Synthesis and Sensory Evaluation of Ring-Substituted Dihydrochalcone Sweeteners†

Murray L. Whitelaw and James R. Daniel*

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana 47907

A series of hesperetin dihydrochalcones, containing electron-donating or -withdrawing substituents para to the proposed hydrogen-bonding AH sites of each aromatic ring, have been synthesized. These novel compounds were evaluated for both total taste potency and percentage sweetness by a trained taste panel. The most potent dihydrochalcone sweetener currently known, 3′-carboxyhesperetin dihydrochalcone (3400 times more potent than 6% w/v sucrose), was synthesized. The characteristic lingering aftertaste of dihydrochalcones remained unimproved by these chemical modifications.

The use of neohesperidin dihydrochalcone (NHDHC, 1,340 times more potent than sucrose) as a broad spectrum sweetener is limited by slow sweetness onset and a characteristic licorice-flavored aftertaste. However, potent sweetness, safety, stability, and potential economic production make the dihydrochalcone class of compounds ideal candidates for sweetener development (Horowitz and Gentili, 1986). Discovery that the aglycon hesperetin dihydrochalcone (**2a**) is also very sweet initiated synthesis of many **2a** analogues in attempts to reduce the aftertaste (DuBois et al., 1981a,b). Sweetness is proposed to result via reciprocal hydrogen bonding between complementary AH (hydrogen donating) and B (hydrogen accepting) functional groups on the sweetener and sweetness receptor (Shallenberger and Acree, 1967). In terms of this model, sweetness of dihydrochalcones could be hypothetically explained by the existence of two potential AH/B "glucophore" units within the molecule (Figure 1). This unusual duality has been suggested as contributing, by a chelation effect, to the lingering aftertaste (DuBois et al., 1981a).

Disruption of either the A-ring or B-ring AH/B unit by deletion of a functional group, or exchange for an alternate functional group, has given primarily tasteless or bitter compounds. It therefore seems that the receptor might specifically recognize these particular AH/B functionalities with little tolerance for mimics. While the sweetness of other structures containing the B-ring isovanillin unit [e.g., flavans, Dick (1981); 2-(3-hydroxy-4-methoxyphenyl)benzodioxanes, Arnoldi et al. (1986); β-(3-hydroxy-4-methoxyphenyl)ethylbenzenes, Yamamoto et al. (1978)] would suggest the B-ring AH/B unit as predominant in eliciting sweetness, it is unclear whether A- or B-ring functionality plays a dominant role in aftertaste. An unexplored strategy in sweetener design is to induce modifications in the electronic constitution of existing AH/B groups via molecular alterations distant from the AH/B groups. Subtle changes in receptor binding strength might therefore be invoked while the functionality proposed to elicit sweetness is maintained. To investigate the influence of both A- and B-rings on sweetness potency and aftertaste, we sought to alter the hydrogen-bonding strength between the assigned AH unit of each ring and the receptor. As both AH units are phenolic in character, this can be achieved by placing substituents para to the

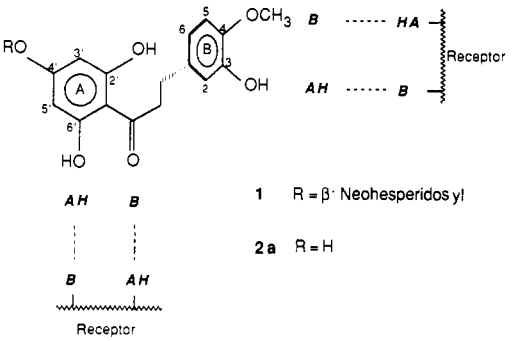

Figure 1. Proposed interaction of the dihydrochalcone structure with the sweetness receptor.

AH hydroxyls. A study of hydrogen bonding between para-substituted phenols and dimethylacetamide showed that H-bond strength is directly proportional to the Hammett sigma value (σ) of the para functional group (Stymne et al., 1973). Similar results were obtained with a para-substituted phenol/pyridine model (Rubin et al., 1964). Hydrogen bond strength is therefore controlled by the electron-donating or -withdrawing power of the para substituent. In assessing the suitability of electron-donating and -withdrawing groups to be placed in the para positions (3′ and 6 of **2a**), it was noted that substituent addition would alter the hydrophile/lipophile balance (HLB) of the molecule. As such variation is liable to contribute taste effects, substituent π values (parametrizing hydrophobicity) were also considered (Martin, 1978). Synthetic selections resulted from the desire to produce a data set well suited for regression analyses (containing broad ranges in nonintercorrelating σ and π values), along with synthetic plausability.

EXPERIMENTAL PROCEDURES

Synthetic Methods. General Procedures. ^{1}H NMR spectra were obtained on a Nicolet NTC-200 spectrometer with tetramethylsilane as an internal reference. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by the Purdue microanalytical service. High-resolution mass spectra were run on a Kratos MS50 mass spectrometer. Workups that involved extraction into an organic solvent used $MgSO_4$ as a drying agent, which was filtered off before the solvent was evaporated in vacuo. Flash chromatography was carried out on silica gel 60 (EM 9385) 230–400 mesh. All other column chromatography was carried out on silica gel 60–200 mesh. For analytical TLC, aluminum-backed silica gel F-254 plates (Merk) were used. Chalcones were visualized on TLC

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plates by appearance of a characteristic red color upon spraying with $KMnO_4/H_2SO_4$ (50 mg/10 mL), while dihydrochalcones gave intense dark brown spots when placed in an iodine chamber. Hydrogenations under pressure were performed with a Parr hydrogenation apparatus. Dry organic solvents were obtained by distillation over the appropriate drying agent (Gordon and Ford, 1972) and stored over activated molecular sieves. All starting materials were purchased from either Aldrich or Sigma Chemical Companies or prepared as stated in the given reference.

General Method for the Benzylation of Phenols. The dry phenol was dissolved in dry DMF to give a 3% w/v solution. Benzyl chloride and anhydrous potassium carbonate (1.5 equiv of each reagent per hydroxyl to be benzylated) were added, and the mixture was stirred at the specified temperature until TLC ($CHCl_3$/EtOAc, 9:1) showed the reaction was complete. The mixture was then cooled to 25 °C and an equivalent volume of water added. The product was extracted into ethyl acetate and the organic layer washed three times with 1% HCl and twice with 3% NaCl. The organic solution was dried and the solvent removed. Evaporation by high-vacuum pump removed excess benzyl chloride to leave the crude solid or oily product.

General Method for the Preparation of Chalcones. Synthetic strategies for chalcone and dihydrochalcone production have been adapted from DuBois et al. (1981a). Equimolar quantities of the appropriately functionalized 2,4,6-tris(benzyloxy)-acetophenones and isovanillins were dissolved in THF/ethanol (1:2) to give an approximate 10% w/v solution. Aqueous KOH (60% w/v, 5 mL/100 mL of reaction solution) was added and the solution stirred at 40–50 °C until reaction was complete by TLC ($CHCl_3$/EtOAc, 5:1, or $CHCl_3$/MeOH, 9:1). An equivalent volume of water was then added and the mixture brought to pH 9 with HCl. The product was extracted into ethyl acetate and the organic layer washed with water and dried. Evaporation of the solvent gave the crude solid product.

General Method for the Preparation of Dihydrochalcones. The appropriate chalcone was dissolved in THF/ethanol (2:1) to give a 10% w/v solution. Palladium on charcoal (10%) was added to give a 1:10 ratio of catalyst to chalcone. After the reaction bottle was twice evacuated and filled, the mixture was hydrogenated at the detailed pressure for the specified length of time. The reaction mixture was filtered through Celite and the solvent evaporated to leave the crude product.

2,4,6-Tris(benzyloxy)acetophenone (3a). Oven-dried (120 °C, 6 h) 2,4,6-trihydroxyacetophenone was benzylated by the standard procedure for 6 h at 80 °C, followed by 5 h at 100 °C. Usual workup gave an orange oil which was triturated with ethanol to precipitate a white solid. Filtration followed by an ethanol wash and air drying gave a 74% yield of **3a** as an off-white powder. Recrystallization from ethyl acetate/ethanol gave white clusters, mp 82–84 °C. Anal. Calcd for $C_{29}H_{28}O_4$: C, 79.43; H, 5.98. Found: C, 79.24; H, 6.04. ^{1}H NMR ($CDCl_3$) δ 2.46 (s, 3 H, CH_3), 4.97 (s, 2 H, OCH_2Ph), 5.01 (s, 4 H, 2 × OCH_2Ph), 6.22 (s, 2 H, 2 × Ar*H*), 7.34 (s, 15 H, 3 × Ph*H*).

