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Synthesis of chlorinated and non-chlorinated biphenyl-2,3- and 3,4-catechols and their [$^2H_3$]-isotopomers†

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A synthetic scheme is described for chlorinated biphenyl-2,3- and 3,4-catechols to be used as standards for structural assignment of metabolites and protein adducts of 2,2′,5,5′-tetrachlorobiphenyl in which both rings retain chlorine substituents. The scheme has general applicability to the synthesis of chlorinated biphenyl catechols. Dimethyl catechol ethers are coupled to dichloroaniline *via* the Cadogan reaction to give a library of isomers, followed by demethylation of the ethers with BBr_3 to yield the target catechols. Separation of pure isomers is accomplished by TLC or HPLC prior to or following demethylation, depending on the isomer mixture. [$^2H_3$]-Isotopomers are generated using 2,5-dichloroaniline-d_3 as the starting arylamine in the coupling reaction. The dichloroaniline-d_3 hydrochloride is obtained as the sole product from nitration of *p*-dichlorobenzene-d_4 followed by Pd/C-catalyzed hydrogenation under strongly acidic conditions. This hydrogenation procedure provides a simple and convenient approach to selective reduction of aryl nitro groups in the presence of halogen ring substituents.

Introduction

Polychlorinated biphenyls (PCBs) are persistent environmental pollutants known to cause adverse health effects, including tumors in the liver and forestomach of rodents.[1–4] The lower chlorinated PCBs are metabolically converted to catechols. Sequential oxidation of the catechols yields semiquinones and quinones[5–7] which are alkylating agents and can form adducts with proteins, RNA, and DNA.[7–10] 2,2′,5,5′-Tetrachlorobiphenyl (TCB, Chart 1), a major component of commercial PCB mixtures, is metabolized to 2,2′,5,5′-tetrachlorobiphenyl-3,4-catechol (3,4-diOH-TCB), and subsequently oxidized to the 3,4-quinone,[7] which binds to cysteinyl residues of liver cytosolic proteins giving di-, tri-, and tetra-chlorinated adducts. A procedure for quantitation of cysteinyl adducts has recently been developed[7] based on GC/high resolution mass spectrometry with selected ion monitoring. Critical to the method is the availability of pure, unambiguously characterized catechols **11**–**16** and their stable isotopomers as standards. In addition, non-chlorinated biphenyl catechols **9** and **10** are required to serve as internal controls for analyte recovery.

Chlorinated hydroxy biphenyls in which the hydroxylated ring is not substituted with chlorine have been synthesized in good yield by palladium-catalyzed cross-coupling of dimethoxyphenylboronic acids with chlorinated aryl bromides or iodides in the presence of base followed by demethylation with boron tribromide.[11,12] However, this strategy is problematic for synthesis of dimethoxy biphenyls in which both rings are chlorinated, since arene boronic acids are prepared from Grignard or lithium salts of halo arene precursors.[13] In addition, Pd-catalyzed cross-coupling of *o*-substituted aryl boronic acids does not work well because of unfavorable steric interactions with the catalyst, a situation that would be exacerbated when synthetic targets are 2,2′-substituted biphenyls.[14,15] A potential alternative route to the biphenyls is Cadogan arylation of arylamines mediated by isopentyl nitrite. The Cadogan coupling reaction has been applied in the synthesis of polychlorinated biphenyls,[16] the synthesis of PCB phenols in moderate to good yields[17–20] and in the synthesis of chlorinated catechols in which the hydroxylated ring is not chlorinated. Cadogan coupling could also be anticipated to be applicable to the synthesis of catechols in which both rings are chlorinated. The lack of regioselectivity inherent in the Cadogan reaction, which might normally be considered a disadvantage, is desirable in this application by allowing simultaneous generation of a library of isomers, provided product mixtures can be readily resolved. Deuterium-labeled isotopomers may be obtained by performing the coupling reaction with the appropriate deuterium-labeled chlorinated anilines.

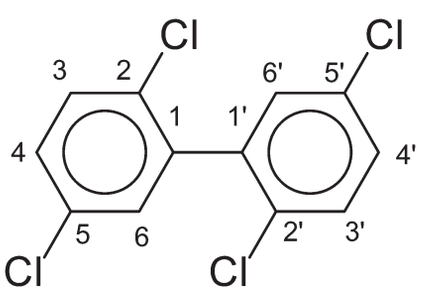

Chart 1 Structure and numbering of tetrachlorobiphenyl.

