed with 20% HCl (2 × 100 mL) and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to yield a semisolid residue. The residue was dissolved in Et_2O (300 mL) and extracted with 15% NaOH (2 × 150 mL). The aqueous portion was washed with Et_2O (100 mL), the pH was adjusted to 1 with 10% HCl, and the mixture was again extracted with Et_2O (2 × 200 mL). The ether solution was

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dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to afford a 3.7 g of semisolid **38** that crystallized upon trituration with $CHCl_3$:[22] NMR ($CDCl_3$) δ 6.3 (br s, 1 H, OH), 7.00–8.15 (m, 3 H, Ar-H), 10.35 (br s, 1 H, COOH). This crude material (2 g, 9.2 mmol) in acetone (50 mL) was heated at reflux with MeI (5.6 g, 39 mmol) and K_2CO_3 (4.4 g, 31 mmol) for 48 h and stirred at room temperature for 64 h. The solid material was removed by filtration, and the solvent was evaporated under reduced pressure. The residual oil was distilled (Kugelrohr bath temperature 52 °C; 0.01 mmHg) to afford 2.3 g (100%) of **39**. Finally, a solution of the ester (2.25 g, 9.18 mmol) in anhydrous Et_2O (15 mL) was reduced by addition to a solution of AlH_3 (generated by the addition of 0.6 g of $AlCl_3$ to a suspension of 0.5 g of $LiAlH_4$ in 15 mL of Et_2O at 0 °C under a nitrogen atmosphere) and allowing the reaction mixture to heat at reflux for 18 h. Excess reducing agent was decomposed by the addition of 15% NaOH at 0 °C. The mixture was filtered, and the filtrate was dried ($MgSO_4$) and evaporated to dryness to yield 0.45 g (23%) of **40** as a colorless oil after distillation (Kugelrohr bath temperature 52 °C; 0.04 mmHg): NMR ($CDCl_3$) δ 3.8–4.0 (m, 5 H, OCH_3, CH_2), 4.6 (br s, 1 H, OH), 7.01–7.11 (m, 3 H, Ar-H). The product[34] was used without further characterization for the preparation of **36a**.

Radioligand Binding. The radioligand binding studies were performed as previously described in detail.[35] Briefly, frontal cortical regions of male Sprague–Dawley rats (200–250 g; Charles River and Harlan–Sprague) were dissected on ice and homogenized (1:10 w/v) in ice-cold 50 mM Tris HCl, 0.5 mM EDTA, and 10 mM $MgCl_2$ at pH 7.4 and centrifuged at 3000*g* for 15 min. The pellet was resuspended in buffer (1:30 w/v) incubated at 37 °C for 15 min and then centrifuged twice at 30000*g* for 10 min (with a resuspension between centrifugations). The final pellet was resuspended in 50 mM Tris HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, 0.1% ascorbate, and 10^{-5} M pargyline.

Assays were performed in triplicate in a 2.0-mL volume containing 5 mg (wet weight) of tissue and 0.4 nM [^{3}H]ketanserin (76 Ci/mmol; New England Nuclear) for 5-HT2 receptor assays, and 10 mg (wet weight) of tissue and 1 nM [^{3}H]mesulergine (75.8 Ci/mmol; Amersham) for 5-HT1C receptor assays. Cinanserin (1.0 μM) was used to define nonspecific binding in the 5-HT2 assay. In the 5-HT1C assays, mianserin (1 μM) was used to define nonspecific binding, and 100 nM spiperone was added to all tubes to block the binding of [^{3}H]mesulergine to 5-HT2 receptors. Tubes were incubated at 37 °C for 15 min, filtered on Schliecher and Schuell (Keene, NH) glass fiber filters (presoaked in 0.1% polyethyleneimine), and washed with 10 mL of ice-cold buffer. The filters were counted at an efficiency of 50%.

Saturation and competition experiments were analyzed using an updated version of the program EBDA[36] to obtain equilibrium dissociation constants (K_D), B_{max}, Hill coefficients, and IC_{50} values. K_i values for competition experiments were obtained using the equation $K_i = IC_{50}/1 + (D^*/K_{D^*})$ where IC_{50} is the experimentally observed concentration of competing drug that inhibits 50% of specific binding, K_{D^*} is the equilibrium dissociation constant determined in saturation studies, and D^* is the concentration of radioactive ligand used in the competition assays.[37] 5-HT hydrogen oxalate and spiperone were obtained from Sigma (St. Louis, MO).

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N,N′-Disubstituted Guanidine High-Potency Sweeteners

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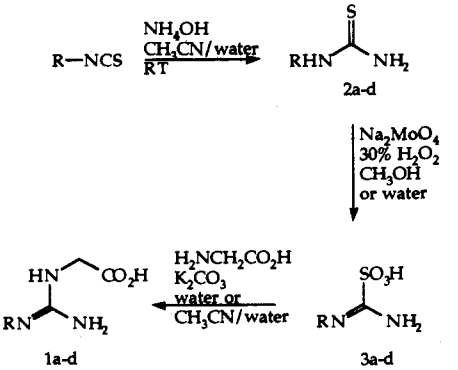
The role and function of the aryl group in the highly potent trisubstituted guanidine sweeteners **7a–d** was investigated. Four disubstituted guanidines, lacking the aryl group, were prepared. These guanidines contain a hydrophobic substituent on one nitrogen and a carboxymethyl group substituted on one of the other nitrogens. They were found to be tasteless or to have a significantly lower sweetness potency than the corresponding trisubstituted compounds. Possible rationales for the effects of structure on the sweet taste activity are discussed.

In 1986, Nofre, Tinti, and their co-workers reported a new series of *N*-(carboxymethyl)guanidines that are high-potency sweeteners.[1] A disubstituted guanidine, *N*-(carboxymethyl)-*N′*-(4-cyanophenyl)guanidine, was reported with a sweetness potency 2400 times that of sucrose relative to a 2% sucrose reference solution ($P_w(2) = 2400$). The same group later reported on a series of *N*-aryl-*N′*-(aryl/alkyl)-*N″*-(carboxymethyl)-trisubstituted guanidines, some of which have substantially increased sweetness potency relative to the earlier described disubstituted analogues. The most potent analogues exhibit sweetness potencies in excess of 100 000 times that of sucrose.[2] In the trisubstituted guanidines, two preferred aryl moieties are 4-cyanophenyl and 4-nitrophenyl. The structure–activity relationships (SAR) in the trisubstituted guanidine

Scheme I. Synthesis of N,N′-Disubstituted Guanidines



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