3-Hydroxy-2′,4′,6′-tris(benzyloxy)-4-methoxychalcone (5a). Reaction of **3a** with isovanillin for 72 h at 40 °C gave, after standard workup, a crude solid. Washing with cold ethyl acetate removed impurities to leave a 58% yield of **5a**. Recrystallization from ethyl acetate/ethanol gave white fluffy crystals, mp 177–179 °C. Anal. Calcd for $C_{37}H_{32}O_6$: C, 77.60; H, 5.63. Found: C, 77.30; H, 5.89. ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3 H, OCH_3), 5.11 (s, 6 H, 3 × OCH_2Ph), 6.50 (s, 2 H, 2 × Ar′*H*), 6.74–7.44 (m, 20 H).

2′,3,4′,6′-Tetrahydroxy-4-methoxydihydrochalcone (Hesperetin Dihydrochalcone, 2a). Hydrogenation of **5a** at 50 psi for 12.5 h followed by standard workup gave a light brown solid. Washing with chloroform and air drying gave a 93% yield of **2a** as an off-white powder. Recrystallization from ethanol/water gave white powdery flakes, mp 188–191 °C. Measured mass (EI) M$^+$ 304.0947 ($C_{16}H_{16}O_6$ requires M$^+$ 304.0947). ^{1}H NMR (acetone-d_6) δ 2.86 (t, 2 H, J = 8 Hz, CH_2Ar), 3.35 (t, 2 H, J = 8 Hz, Ar′$COCH_2$), 3.79 (s, 3 H, OCH_3), 5.94 (s, 2 H, 2 × Ar′*H*), 6.67–6.85 (m, 3 H, 3 × Ar*H*). This compound can also be prepared by hydrogenation of hesperetin in aqueous KOH (DuBois et al., 1977).

2-Methylphloroglucinol (9) was prepared by Clemmensen reduction of phloroglucinol carboxaldehyde. Column chromatography ($CHCl_3$/EtOAc, 1:1) and recrystallization from ethyl acetate/benzene gave light yellow clusters, mp 215–216 °C [lit. 214 °C (Robertson and Whalley, 1951)]. Measured mass (EI) M$^+$ 140.0473 ($C_7H_8O_3$ requires M$^+$ 140.0473). ^{1}H NMR (DMSO-d_6) δ 1.80 (s, 3 H, CH_3), 5.77 (s, 2 H, 2 × Ar*H*), 8.69 (s, 1 H, O*H*), 8.82 (s, 2 H, 2 × O*H*).

3-Methylphloroacetophenone (10). Oven-dried (120 °C, 6 h) **9** underwent a standard Hoesch reaction to give an 83% yield of **10** on the basis of the amount of **9** reacted. Recrystallization from ethanol/water gave light yellow fibrous needles, mp 210–211 °C [lit. 210–211 °C (Robertson and Whalley, 1951)]. Measured mass (EI) M$^+$ 182.0580 ($C_9H_{10}O_4$ requires M$^+$ 182.0579). ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 1.83 (s, 3 H, CH_3), 2.55 (s, 3 H, $COCH_3$), 6.01 (s, 1 H, Ar*H*), 10.34 (br s, 1 H, O*H*), 10.52 (br s, 1 H, O*H*), 13.96 (s, 1 H, O*H*).

2,4,6-Tris(benzyloxy)-3-methylacetophenone (3b). Oven-dried (120 °C, 6 h) **10** was benzylated for 17 h at 80 °C, followed by 6 h at 100 °C. Standard workup gave a red oil which crystallized on standing. The solid was washed with ethanol and air-dried to yield 51% of **3b**. Recrystallization from ethyl acetate/ethanol gave transparent cubes, mp 95–97 °C. Anal. Calcd for $C_{30}H_{28}O_4$: C, 79.62; H, 6.24. Found: C, 79.42; H, 6.37. ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3 H, $COCH_3$), 2.46 (s, 3 H, OCH_3), 4.84 (s, 2 H, OCH_2Ph), 5.02 (s, 4 H, 2 × OCH_2Ph), 6.37 (s, 1 H, Ar*H*), 7.37–7.41 (m, 15 H, 3 × Ph*H*).

3-Hydroxy-2′,4′,6′-tris(benzyloxy)-4-methoxy-3′-methylchalcone (5b). Reaction of **3b** with isovanillin for 48 h at 40 °C, followed by usual workup, gave a red oil. Addition of ethanol precipitated a solid which was filtered and air-dried to give an 82% yield of **5b** on the basis of the amount of **3b** reacted. Recrystallization from ethyl acetate/benzene gave yellow fluffy needles, mp 129–131 °C. Anal. Calcd for $C_{38}H_{34}O_6 \cdot C_6H_6$: C, 79.50; H, 6.07. Found: C, 79.40; H, 6.11. ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3 H, CH_3), 3.92 (s, 3 H, OCH_3), 4.85 (s, 2 H, OCH_2Ph), 5.03 (s, 2 H, OCH_2Ph), 5.05 (s, 2 H, OCH_2Ph), 5.61 (s, 1 H, O*H*), 6.40 (s, 1 H, Ar′*H*), 6.81 (d, 1 H, J = 8 Hz, H_5), 6.89 (d, 1 H, J = 16 Hz, C*H*Ar), 6.98 (dd, 1 H, J = 8 Hz, 2 Hz, H_6), 7.11 (d, 1 H, J = 2 Hz, H_2), 7.23–7.42 (m, 16 H).

2′,3,4′,6′-Tetrahydroxy-4-methoxy-3′-methyldihydrochalcone (2b). Hydrogenation of **5b** at 50 psi for 10 h followed by standard workup gave an off-white solid. After washing with chloroform, a 98% yield of **2b** was obtained. Recrystallization from ethanol/water gave a white powder, mp 214–216 °C. Measured mass (EI) M$^+$ 318.1107 ($C_{17}H_{18}O_6$ requires M$^+$ 318.1103). ^{1}H NMR (DMSO-d_6) δ 1.84 (s, 3 H, Ar′CH_3), 2.74 (t, 2 H, J = 8 Hz, CH_2Ar), 3.23 (t, 2 H, J = 8 Hz, Ar′$COCH_2$), 3.72 (s, 3 H, OCH_3), 6.01 (s, 1 H, Ar′*H*), 6.57–6.82 (m, 3 H, 3 × Ar*H*), 8.81 (s, 1 H, O*H*), 10.32 (br s, 1 H, O*H*), 10.57 (br s, 1 *H*, O*H*), 13.98 (s, 1 H, O*H*).

3-Chlorophloroacetophenone (11). Phloroacetophenone monohydrate (5.00 g, 26.9 mmol) was oven-dried (120 °C, 6 h) and dissolved in anhydrous ether (150 mL). The solution was cooled to 0 °C, and then sulfuryl chloride (4.00 g, 29.6 mmol) in anhydrous ether (20 mL) was added dropwise with stirring over 20 min. The reaction was stirred at 0–5 °C for 45 min and then allowed to slowly warm to room temperature over 5 h. The solution was poured into ice (200 g) and stirred until the ice was melted, and the organic layer was separated. After washing with water (2 × 200 mL), the organic layer was dried and the solvent evaporated to leave a yellow solid. Recrystallization from ethanol/water and drying in vacuo gave **11** (5.12 g, 86% yield) as a light yellow powder. Further purification by column chromatography on silica ($CHCl_3$/EtOAc, 2:1) and recrystallization from ethyl acetate/hexane gave yellow needles, mp 215–218 °C. Measured mass (EI) M$^+$ 202.0033 ($C_8H_7ClO_4$ requires M$^+$ 202.0033). ^{1}H NMR (acetone-d_6) δ 2.65 (s, 3 H, $COCH_3$), 6.19 (s, 1 H, Ar*H*), 9.65 (s, 1 H, O*H*), 10.60 (s, 1 H, O*H*), 13.49 (s, 1 H, O*H*).

2,4,6-Tris(benzyloxy)-3-chloroacetophenone (3c). Benzylation of **11** for 10 h at 70 °C, followed by 12 h at 90 °C, produced a red oil on workup. Trituration with ethanol precipitated a light brown solid which was filtered and air-dried to give a 43% yield of **3c**. Recrystallization from ethyl acetate/ethanol gave transparent cubes, mp 106–108 °C. Anal. Calcd

for $C_{29}H_{25}ClO_4$: C, 73.65; H, 5.33; Cl, 7.50. Found: C, 73.72; H, 5.30; Cl, 7.32. ^1H NMR ($CDCl_3$) δ 2.42 (s, 3 H, $COCH_3$), 5.01 (s, 4 H, 2 × OCH_2Ph), 5.12 (s, 2 H, OCH_2Ph), 6.41 (s, 1 H, Ar*H*), 7.33–7.47 (m, 15 H, 3 × Ph*H*).