We report a synthetic route generally applicable to obtaining chlorinated and non-chlorinated biphenyl catechols *via* Cadogan coupling of appropriately substituted catechol dimethyl ethers (Scheme 1) followed by demethylation (Scheme 2). Deuterium-labeled chlorinated anilines, synthons in the preparation of deuterium isotopomers of the chlorinated catechols, are synthesized by a novel, selective catalytic hydrogenation of chlorinated aniline in acidic medium (Scheme 3).

Results and discussion

Synthesis of biphenyl catechols

Attempts to generate trichlorocatechol isomers **13**–**15** by coupling 2,5-dichloroaniline directly with 3-chlorocatechol gave a moderate yield of the target mixture of biphenyl catechols accompanied by

† Electronic supplementary information (ESI) available: preparation of compounds **1**–**16**; ^1H NMR spectra of compounds **11**–**16**; [^1H,^{13}C] HMBC and HSQC spectra of compounds **11**–**15**; mass spectra of compounds **19** and **21**. See http://www.rsc.org/suppdata/ob/b4/b409373a/

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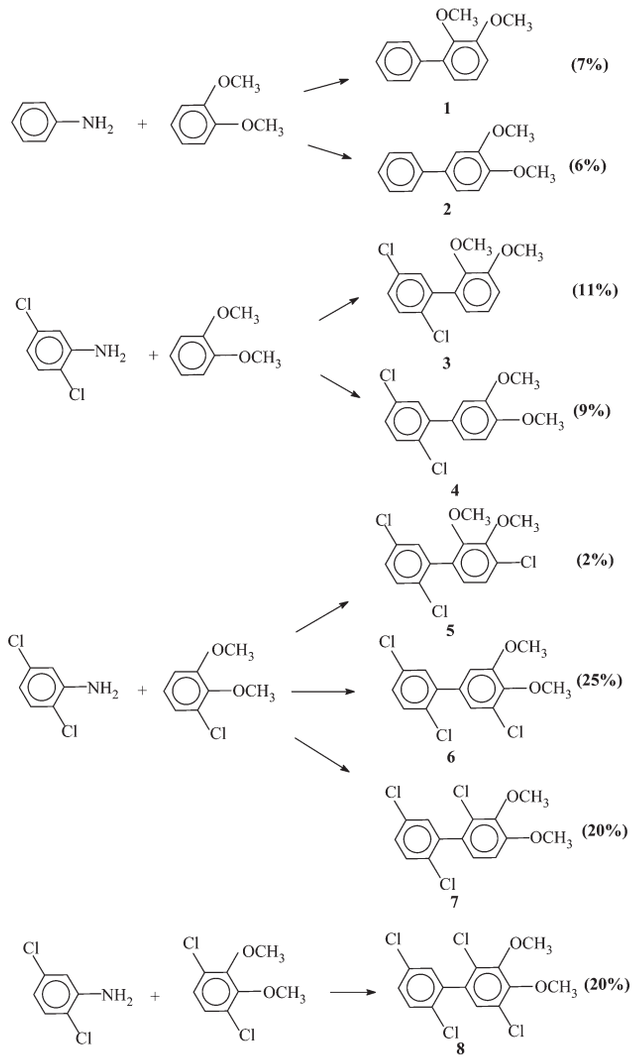
a substantial quantity of byproducts from which separation of the products proved difficult. Protection of the starting catechol by acetylation to 3-chloro-1,2-diacetoxybenzene gave a mixture of coupling products containing only traces of chlorinated diactetoxybiphenyls, which also proved difficult to purify.