3-Hydroxy-2′,4′,6′-tris(benzyloxy)-4-methoxy-3′-chlorochalcone (5c). Reaction of **3c** with isovanillin for 18 h at 40 °C gave, after workup, a 72% yield of **5c**. Recrystallization from ethyl acetate/ethanol gave yellow cubes, mp 115–117 °C. Anal. Calcd for $C_{37}H_{31}ClO_6$: C, 73.20; H, 5.15; Cl, 5.84. Found: C, 73.04; H, 5.08; Cl, 5.94. ^1H NMR ($CDCl_3$) δ 3.87 (s, 3 H, OCH_3), 5.01 (s, 4 H, 2 × OCH_2Ph), 5.12 (s, 2 H, OCH_2Ph), 5.69 (s, 1 H, O*H*), 6.44 (s, 1 H, Ar′*H*), 6.79–7.41 (m, 20 H).

2′,3,4′,6′-Tetrahydroxy-4-methoxy-3′-chlorodihydrochalcone (2c). To inhibit dehalogenation, hydrogenation of **5c** was carried out with three drops of glacial acetic acid added to the reaction mixture. After 2 h at 5 psi, the reaction was complete and gave a crude green solid upon workup. Washing with chloroform and air-drying left a 91% yield of **2c** as an off-white solid. Recrystallization from ethanol/water gave white powdery flakes, mp 180–182 °C. Measured mass (EI) M^+ 338.0553 ($C_{16}H_{15}ClO_6$ requires M^+ 338.0557). ^1H NMR (acetone-d_6) δ 2.88 (t, 2 H, J = 8 Hz, CH_2Ar), 3.39 (t, 2 H, J = 8 Hz, Ar′$COCH_2$), 3.80 (s, 3 H, OCH_3), 6.20 (s, 1 H, Ar′*H*), 6.67–6.86 (m, 3 H, 3 × Ar*H*).

3-Formylphloroacetophenone (12). Oven-dried (120 °C, 6 h) phloroacetophenone underwent a Gatterman formylation with zinc cyanide and HCl_g similar to that of Shah and Shah (1939), but with omission of aluminum chloride. Recrystallization of **12** from methanol/water with decolorizing charcoal gave fine white needles in 73% yield (on the basis of reacted phloroacetophenone), mp 185–188 °C [lit. 182–183 °C (Robertson and Whalley, 1951)]. Measured mass (EI) M^+ 196.0372 ($C_9H_8O_5$ requires M^+ 196.0372). ^1H NMR (DMSO-d_6) δ 2.61 (s, 3 H, $COCH_3$), 5.88 (s, 1 H, Ar*H*), 9.99 (s, 1 H, C*H*O), 12.40 (br s, 1 H, O*H*), 13.65 (br s, 1 H, O*H*), 14.85 (br s, 1 H, O*H*).

3-Formyl-2,4,6-tris(benzyloxy)acetophenone (3d). Oven-dried (120 °C, 6 h) **12** was benzylated for 15 h at 75 °C, followed by 10 h at 100 °C, to give a yellow solid upon workup. Washing with ethanol and air-drying left a 61% yield of **3d**. Recrystallization from ethyl acetate/ethanol gave white semitransparent clusters, mp 139–141 °C. Anal. Calcd for $C_{30}H_{26}O_5$: C, 77.24; H, 5.62. Found: C, 77.16; H, 5.46. ^1H NMR ($CDCl_3$) δ 2.39 (s, 3 H, $COCH_3$), 4.97 (s, 2 H, OCH_2Ph), 5.08 (s, 2 H, OCH_2Ph), 5.13 (s, 2 H, OCH_2Ph), 6.38 (s, 1 H, Ar*H*), 7.33–7.40 (m, 15 H, 3 × Ph*H*), 10.40 (s, 1 H, C*H*O).

2,4,6-Tris(benzyloxy)-3-(hydroxymethyl)acetophenone (3n). A solution of **3d** (5.00 g, 10.7 mmol) in dry THF (55 mL) was cooled to 5 °C in an ice bath. Under a nitrogen atmosphere 0.5 M sodium borohydride in diglyme (10.7 mL, 5.4 mmol) was added dropwise with stirring. The solution was stirred for 6 h as the temperature was slowly increased to 15 °C. The cloudy light yellow solution was poured into a dilute HCl/ice mixture (200 mL) and then extracted into ethyl acetate (100 mL). The organic layer was washed with water (3 × 200 mL) and dried. The solvent was evaporated to leave a yellow oil which solidified on standing. Recrystallization from benzene/petroleum ether gave light yellow semitransparent clusters of **3n** (4.70 g, 94% yield), mp 87–89 °C. Anal. Calcd for $C_{30}H_{28}O_5$: C, 76.90; H, 6.02. Found: C, 76.84; H, 6.11. ^1H NMR ($CDCl_3$) δ 2.45 (s, 3 H, Ar$COCH_3$), 4.69 (d, 2 H, J = 7 Hz, CH_2OH), 4.95 (s, 2 H, OCH_2Ph), 5.05 (s, 2 H, OCH_2Ph), 5.07 (s, 2 H, OCH_2Ph), 6.39 (s, 1 H, Ar*H*), 7.35–7.41 (m, 15 H, 3 × Ph*H*).

2′,3,4′,6′-Tetrakis(benzyloxy)-4-methoxy-3′-(hydroxymethyl)chalcone (5n). Reaction of **3n** and **4b** at 40 °C for 26 h, followed by standard workup, gave a 90% yield of **5n**. Recrystallization from benzene/petroleum ether gave light yellow clusters, mp 141–144 °C. Anal. Calcd for $C_{45}H_{40}O_7$: C, 78.02; H, 5.82. Found: C, 78.05; H, 5.79. ^1H NMR ($CDCl_3$) δ 3.90 (s, 3 H, OCH_3), 4.69 (br s, 2 H, CH_2OH), 4.95 (s, 2 H, OCH_2Ph), 5.04 (s, 2 H, OCH_2Ph), 5.07 (s, 2 H, OCH_2Ph), 5.11 (s, 2 H, OCH_2Ph), 6.42 (s, 1 H, Ar′*H*), 6.81–7.45 (m, 25 H).

3′-Formyl-2′,3,4′,6′-tetrakis(benzyloxy)-4-methoxychalcone (5d). To a solution of **5n** in benzene (500 mL) was added pyridinium chlorochromate on alumina (50 g; Cainelli and Cardillo, 1984) with stirring. After stirring for 2.5 h at 25 °C, a further 50 g of PCC on alumina was added to the black mixture. Stirring at 25 °C was continued for another 3.5 h, after which time the mixture was filtered through Celite. The solvent was evaporated to 200 mL and then refiltered to remove precipitate. The filtrate was evaporated to leave a brown sticky oil which was purified by column chromatography ($CHCl_3$) to give a yellow oil. Crystallization from benzene/petroleum ether yielded 7.77 g (78%) of **5d** as a white powder, mp 93–95 °C. Anal. Calcd for $C_{45}H_{38}O_7$: C, 78.24; H, 5.55. Found: C, 78.41; H, 5.86. ^1H NMR ($CDCl_3$) δ 3.91 (s, 3 H, OCH_3), 4.98 (s, 2 H, OCH_2Ph), 5.09 (s, 2 H, OCH_2Ph), 5.11 (s, 2 H, OCH_2Ph), 5.15 (s, 2 H, OCH_2Ph), 6.40 (s, 1 H, Ar′*H*), 6.78 (d, 1 H, J = 16 Hz, C*H*Ar), 6.88–7.06 (m, 3 H, 3 × Ar*H*), 7.19–7.45 (m, 21 H), 10.40 (s, 1 H, C*H*O).

3′-Formyl-2′,3,4′,6′-tetrahydroxy-4-methoxydihydrochalcone (2d). Hydrogenation of **5d** at 50 psi for 4.5 h followed by the usual workup gave a cream solid. The product was washed with chloroform and then dissolved in THF and applied to a column of silica which was eluted with ethyl acetate. A yield of 59% was obtained for pure **2d**, this compound being sparingly soluble in most solvents except THF and DMSO, mp 229–230 °C, after changing crystal structure and turning red at 220 °C. Measured mass (EI) M^+ 332.0908 ($C_{17}H_{16}O_7$ requires M^+ 332.0896). ^1H NMR (DMSO-d_6) δ 2.76 (t, 2 H, J = 7 Hz, CH_2Ar), 3.27 (t, 2 H, J = 7 Hz, Ar′$COCH_2$), 3.72 (s, 3 H, OCH_3), 5.90 (s, 1 H, Ar′*H*), 6.58–6.83 (m, 3 H, 3 × Ar*H*), 9.99 (s, 1 H, C*H*O).

3′-Carboxy-2′,3,4′,6′-tetrakis(benzyloxy)-4-methoxychalcone (5e). The oxidation procedure was adapted from that of Bal et al. (1981). To a solution of **5d** (4.00 g, 5.8 mmol) in THF (40 mL) and *tert*-butyl alcohol (80 mL) was added 2-methyl-2-butene (32 mL). Dropwise addition of sodium chlorite (4.80 g, 53.1 mmol) and sodium dihydrogen phosphate (4.80 g, 34.8 mmol) in H_2O (20 mL) then followed. The clear solution was stirred vigorously for 30 min, and then the aqueous layer was discarded and the organic layer evaporated. The residue was dissolved in ethyl acetate (100 mL), washed with H_2O (2 × 50 mL), dried, and evaporated to leave a light yellow oil which solidified on standing. Recrystallization from benzene/petroleum ether gave 3.54 g (87% yield) of **5e** as a white powder, mp 164–167 °C. Anal. Calcd for $C_{45}H_{38}O_8$: C, 76.47; H, 5.42. Found: C, 76.77; H, 5.57. ^1H NMR (DMSO-d_6) δ 3.81 (s, 3 H, OCH_3), 4.93 (s, 2 H, OCH_2Ph), 5.12 (s, 2 H, OCH_2Ph), 5.22 (s, 2 H, OCH_2Ph), 5.26 (s, 2 H, OCH_2Ph), 6.93 (s, 1 H, Ar′*H*), 7.00–7.46 (m, 25 H), 13.10 (br s, 1 H, COO*H*).

3′-Carboxy-2′,3,4′,6′-tetrahydroxy-4-methoxydihydrochalcone (2e). Hydrogenation of **5e** at 50 psi for 3.5 h followed by the usual workup gave a creamy brown solid. Washing with chloroform produced an 85% yield of **2e** as an off-white powder. Trace impurities were removed by chromatographing through a column of silica eluted with EtOAc/EtOH/AcOH (200:10:0.5). A pure white powder was obtained, mp 185 °C (decomposing to a sticky orange tar). Measured mass (FAB) $[M + H]^+$ 349.0924 ($C_{17}H_{16}O_8$ requires $[M + H]^+$ 349.0923). ^1H NMR (DMSO-d_6) δ 2.74 (t, 2 H, J = 8 Hz, CH_2Ar), 3.26 (t, 2 H, J = 8 Hz, Ar′$COCH_2$), 3.72 (s, 3 H, OCH_3), 5.57 (s, 1 H, Ar′*H*), 6.59–6.83 (m, 3 H, 3 × Ar*H*).

3′-Carbomethoxy-2′,3,4′,6′-tetrakis(benzyloxy)-4-methoxychalcone (5f). A mixture of **5e** (1.0 g, 1.4 mmol), dimethyl sulfate (0.15 mL, 1.6 mmol), and anhydrous potassium carbonate (245 mg, 1.8 mmol) in acetone (30 mL) was stirred at 45 °C for 1 h. The mixture was then cooled to 25 °C, poured into 0.1 M HCl (50 mL), and extracted with ethyl acetate (2 × 50 mL). After washing with water (2 × 20 mL), the combined organic layers were dried and the solvent was evaporated to leave a yellow oil (900 mg). Flash chromatography ($CHCl_3$) gave 730 mg (72% yield) of pure **8f** as a light yellow oil. Crystallization from benzene/petroleum ether gave transparent cubes, mp 165–166 °C. Anal. Calcd for $C_{46}H_{40}O_8$: C, 76.65; H, 5.59. Found: C, 76.85; H, 5.54. ^1H NMR ($CDCl_3$) δ 3.78 (s, 3 H, CO_2CH_3), 3.91 (s, 3 H, ArOCH_3), 4.98 (s, 2 H, OCH_2Ph), 5.03 (s, 2 H, OCH_2Ph), 5.08 (s, 2 H, OCH_2Ph), 5.11 (s, 2 H, OCH_2Ph), 6.36 (s, 1 H, Ar′*H*), 6.81 (d, 1 H, J = 16 Hz, C*H*Ar), 6.87 (d, 1 H, J = 8 Hz, H_5), 7.06–7.46 (m, 23 H).

3′-Carbomethoxy-2′,3,4′,6′-tetrahydroxy-4-methoxydihydrochalcone (2f). Hydrogenation of **5f** was performed in THF/methanol (2:1) under a balloon of hydrogen for 9 h. Stan-

dard workup gave a light brown solid which was purified by flash chromatography ($CHCl_3$) to yield 86% of **2f** as a white powder. Recrystallization from acetone/water gave fine white needles, mp 164–165 °C. Measured mass (EI) M^+ 362.0996 ($C_{18}H_{18}O_8$ requires M^+ 362.1001). ^1H NMR (DMSO-d_6) δ 2.75 (t, 2 H, J = 7 Hz, CH_2Ar), 3.25 (t, 2 H, J = 7 Hz, Ar'$COCH_2$), 3.72 (s, 3 H, $ArOCH_3$), 3.78 (s, 3 H, CO_2CH_3), 5.99 (s, 1 H, Ar'H), 6.57–6.82 (m, 3 H, 3 × ArH).

3-Hydroxy-2′,4′,6′-tris(benzyloxy)-4-methoxy-6-methylchalcone (5g). Reaction of **3a** with 6-methylisovanillin (**4g**; Kozlova et al., 1981) for 36 h at 40 °C gave, after standard workup, crude product. Impurities were removed by washing with cold diethyl ether/ethyl acetate (10:1) to provide a 66% yield of **5g**. Recrystallization from ethyl acetate/ethanol gave light yellow fluffy crystals, mp 181–183 °C. Anal. Calcd for $C_{38}H_{34}O_6$: C, 77.80; H, 5.84. Found: C, 77.51; H, 5.86. ^1H NMR (DMSO-d_6) δ 2.07 (s, 3 H, $ArCH_3$), 3.75 (s, 3 H, OCH_3), 5.07 (s, 4 H, 2 × OCH_2Ph), 5.08 (s, 2 H, OCH_2Ph), 6.47 (s, 2 H, 2 × Ar'H), 6.62 (d, 1 H, J = 15 Hz, $CHAr$), 6.77 (s, 1 H, ArH), 7.08 (s, 1 H, ArH), 7.23–7.42 (m, 16 H).

2′,3,4′,6′-Tetrahydroxy-4-methoxy-6-methyldihydrochalcone (2g). Hydrogenation of **5g** at 40 psi for 14 h gave a gray/green oily product upon workup. The oil was chromatographed on a column of silica (toluene/CH_2Cl_2/acetone, 3:2:2) to give a 98% yield of **2g** as a white powder. Recrystallization from ethanol/water gave white flakes, mp 184–186 °C. Measured mass (EI) M^+ 318.1102 ($C_{17}H_{18}O_6$ requires M^+ 318.1103). ^1H NMR (acetone-d_6) δ 2.24 (s, 3 H, $ArCH_3$), 2.85 (t, 2 H, J = 8 Hz, CH_2Ar), 3.30 (t, 2 H, J = 8 Hz, Ar'$COCH_2$), 3.79 (s, 3 H, OCH_3), 5.94 (s, 2 H, 2 × Ar'H), 6.72 (s, 1 H, ArH), 6.73 (s, 1 H, ArH).

3-(Benzyloxy)-4-methoxy-6-bromobenzaldehyde (4h). Benzylation of 6-bromoisovanillin (Saraf, 1983) at 70 °C for 2 h produced upon workup a brown solid. Washing with ethanol and air-drying left an 85% yield of **4h**. Recrystallization from ethyl acetate/ethanol gave transparent needles, mp 144–146 °C [lit. 144–145 °C (Battersby et al., 1965)]. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.96 (s, 3 H, OCH_3), 5.14 (s, 2 H, OCH_2Ph), 7.16 (s, 1 H, ArH), 7.35–7.43 (m, 6 H, ArH, PhH), 10.11 (s, 1 H, CHO).

2′,3,4′,6′-Tetrakis(benzyloxy)-4-methoxy-6-bromochalcone (5h). Reaction of **3a** with **4h** at 60 °C for 10 h, followed by the regular workup, gave an 86% yield of **5h**. Recrystallization from ethyl acetate/ethanol gave light yellow needles, mp 164–165 °C. Anal. Calcd for $C_{44}H_{37}BrO_6$: C, 71.26; H, 5.03; Br, 10.77. Found: C, 71.50; H, 5.12; Br, 10.88. ^1H NMR (DMSO-d_6) δ 3.83 (s, 3 H, OCH_3), 5.13–5.16 (m, 8 H, 4 × OCH_2Ph), 6.54 (s, 2 H, 2 × Ar'H), 7.09 (d, 1 H, J = 15 Hz, $CHAr$), 7.23–7.44 (m, 21 H), 7.55 (d, 1 H, J = 15 Hz, Ar'$COCH$), 7.59 (s, 1 H, ArH).