Chloroanisoles have been used as nucleophiles in Cadogan coupling to form hydroxy PCBs. In addition to protecting the phenol functionality, methoxylation may facilitate coupling by enhancing the nucleophilicity of the catechol moiety. Coupling of 1,2-dimethoxybenzene with aniline or 2,5-dichloroaniline gave moderate yields of dimethoxybiphenyl ethers **1** and **2** or dichlorodimethoxybiphenyl isomers **3** and **4**, respectively. In both reactions, the yield of the two possible isomers was equivalent, in accord with expectation based on the *o*,*p*-directing properties of the methoxy substituents. The isomers were readily isolated by column chromatography. Cadogan coupling of 3-chloro-1,2-dimethoxy benzene with 2,5-dichloroaniline gave a mixture of the trichlorodimethoxybiphenyl isomers **5**–**7**, which were most readily separated by semipreparative HPLC following demethylation. Based on partial resolution and ^{1}H NMR analysis, yields of compounds **6** and **7** were estimated to be approximately equivalent and **5**, from coupling at the least activated arene position, was formed in 10-fold lower yield. Coupling of 2,5-dichloroaniline with 1,4-dichloro-2,3-dimethoxybenzene yielded 3,4-dimethoxy-2,2′,5,5′-tetrachlorobiphenyl **8**. The reactions and yields are summarized in Scheme 1. The dimethyl ethers used in this study were conveniently prepared from commercially available chlorocatechols by treatment with methyl sulfate; demethylation of the coupled products was accomplished by treatment of the ethers with BBr_3 (Scheme 2). Yields from demethylation were variable, presumably as a consequence of the lability of the final catechol products towards oxidative degradation under conditions of atmospheric work-up used in this study.



Scheme 1

2,5-Dichloroaniline-d_3 by selective hydrogenation of 2,5-dichloronitrobenzene-d_3

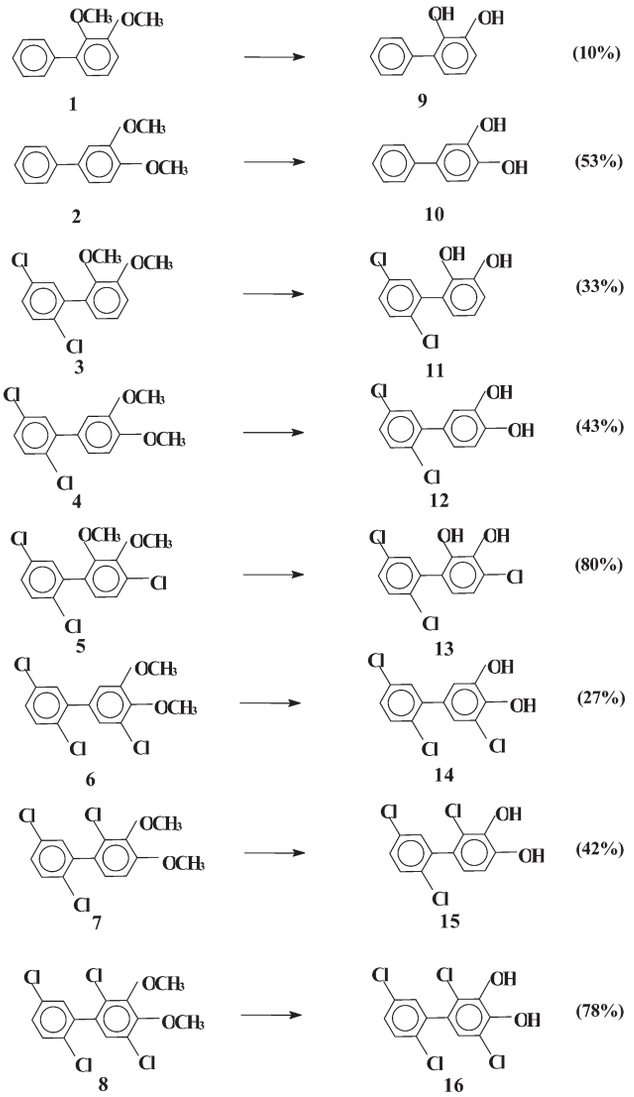
For isotope dilution standards, isotopomers of the analytes differing in molecular weight from the natural abundance products by 2 or more mass units are preferable. The most straightforward approach to obtaining isotopomers of the TCB-derived catechols was to perform the coupling reaction with [3,4,6-$^2H_3$]-2,5-dichloroaniline. Nitration of commercially available *p*-dichlorobenzene-d_4 by a published procedure[21] yielded [3,4,6-$^2H_3$]-2,5-dichloronitrobenzene (**18**). A concern in selecting a procedure for reduction of the nitro group was over-reduction leading to dehalogenation. Metal-catalyzed hydrogenation under acidic conditions has been suggested[22–25] as a means of avoiding this side reaction; however, the procedures are cumbersome and require extensive work-up. As illustrated in Scheme 3, protonation of the chloroaniline product in acid media can inhibit dehalogenation by blocking formation of imide tautomers susceptible to further reduction to cyclohexadienes that re-aromatize *via* dehydrohalogenation. Based on this rationale, the simple expedient of hydrogenation catalyzed by Pd/C in methanol/HCl was adopted. At nominal pH 1, [3,4,6-$^2H_3$]-2,5-dichloroaniline hydrochloride was isolated as the sole reduction product following filtration to remove the catalyst and evaporation of solvent. Complete retention of the deuterium label in the free aniline base **19**, isolated by neutralization of an aqueous solution of the hydrochloride salt and extraction into ether, was confirmed by mass spectrometry (ESI† Fig. S17). Compound **19** was then used to prepare the d_3 isotopomer **21**. The low-resolution cluster at the mass-to-charge ratio of the molecular ion in the FAB-MS of **21** shows complete retention of label in the synthesis (ESI† Fig. S18). This hydrogenation procedure provides a simple, convenient and generally-applicable approach to selective reduction of aryl nitro groups in the presence of halogen ring substituents.

Structural characterization of regioisomers

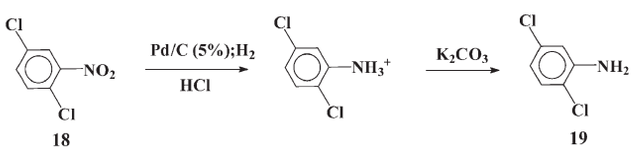
The non-chlorinated biphenylcatechols **9** and **10** are known, and well characterized.[26–29] Because of the importance of correctly establishing the structures of the chlorinated catechol isomers, structural assignments for **11**–**16** have been confirmed by assignment of ^{1}H and ^{13}C resonances through analysis of 1D ^{1}H NMR spectra, and one-bond (HSQC) and multiple bond (HMBC) ^{1}H–^{13}C shift correlation experiments. These data are available as electronic supplementary information (ESI).†

For isomers **11**–**16**, the 2′,5′-dichloro substitution pattern of the dichloro-substituted ring was established by 1D ^{1}H NMR and HSQC experiments. Compound **13** serves as an illustrative example, and the same strategy was applied for all remaining chlorinated catechol isomers. In the 1D spectrum, three one-proton signals were identified between 7.2 and 7.4 ppm: an AX quartet, accounting for two protons, and a one-proton doublet, *meta*-coupled (J_{meta} = 2.1 Hz) to one of the AX signals. The AX vicinal proton signals H3′ and H4′ were identified by the presence of weak cross-peaks arising from $^2J(^{13}C,^1H)$ coupling detected in the HSQC experiment. The signal from the *meta*-coupled AX proton could then be assigned to H4′, which is *meta*-coupled to H6′, and the remaining AX signal was assigned to H3′. Coupling of 1,4-dichloro-2,3-dimethoxybenzene and 1,4-dichloroaniline leads to a unique structure, 3,4-dihydroxy-2,2′,5,5′-tetrachlorobiphenyl (**16**). The catechol ring has a single unsubstituted position at C6; hence, the ^{1}H NMR signature of the catechol ring of **16** is a singlet. Substitution patterns on the catechol rings of the trichloro and dichloro biphenylcatechol isomers were verified as follows.

Trichloro catechols: 2,3-dihydroxy-2′,4,5′-trichlorobiphenyl (13) 3,4-dihydroxy-2′,5,5′-trichlorobiphenyl (14) and 3,4-dihydroxy-2,2′,5′-trichlorobiphenyl (15). The substitution pattern of the catechol rings of **13**–**15** was established by 1D ^{1}H