2′,3,4′,6′-Tetrakis(benzyloxy)-4-methoxy-6-bromodihydrochalcone (6h). A solution of **5h** (200 mg, 0.27 mmol) in dry pyridine (8 mL) was cooled to 5 °C in an ice bath. Sodium borohydride in diglyme (0.5 M, 2.2 mL, 1.08 mmol) was added dropwise with stirring under a nitrogen atmosphere. The solution was allowed to warm to 25 °C and then stirred at 60 °C for 4 h. After cooling, the clear yellow solution was poured into 5% HCl/ice (10 mL) and extracted into toluene (20 mL). The organic layer was washed with 5% HCl (2 × 20 mL) and H_2O (2 × 20 mL) and then dried and evaporated to leave a yellow oil. Crystallization from ethyl acetate/ethanol gave 140 mg (70% yield) of **6h** as fine white needles, mp 110–111 °C. Anal. Calcd for $C_{44}H_{39}BrO_6$: C, 71.06; H, 5.29; Br, 10.74. Found: C, 70.77; H, 5.34; Br, 10.83. ^1H NMR (DMSO-d_6) δ 2.84–2.92 (m, 4 H, CH_2CH_2), 3.72 (s, 3 H, OCH_3), 4.95 (s, 2 H, OCH_2Ph), 5.11 (s, 6 H, 3 × OCH_2Ph), 6.49 (s, 2 H, 2 × Ar'H), 6.95 (s, 1 H, ArH), 7.09 (s, 1 H, ArH), 7.34–7.42 (m, 20 H, 4 × PhH).

2′,3,4′,6′-Tetrahydroxy-4-methoxy-6-bromodihydrochalcone (2h). To a stirred solution of **6h** (800 mg, 1.1 mmol) in glacial acetic acid (40 mL) at 80 °C was added concentrated HCl (20 mL) over 30 min. After 3 h of stirring at 80 °C, water (200 mL) was added and the product extracted into ethyl acetate (2 × 75 mL). The combined organic layers were washed with H_2O (2 × 75 mL) and 5% $NaHCO_3$ (75 mL). The ethyl acetate was evaporated and the residue dissolved in 5% K_2CO_3 (150 mL) and washed with $CHCl_3$ (150 mL), and then the aqueous layer was acidified to pH 5 with concentrated HCl. The product was extracted into ethyl acetate (2 × 75 mL) and then dried and evaporated to leave a brown oil which solidified on standing. Purification by column chromatography ($CHCl_3$/EtOAc, 2:1) gave 320 mg (78% yield) of **2h** as a white solid. Recrystallization from ethyl acetate/chloroform gave a white powder, mp 163–167 °C. Measured mass (CI) $[M + H]^+$ 383.0130 (^{79}Br), 385.0110 (^{81}Br) ($C_{16}H_{15}BrO_6$ requires $[M + H]^+$ 383.0130 (^{79}Br), 385.0110 (^{81}Br)). ^1H NMR (DMSO-d_6) δ 2.83 (t, 2 H, J = 7 Hz, CH_2Ar), 3.23 (t, 2 H, J = 7 Hz, Ar'$COCH_2$), 3.76 (s, 3 H, OCH_3), 5.82 (s, 2 H, 2 × Ar'H), 6.78 (s, 1 H, ArH), 7.07 (s, 1 H, ArH).

3-Hydroxy-2′,4′,6′-tris(benzyloxy)-4-methoxy-6-nitrochalcone (5i). Reaction of **3a** with 6-nitroisovanillin (**4i**; McDonald and Suksamran, 1978) at 40 °C for 12 h gave a crude solid upon workup. Washing with cold diethyl ether removed impurities to give a 65% yield of **5i**. Recrystallization from ethyl acetate/ethanol with decolorizing charcoal gave light yellow fluffy crystals, mp 196–198 °C. Anal. Calcd for $C_{37}H_{31}NO_8$: C, 71.95; H, 5.06; N, 2.27. Found: C, 72.19; H, 4.95; N, 2.24. ^1H NMR (DMSO-d_6) δ 3.92 (s, 3 H, OCH_3), 5.13 (s, 4 H, 2 × OCH_2Ph), 5.15 (s, 2 H, OCH_2Ph), 6.55 (s, 2 H, 2 × Ar'H), 6.79 (d, 1 H, J = 16 Hz, $CHAr$), 7.16 (s, 1 H, ArH), 7.26–7.72 (m, 17 H).

3-Hydroxy-2′,4′,6′-tris(benzyloxy)-4-methoxy-6-nitrodihydrochalcone (6i). A solution of **5i** (0.50 g, 0.81 mmol) in dry pyridine (20 mL) was cooled to 5 °C under a nitrogen atmosphere. Sodium borohydride in diglyme (0.5 M, 6.5 mL, 3.24 mmol) was added to give an orange solution which was stirred and warmed to 25 °C over 30 min. The reaction was stirred at 70 °C for 6.5 h and then cooled and added to 5% HCl/ice (20 mL). The product was extracted into toluene (40 mL), washed with 5% HCl (3 × 20 mL) and water (2 × 20 mL), and then dried. Evaporation of the solvent left a red-orange oil, which yielded a yellow solid upon addition and evaporation of ethanol. The solid was washed with ethanol (2 × 10 mL) and purified by flash chromatography ($CHCl_3$/EtOAc, 20:1) to give 260 mg (52% yield) of **6i** as an off-white powder. Recrystallization from ethyl acetate/ethanol gave pale yellow needles, mp 124–126 °C. Measured mass (FAB) $[M + H]^+$ 620.2198 ($C_{37}H_{33}NO_8$ requires $[M + H]^+$ 620.2284). ^1H NMR ($CDCl_3$) δ 3.15–3.22 (m, 4 H, CH_2CH_2), 3.89 (s, 3 H, OCH_3), 4.96 (s, 2 H, OCH_2Ph), 5.01 (s, 4 H, 2 × OCH_2Ph), 6.19 (s, 2 H, 2 × Ar'H), 6.80 (s, 1 H, H_2), 7.32 (s, 10 H, 2 × PhH), 7.36 (s, 5 H, PhH), 7.54 (s, 1 H, H_5).

2′,3,4′,6′-Tetrahydroxy-4-methoxy-6-nitrodihydrochalcone (2i). To 600 mg (0.97 mmol) of **6i** in glacial acetic acid (30 mL) at 70 °C was added concentrated HCl (7 mL) with stirring. After 15 min at 70 °C, a further 8 mL of concentrated HCl was added and stirring continued for 5 h. The solution was diluted with water (100 mL) and cooled to 10 °C to precipitate the product. The precipitate was filtered, washed with water (3 × 10 mL), and air-dried. Purification by flash chromatography ($CHCl_3$/EtOAc, 1:1) gave 150 mg (44% yield) of **2i** as a yellow solid. Recrystallization from ethyl acetate/chloroform gave a light yellow powder, mp 211 °C (dec). Measured mass (CI) $[M + H]^+$ 350.0875 ($C_{16}H_{15}NO_8$ requires $[M + H]^+$ 350.0875). ^1H NMR (DMSO-d_6) δ 3.09 (t, 2 H, J = 7 Hz, CH_2Ar), 3.31 (t, 2 H, J = 7 Hz, Ar'$COCH_2$), 3.84 (s, 3 H, OCH_3), 5.81 (s, 2 H, 2 × Ar'H), 6.84 (s, 1 H, H_2), 7.60 (s, 1 H, H_5).

2′,3,4′,6′-Tetrahydroxy-6-amino-4-methoxydihydrochalcone (2m). Hydrogenation of **5i** for 12 h at 40 psi gave, after filtration and evaporation of solvent, a yellow/green oil. Purification by column chromatography (toluene/CH_2Cl_2/acetone, 3:2:1) gave an 83% yield of **2m** as a yellow solid. Yellow crystals of **2m**, which appeared as the chromatography eluent evaporated, had mp 205 °C (dec). Measured mass (FAB) $[(M + H) - H_2O]^+$ 302.1038 ($C_{16}H_{17}NO_2$ requires $[(M + H) - H_2O]^+$ 302.1028). ^1H NMR (DMSO-d_6) δ 2.61 (t, 2 H, J = 8 Hz, CH_2Ar), 3.09 (t, 2 H, J = 8 Hz, Ar'$COCH_2$), 3.78 (s, 3 H, OCH_3), 5.77 (s, 2 H, 2 × Ar'H), 6.60 (s, 1 H, ArH), 6.74 (s, 1 H, ArH).