Scheme 2



Scheme 3

NMR spectra and ^{13}C,^{1}H shift correlations in HSQC and HMBC experiments, taking into account expected substituent effects on ^{13}C shifts. Signals of phenyl- and hydroxyl-substituted carbons will experience a large down-field shift, and signals of carbons *ortho* to hydroxy-substituted positions will experience a large up field shift, while the effect of chlorine substitution on the signal of carbon *ortho* to a hydroxyl-substituted position will be a smaller up-field shift relative to the corresponding protonated carbon. Among isomers **13**–**15**, 3,4-dihydroxy-2′,5,5′-trichlorobiphenyl (**14**) is readily distinguished by 1D ^{1}H NMR through the presence of *meta*-coupled doublets (J = 1.2 Hz) from catechol ring protons H2 and H6. The highest field carbon signal (115.5 ppm) was assigned to C2, *ortho* to hydroxylated C3. The *meta*-coupled doublet at 6.95 ppm has one-bond connectivity with C2, and was therefore assigned to H2. H6 and C6 were assigned on the basis of one bond connectivity between the carbon signal at 121.2 ppm and the *meta*-coupled doublet at 6.91 ppm. In the HMBC experiment, assignment of down-field carbons at 144.5 and 139.3 to hydroxy-substituted C3,C4 followed from $^{2/3}J$(C,H) coupling with hydroxy protons at 5.47 and 5.56 ppm. Assignment of the proton signal at 5.47 ppm to O*H*3 and the carbon signal at 144.5 ppm to C3 follow from connectivity of the proton signal at 5.47 ppm with C2 and the carbon signal at 144.5 ppm. The assignments of O*H*4, C4 and C5 were similarly based on connectivity observed for the proton signal at 5.56 ppm with carbon signals at 139.3 and 119.5 ppm, respectively. C1 is assigned to the carbon signal at 139.8 through ^{2}J(C,H) coupling with H2 and H6. C5 and C6 of isomers **13** and **15** are protonated and were readily identified in the HSQC experiment through ^{1}J(H,C) coupling with the protons of an AX quartet between 6.7 and 7.0 ppm. In both compounds, the vicinal relation of the protonated carbons was confirmed by weak cross peaks from ^{2}J(C,H) coupling. Isomer **15** was identified on the basis of $^{1/2}J$(C,H) coupling between the strongly up-field shifted carbon signal at 116.3 ppm, assigned to C5 *ortho* to hydroxylated C4, and the protons of the AX quartet. Isomer **13** does not have a protonated carbon *ortho* to a hydroxy-substituted position, and no up-field carbon signal was detected in the HSQC experiment. The remaining catechol ring carbon resonances of **15** were readily identified through the following ^{3}J(C,H) connectivities: C1 at 131.3 ppm, with H5 and H6′; C1′ at 141.6 ppm, with H3′ and H6 and C2 at 121.6 ppm, with O*H*4 and H6. Four-bond connectivity was observed between C2 and H5. Connectivity between catechol ring carbons and hydroxyl protons, helpful in making carbon assignments in the case of **15**, was absent in **13**, and assignments have been based on an alternative analysis of connectivities. In the HMBC experiment, the high-field doublet (6.74 ppm) of the H5,H6 AX pattern showed connectivity with carbons at 122.4, 139.6 and 144.6 ppm. Based on the expectation that the cross peaks in the HMBC represent $^{2/3}J$(C,H) couplings, this series of connectivities was consistent with assignment of the proton resonance at 6.74 ppm to H6, and the carbon resonances to C5, C1′ and C2, respectively. From these assignments followed assignment of the low field component of the AX quartet at 6.97 ppm to H5, and carbon signals at 125.0, 126.9 and 142.1 ppm to C6, C1 and C3, respectively. A strong cross peak in the HMBC spectrum was observed between H5 and a carbon signal at 122.4 ppm, coinciding with C5. Since 1-bond connectivities are not observed in the HMBC experiment, this cross peak was attributed to 2-bond coupling of H5 with C4. This analysis results in identification of twelve carbon signals, supporting the carbon signal assignments.