5-(Benzyloxy)-6-methoxyphthalaldehydic Acid (7). A procedure modified from that of Sinhabubu and Borchardt (1983) was employed. Morpholine (1.37 mL, 15.7 mmol, dried over NaOH under a nitrogen atmosphere for 48 h) and dry THF (20 mL) were placed in a flamed out round-bottom flask under a nitrogen atmosphere. A solution of **4h** (4.20 g, 13.1 mmol) in dry THF (80 mL) was placed in a flamed out dropping funnel attached to the flask. The morpholine solution was cooled to −50 °C in an acetone/dry ice bath, and the n-butyllithium (9.80 mL of a 1.6 M solution in hexane, 15.7 mmol) was added with stirring. After 5 min, the solution of **4h** was added dropwise

over 20 min with the temperature at −50 °C. The clear yellow solution was cooled to −75 °C, and *n*-butyllithium (12.26 mL, 19.6 mmol) was added dropwise over 20 min with the temperature kept below −70 °C. The solution was stirred below −70 °C for 35 min, and then a large excess of crushed dry ice was added. After 1 h at −70 °C the mixture was allowed to warm to 25 °C and acidified to pH 1 with 6 M HCl. Brine (100 mL) was added and the mixture extracted with ether (2 × 50 mL) followed by ethyl acetate (50 mL). The combined organic layers were washed with brine (50 mL), and the solvent was evaporated in vacuo. The residue was dissolved in 1 M NaOH (100 mL) and washed with ether (50 mL). The aqueous layer was acidified to pH 1 with 6 M HCl and the product extracted into ethyl acetate (100 mL). The organic layer was washed with water (2 × 50 mL), dried, and evaporated to leave a yellow-orange solid. Purification by flash chromatography ($CHCl_3$/EtOAc, 10:1, followed by $CHCl_3$/MeOH, 10:1) gave 2.73 g (73% yield) of **7** as a light tan solid. Recrystallization from ethyl acetate/hexane gave white crystals, mp 175–178 °C. Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 66.79; H, 5.07. ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3 H, OCH_3) 5.23 (s, 2 H, OCH_2Ph) 6.53 (br s, 1 H, C*H*OH) 7.32–7.46 (m, 7 H, 2 × Ar*H*, Ph*H*).

3-[2-Oxo-2-[2′,4′,6′-tris(benzyloxy)phenyl]ethyl]-5-(benzyloxy)-6-methoxy-1(3*H*)-isobenzofuranone (8). Dry diisopropylamine (2.5 mL, 17.5 mmol, dried over 3-Å molecular sieves) and dry THF (10 mL) were introduced to a flamed out round-bottom flask under a nitrogen atmosphere. The solution was cooled to −50 °C in an acetone/dry ice bath, and *n*-butyllithium (11.0 mL of a 1.6 M solution in hexane) was added dropwise with stirring. After the mixture was allowed to warm to −30 °C over 5 min, a solution of **3a** (3.06 g, 7.0 mmol) in dry THF (15 mL) was added dropwise. The deep orange solution was warmed to −10 °C over 20 min, followed by the dropwise addition of **7** (2.00 g, 7.0 mmol) in dry THF (20 mL) over 10 min. The solution was slowly warmed to 10 °C over 2 h and then allowed to warm to 25 °C and stirred for a further 4 h. A 5% $NaHCO_3$ solution (50 mL) was used to quench the reaction, and the product was extracted into ethyl acetate (2 × 50 mL). The combined organic layers were washed with water (2 × 50 mL), dried, and evaporated to leave an orange oil. TLC ($CHCl_3$/MeOH, 9:1) indicated this was chalcone **5j** by the red color induced by $KMnO_4/H_2SO_4$ spray. The product began to spontaneously convert to the higher R_f 8 on standing. The oil was dissolved in benzene (70 mL) and gently heated with *p*-toluenesulfonic acid (10 mg) to totally convert the chalcone to 8. The *p*-toluenesulfonic acid was washed out with water and the product purified by flash chromatography ($CHCl_3$/EtOAc, 20:1) to give 2.70 g (55% yield) of **8** as a yellow oil. Unreacted **3a** (640 mg) was also recovered from the column. Crystallization of **8** from ethyl acetate/hexane gave fine white crystals, mp 128–130 °C. Anal. Calcd for $C_{46}H_{38}O_8$: C, 76.47; H, 5.42. Found: C, 76.23; H, 5.12. ^{1}H NMR ($CDCl_3$) δ 3.19 (dd, 1 H, J = 18 Hz, 8 Hz, Ar′COC*H*), 3.51 (dd, 1 H, J = 18 Hz, 4 Hz, Ar′COC*H*), 3.87 (s, 3 H, OCH_3), 4.87 (s, 2 H, OCH_2Ph), 4.97 (s, 6 H, 3 × OCH_2Ph), 5.89 (m, 1 H, H_3), 6.25 (s, 2 H, 2 × Ar′*H*), 6.97 (s, 1 H, Ar*H*), 7.20–7.34 (m, 21 H).

6-Carboxy-2′,3,4′,6′-tetrahydroxy-4-methoxydihydrochalcone (2j). To a solution of **8** (300 mg, 0.52 mmol) in THF (18 mL) and methanol (12 mL) was added 20% NaOH (0.21 mL, 1.0 mmol). The solution became bright yellow upon addition of the base, and after 5 min of stirring, TLC ($CHCl_3$/MeOH, 9:1) showed complete conversion of the starting material to the chalcone form **5j**. Palladium on charcoal (75 mg) was added and the mixture stirred under a balloon of hydrogen for 6.5 h. After addition of 6 M HCl (0.21 mL) and filtration through Celite, the solvent was evaporated to leave an off-white solid. Purification by flash chromatography ($CHCl_3$/MeOH, 9:1) gave 125 mg (85% yield) of **2j** as a white powder, mp 155 °C (dec). Measured mass (FAB) $[M + H]^+$ 349.0917 ($C_{17}H_{16}O_8$ requires $[M + H]^+$ 349.0928). ^{1}H NMR (DMSO-d_6) δ 3.06 (t, 2 H, J = 6 Hz, CH_2Ar), 3.18 (t, 2 H, J = 6 Hz, Ar′COCH_2), 3.72 (s, 3 H, OCH_3), 5.75 (s, 2 H, 2 × Ar′*H*), 6.68 (s, 1 H, Ar*H*), 7.34 (s, 1 H, Ar*H*).

6-Carbomethoxy-2′,3,4′,6′-tetrakis(benzyloxy)-4-methoxychalcone (5k). A procedure modified from that of Grundy et al. (1972) was employed. To a solution of **8** (500 mg, 0.71 mmol) in THF (20 mL) was added NaOH (31 mg in 0.1 mL of H_2O, 0.78 mmol). The deep yellow solution was stirred for 15 min before dimethyl sulfate (74 μL, 0.78 mmol) was added. After refluxing for 3 h, the solution was cooled, poured into 0.1 M HCl (20 mL), and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine (50 mL) and dried, and the solvent was evaporated to give a yellow oil. Crystallization from ethyl acetate/methanol gave 460 mg (90% yield) of **5k** as clustered needles, mp 145–146 °C. Anal. Calcd for $C_{46}H_{40}O_8$: C, 76.65; H, 5.59. Found: C, 76.46; H, 5.78. ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3 H, CO_2CH_3), 3.94 (s, 3 H, $ArOCH_3$), 5.01 (s, 2 H, OCH_2Ph), 5.05 (s, 4 H, 2 × OCH_2Ph), 5.11 (s, 2 H, OCH_2Ph), 6.27 (s, 2 H, 2 × Ar′*H*), 6.67 (d, 1 H, J = 16 Hz, C*H*Ar), 7.13 (s, 1 H, Ar*H*), 7.21–7.40 (m, 20 H), 7.47 (s, 1 H, Ar*H*), 8.18 (d, 1 H, J = 16 Hz, Ar′COC*H*).

6-Carbomethoxy-2′,3,4′,6′-tetrahydroxy-4-methoxydihydrochalcone (2k). A solution of **5k** in THF/methanol (2:1) was hydrogenated under a balloon of hydrogen for 6 h. Standard workup followed by flash chromatography ($CHCl_3$/MeOH, 9:1) gave a 95% yield of **2k** as a white powder, mp 225–227 °C. Measured mass (CI) $[M + H]^+$ 363.1073 ($C_{18}H_{18}O_8$ requires $[M + H]^+$ 363.1079). ^{1}H NMR (DMSO-d_6) δ 3.03 (t, 2 H, J = 6 Hz, CH_2Ar), 3.18 (t, 2 H, J = 6 Hz, Ar′COCH_2), 3.70 (s, 3 H, CO_2CH_3), 3.73 (s, 3 H, $ArOCH_3$), 5.75 (s, 2 H, 2 × Ar′*H*), 6.70 (s, 1 H, Ar*H*), 7.33 (s, 1 H, Ar*H*).

3,6-Bis(benzyloxy)-4-methoxybenzaldehyde (4l). Benzylation of 6-hydroxyisovanillin (Daly et al., 1961) at 70 °C for 30 h produced upon workup a light brown powder. After the powder was washed with ethanol and air-dried, an 86% yield of **4l** was obtained. Recrystallization from ethyl acetate/ethanol gave white needles, mp 132–134 °C. Anal. Calcd for $C_{22}H_{20}O_4$: C, 75.85; H, 5.79. Found: C, 76.19; H, 5.95. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 3.93 (s, 3 H, OCH_3), 5.06 (s, 2 H, OCH_2Ph), 5.23 (s, 2 H, OCH_2Ph), 6.72 (s, 1 H, Ar*H*), 7.31–7.45 (m, 11 H, Ar*H*, 2 × Ph*H*), 10.29 (s, 1 H, C*H*O).

2′,3,4′,6,6′-Pentakis(benzyloxy)-4-methoxychalcone (5l). Reaction of **3a** with **4l** at 60 °C for 48 h, followed by the usual workup, gave an orange oil. Crystallization from benzene/petroleum ether gave a crude solid which was filtered and washed clean with ethanol to give an 80% yield of **5l**. Recrystallization from benzene/petroleum ether gave light yellow clusters, mp 129–131 °C. Anal. Calcd for $C_{51}H_{44}O_7$: C, 79.67; H, 5.77. Found: C, 79.63; H, 6.08. ^{1}H NMR ($CDCl_3$) δ 3.87 (s, 3 H, OCH_3), 4.97 (s, 8 H, 4 × OCH_2Ph), 5.06 (s, 2 H, OCH_2Ph), 6.24 (s, 2 H, 2 × Ar′*H*), 6.52 (s, 1 H, H_5), 6.81 (d, 1 H, J = 16 Hz, C*H*Ar), 7.15–7.45 (m, 16 H), 7.79 (d, 1 H, J = 16 Hz, Ar′COC*H*).

2′,3,4′,6,6′-Pentahydroxy-4-methoxydihydrochalcone (2l). Hydrogenation of **5l** by stirring under a balloon of hydrogen for 6 h gave, after the standard workup, a green solid. Washing with chloroform removed trace impurities to leave an 85% yield of **2l** as a white powder, mp 170 °C (dec). This compound was unstable in solution, converting to less polar species by TLC analysis. Measured mass (FAB) $[M + H]^+$ 321.0971 ($C_{16}H_{16}O_7$ requires $[M + H]^+$ 321.0974). ^{1}H NMR (DMSO-d_6) δ 2.66 (t, 2 H, J = 7 Hz, CH_2Ar), 3.17 (t, 2 H, J = 7 Hz, Ar′COCH_2), 3.67 (s, 3 H, OCH_3), 5.79 (s, 2 H, 2 × Ar′*H*), 6.39 (s, 1 H, Ar*H*), 6.51 (s, 1 H, Ar*H*).

Sensory Evaluation. Evaluations of taste for the novel compounds were performed by a human taste panel following the guidelines of Swartz and Furia (1977). Pure compounds were assessed as nonmutagenic to three strains (TA 98, TA 100, and TA 102) of *Salmonella typhimurium* following the protocol of Marion and Ames (1983). None of the compounds showed any single-dose acute toxicity when fed to mice at 200 and 400 mg/kg. Twenty nonsmoking volunteer panelists were initially screened for their ability to distinguish the four basic tastes (sweet, salt, sour, and bitter) and rank sucrose solutions of varying concentration. Training sessions were conducted over a number of weeks during which panelists performed triangle tests (identifying a sucrose solution of different concentration from two others of identical concentration), magnitude estimations (estimating potencies of varying sucrose and NHDHC concentrations against a sucrose reference), and taste quality tests (estimating the percentage of sweet and nonsweet taste components of varying concentrations of NHDHC). For magnitude estimations, panelists first assigned a number to the reference and then rated the perceived taste intensities of test solutions

against this number. Perceived intensities given by the panelists were then "normalized" to bring parity to individual scaling systems. A normalized intensity is the ratio of a rated intensity to the geometric mean of all intensities scored by the panelist for the session (e.g., normalized intensity of compound A given by eq 1). Normalized intensities (I_N) were cal-

$$I_{N(A)} = I_A/(I_A I_B I_C \dots I_n)^{1/n} \quad (1)$$

culated for each solution, as determined by each individual panelist. I_N values were averaged across the panel to give a single value for each solution. Calculation of normalized intensities in this way gave highly consistent evaluations when various concentrations of NHDHC were repeatedly given to the panel.

The eight most consistent performers during training were selected as final panelists to evaluate the new compounds. All testing was carried out by the standard "sip and spit" procedure using dilute dihydrochalcone concentrations (between 20 and 100 ppm) of similar intensity to the 6% sucrose reference. Copious rinsing and chewing of breadsticks relieved aftertaste between tastings. Panelists held the test solution in their mouths until maximum intensity was reached and then rated this peak taste intensity. Taste quality of each compound was then assessed by dividing the taste into the percentage of sweet versus nonsweet components. Nonsweetness was comprised of bitterness, licorice, or menthollike taste, and any other sidetaste. A maximum of four compounds were tested per session against the reference. Compounds of reasonable sweetness were subjected to repeated evaluations, while poor quality compounds were only tested once by the panel. After each session, the geometric mean of intensity assessments for each individual panelist was calculated. Each assessment was divided by this geometric mean to give the normalized intensity for each solution, as determined by the particular panelist. Average normalized intensity (I_N) values for each solution were then calculated from all panelists. These averaged I_N values were corrected to the scale of reference = 1 and multiplied by the dilution factor (concentration of sucrose reference/concentration of sweetener) to give the reported taste potency for each compound (eq 2). Data widely outside the 95% confidence limits were rejected by the t-value method (Gordon and Ford, 1972).

$$\text{taste potency} = (I_N \text{ sweetener}/I_N \text{ reference}) \times (60\,000 \text{ ppm}/\text{sweetener ppm}) \quad (2)$$

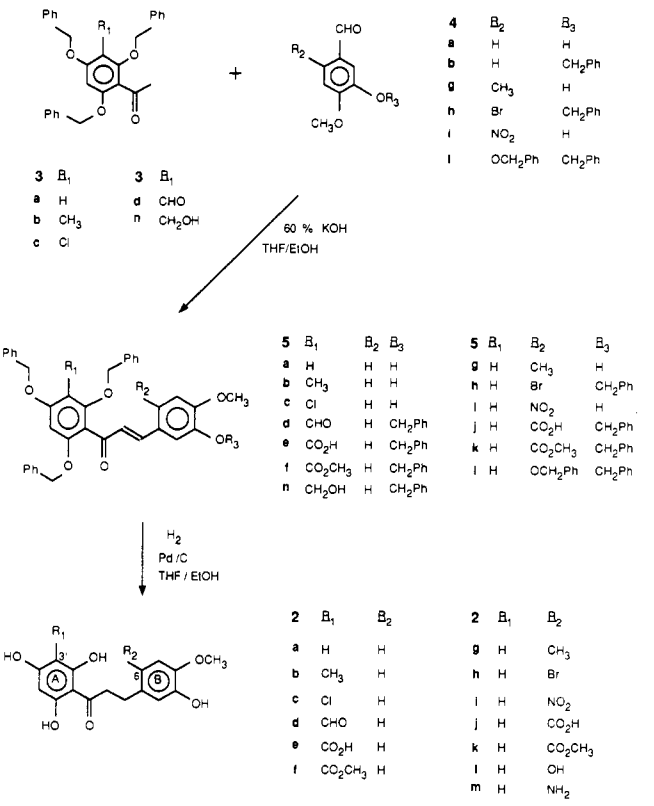
RESULTS AND DISCUSSION

Synthesis. Analogues of **2a** were synthesized by the general route outlined in Scheme I. Aldol condensations between appropriately functionalized derivatives of 2,4,6-tris(benzyloxy)acetophenone (**3a**) and isovanillin (**4a**) gave the corresponding chalcones **5** in reasonable to high yield. Hydrogenation then saturated the olefin and concomitantly deprotected the phenolic ethers to provide the desired dihydrochalcones **2** in excellent yield.

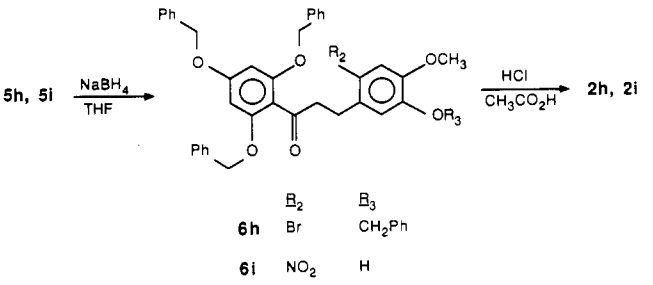
Chalcones containing functional groups labile to the hydrogenation conditions (**5h**, **5i**; $R_2 = NO_2$ or Br) were reduced to the benzylated dihydrochalcones **6h** and **6i** with sodium borohydride. Debenzylation proceeded in warm HCl/acetic acid to give the dihydrochalcones **2h** and **2i** (Scheme II).

During the preparation of the many chalcones it was discovered that the aldol condensation produced higher yields of cleaner product when the isovanillin unit **4** had its hydroxyl masked as a benzyl ether. The formyl derivative of the masked phloroacetophenone **3d** failed to produce the target chalcone by aldol condensation, possibly because of a competing Cannizzaro reaction. Compound **3d** was therefore reduced to the hydroxymethyl derivative **3n**, which reacted smoothly under the standard aldol conditions. The resulting chalcone (**5n**) was oxidized by pyridinium chlorochromate to give formylchalcone **5d**.

Scheme I. Synthesis of Dihydrochalcones



Scheme II. Synthetic Route to Dihydrochalcones 2h and 2i



Hydrogenation of **5n** produced a mixture of compounds from which the (hydroxymethyl)dihydrochalcone could not be isolated. While standard aldol conditions failed for reaction of **3a** with phthalaldehydic acid **7**, modified conditions using lithium diisopropylamide as the base in anhydrous THF successfully gave chalcone **5j**. This chalcone readily converted to isobenzofuranone **8**. Under alkaline conditions **8** was opened up to the chalcone form and hydrogenated to dihydrochalcone **2j** (Scheme III).

Sensory Evaluation. The novel dihydrochalcones were generally found to have low water solubilities (<100 ppm). Formation of the monobasic metal salts of dihydrochalcones has been reported to improve sweetness and decrease persistence (Westall and Messing, 1977). Triangle tests by our taste panel showed that 100 ppm solutions of NHDHC (**1**) and hesperetin dihydrochalcone (**2a**) could not be distinguished from their counterpart solutions containing 1 molar equivalent of NaOH. It therefore seemed that minor addition of base to dihydrochalcones would enhance solubility without inducing any fundamental changes in taste. Aqueous solutions of the dihydrochalcones containing 1 molar equivalent of NaOH were prepared, enabling sufficient concentrations for taste panel evaluation to be obtained. Sonication also helped where dissolution proved difficult.

Scheme III. Synthetic Route to Dihydrochalcone 2j

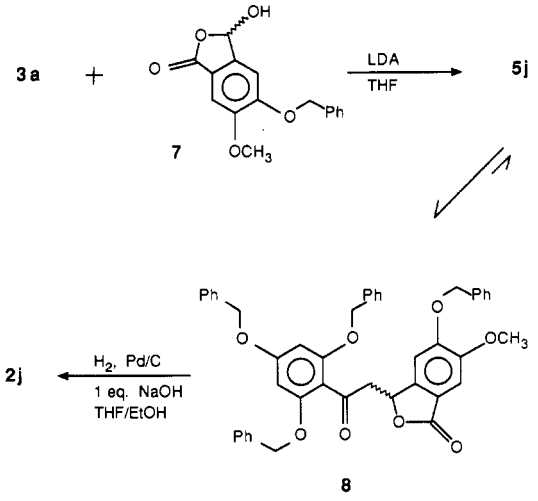


Table I. Taste Potencies and Sweetness Character of Hesperetin Dihydrochalcone Analogues

compd[a]	R_1, R_2	taste potency[b,c]	% sweetness[c]	n[d]
1	NHDHC	390 (70)	90 (4)	14
2a	H, H	240 (60)	61 (7)	14
2b	CH_3, H	90 (160)	0	6
2c	Cl, H	60 (50)	20 (12)	14
2d	CHO, H	740 (350)	4 (5)	6
2e	COOH, H	3400 (1100)	57 (24)	14
2f	$COOCH_3$, H	tasteless		
2g	H, CH_3	150 (60)	46 (8)	14
2h	H, Br	140 (190)	0	6
2i	H, NO_2	400 (140)	18 (16)	8
2j	H, COOH	370 (170)	0	6
2k	H, $COOCH_3$	tasteless		
2l	H, OH	unstable		
2m	H, NH_2	unstable		

[a] All compounds were tested at 100 ppm with 1 equiv of NaOH added, except 1, 2f, and 2k (no NaOH), 2e (20 ppm, no NaOH), and 2i (50 ppm). [b] Weight basis. [c] Values compared to 6% aqueous sucrose (±2 SEM). [d] Number of evaluations.

Taste evaluations of the A-ring analogues of **2a** are given in Table I. The panel's judgement of NHDHC was in close agreement with literature values (taste potency = 340, sweet character = 77%; DuBois et al., 1981a). In our judgment **2a**, however, is of much lower potency and sweetness than previously reported (potency = 850, 83% sweet; DuBois et al., 1981a). The susceptibility of taste properties to minor structural changes is illustrated by the A-ring methyl and chloro analogues (**2b** and **2c**), where both taste potency and sweetness character are substantially lowered. Addition of an aldehyde to the A-ring (**2d**) gave a strongly bitter compound. The carboxylic acid **2e** displays an enormous increase in potency, while retaining the sweet character of the unsubstituted **2a**. Despite compound **2e** being the most potent dihydrochalcone sweetener reported thus far, the unimproved taste quality remains a drawback to commercial utility. High solubility of **2e** alleviated the need for added base, while comparative tasting showed that 1 equiv of NaOH had no effect on taste. Tastes of the B-ring analogues are also given in Table I. The B-ring carboxylic acid analogue **2j** had an ill-defined side-taste, while the nitro compound **2i** showed strong bitterness. The 6-hydroxy and 6-amino derivatives (**2l** and **2m**) were found to be unstable in solution and therefore not submitted to the taste panel. Preliminary screening, however, found them to be very bitter at 100 ppm (1 equiv of NaOH added). The 6-methyl and 6-bromo substituted compounds both showed decreases in taste potency and percent sweetness. The base-labile methyl ester derivatives (**2f** and **2k**) were of lower water solubility than the other dihydrochalcones. Aqueous solutions of **2f** and **2k** were saturated at 10 ppm, both being tasteless. Characteristic dihydrochalcone aftertaste was strong for all compounds in Table I. Extinction times (time taken for a level of peak intensity to decline to a predetermined "faint" level; DuBois and Lee, 1983) were not assessed due to our panel reporting an obvious lack of improvement in temporal properties.

Structure–Activity Relationships. The variance seen in some of our taste panel evaluations led to wide confidence intervals surrounding taste potency and percent sweetness values, thus precluding opportunities for statistically viable quantitative structure–activity relationships. Some general structure–activity relationships, however, can still be drawn from the trends seen in Table I. It is noted that hesperetin structures containing strong electron withdrawing substituents (CHO, NO_2) give compounds of high taste potency. This may be reflecting the ability of electron-withdrawing substituents to create a phenoxide ion at the para hydroxyl group. All modifications to the 6-position of the B-ring, whether they are electron donating or withdrawing or contribute hydrophilic or hydrophobic character to the molecule, are detrimental to sweetness quality. This suggests that substitutions at the 6-position are inhibiting interaction with the sweetness receptor. Decreases in sweetness have been found whenever the B-ring is functionalized with anything other than the basic isovanillyl (3-hydroxy-4-methoxyphenyl) unit, thereby implying a strict specificity for this side of the molecule. An exception to this rule is a reported potent sweetness in the 4-*n*-propoxy analogue of NHDHC (Krbechek et al., 1968). Subsequent 4-*n*-propoxydihydrochalcone analogues, however, have shown bitterness or tastelessness (DuBois et al., 1981a).

While the B-ring functionality seems cricual for invoking sweetness, it is the addition of a carboxylic acid to the A-ring (**2e**) that gives a dramatic rise in potency. The reason for this enhancement remains unclear, although a possible explanation lies with an increased polarity effecting a more optimum hydrophile/lipophile balance. The carboxylic acid potentially provides alternative AH/B sites, and with these the molecule may orient itself differently at the receptor. Hypothetically, the A-ring functionality may now become dominant in receptor interaction. We are currently inducing further structural changes to **2e** in the hope of improving its temporal properties and understanding its increased potency.

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Registry No. **2a**, 35400-60-3; **2b**, 130435-02-8; **2c**, 130435-03-9; **2d**, 130435-04-0; **2e**, 130435-05-1; **2f**, 130435-06-2; **2g**, 130468-39-2; **2h**, 130435-07-3; **2i**, 130435-08-4; **2j**, 130435-09-5; **2k**, 130435-10-8; **2l**, 130435-11-9; **2m**, 130435-12-0; **3a**, 61497-71-0; **3b**, 130435-25-5; **3c**, 130435-26-6; **3d**, 130435-27-7; **3n**, 130435-28-8; **4a**, 621-59-0; **4b**, 6346-05-0; **4g**, 7721-61-1; **4h**, 6451-86-1; **4i**, 58749-47-6; **4l**, 89635-08-5; **5a**, 130435-13-1; **5b**, 130435-14-2; **5c**, 130435-15-3; **5d**, 130435-16-4; **5e**, 130435-17-5; **5f**, 130435-18-6; **5g**, 130435-19-7; **5h**, 130435-20-0; **5i**, 130435-21-1; **5j**, 130435-22-2; **5k**, 130435-23-3; **5l**, 130435-24-4; **5n**, 130468-40-5; **6h**, 130435-30-2; **6i**, 130435-31-3; **7**, 130435-32-4; **8**, 130435-33-5; **9**, 88-03-9; **10**, 2657-28-5; **11**, 130435-29-9; **12**, 62018-55-7; phloroglucinolcarboxaldehyde, 487-70-7; phloroacetophenone monohydrate, 480-66-0.