Dichlorocatechols: 2,5-dichloro-2′,3′-dihydroxybiphenyl (11) and 2,5-dichloro-3′,4′-dihydroxybiphenyl (12). Assignments of proton and carbon signals from the 2,5-dichloro-substituted rings of **11** and **12** were made as described above for the trichloro catechols. In the region where the chlorinated carbons C2 or C5 of **11** are expected, only one cross peak could be resolved. Since the chlorinated carbons in the 2,5-substituted ring have similar shifts, it is possible that the shifts coincide; however, connectivity of the carbon signal is observed with H3 only, thus a definitive assignment cannot be made. In the ^{1}H NMR spectrum of **11**, 2′,3′-substitution of the catechol ring gives an AXY coupling pattern for the three ring protons: two *meta*-coupled doublets-of-doublets at 6.94 and 6.71 ppm and an unresolved doublet-of-doublets appearing as an accidental triplet at 6.88 ppm. In the HSQC spectrum, the highest field carbon resonance, at 115.4 ppm, can be assigned to C4′, adjacent to the hydroxy-substituted 3′-carbon. The proton signal at 6.94 ppm, ^{1}J(C,H)-coupled to C4′, is assigned to H4′. The proton signal at 6.88 ppm shows one-bond coupling to the carbon signal at 120.9 ppm and ^{2}J(C,H) coupling with H4′ and the proton signal at 6.71 ppm. This permits the assignment of the proton signals at 6.88 and 6.71 ppm to H5′ and H6′, respectively, and the carbon resonance at 120.9 ppm to C5′. A carbon signal at 122.0 ppm with ^{1}J(C,H) coupling to H6′ is assigned to C6′. In the HMBC spectrum, carbon signals at 144.0 and 140.2 ppm can be assigned to the hydroxy-substituted carbons of the catechol ring. The signal at 140.2 ppm is coupled to both hydroxy protons, H4′ and H6′ and weakly coupled to H5′, while the signal at 144.0 ppm is coupled to H4′, H5′ and the hydroxy protons. Assuming that ^{4}J(C,H) couplings will be weak, this pattern is consistent with assignment of the carbon resonance at 144.0 ppm to C3′ and 140.2 ppm to C2′, the hydroxy signal at 5.44 ppm to O*H*3′, and at 4.98 ppm to O*H*2′. Similarly, a carbon signal at 125.2 ppm is assigned to C1′, based on connectivities with O*H*2′, H5′ and H6, and a weak ^{4}J(C,H) coupling to H3.

The AX pattern of catechol ring protons H6′ and H5′ of **12** was identified by weak ^2J(C,H) coupling detected in the 1-bond correlation spectrum. Assignment of the high field doublet (6.80 ppm) of the AX pattern to H6′ followed from *meta*-coupling to a doublet at 6.96 ppm (J = 2.1 Hz), which could be assigned to H2′. The low field doublet of the AX pattern (6.92 ppm) was then identified as H5′. ^1J(C,H) coupling of H5′ with a high field carbon signal at 118.9 ppm and H2′ with a second high field carbon signal at 120.3 ppm allowed assignment of the carbon signals to C5′ and C2′, respectively. These carbon resonances are the highest field carbon signals in the spectrum, and the assignments are consistent with the positions of C2′ and C5′ *ortho* to hydroxylated carbons. In the multi-bond correlation spectrum, assignment of the lowest field carbon signal at 149.3 ppm to C4′and an O*H* proton at 8.14 ppm to O*H*4′ follow from cross peaks between the carbon signal and H6′, H5′, H2′ and the O*H* signal. A second low-field carbon at 148.6 ppm is assigned to C3′ and an OH resonance at 8.18 ppm, to O*H*3′ on the basis of cross peaks between the carbon signal and H5′, H2′ and the O*H* signal at 8.18 ppm. The carbon signal at 133.6 ppm is identified as C1′ through ^3J(C,H) coupling with H6 and H6′.

Experimental section

Chemicals

Aniline, 2,5-dichloroaniline, catechol, isoamyl nitrite, methyl sulfate and boron tribromide, were purchased from Sigma-Aldrich (Milwaukee, WI); 3-chloro-1,2-dihydroxybenzene, 3,6-dichloro-1,2-dihydroxybenzene, from Helix Biotech (Richmond, BC) and [$^2H_4$]-1,4-dichlorobenzene, from Cambridge Isotope Laboratories (Cambridge, MA). All chemicals were used as received unless stated otherwise.

Chromatography

Preparative thin layer chromatography was performed on glass-backed silica gel plates (1000 µm) from Sigma-Aldrich (Milwaukee, WI). HPLC was performed on a Waters 600E system (Medford, MA) equipped with a Waters 900 photodiode array detector. For analytical separations, a 5 µm ODS, 120 Å column (4.6 × 250 mm, Alltech, Deerfield, IL) was eluted at 1 mL min^{-1} with 50% methanol in deionized water for 3 min increasing linearly to 95% methanol over 30 min. Semipreparative HPLC separations were performed on a 10 µm ODS, 120 Å column (10 × 250 mm, Alltech, Deerfield, IL) at a flow rate of 3 mL min^{-1} with the same gradient.

NMR

^{1}H-NMR spectra were recorded on a Varian Inova 500 spectrometer at 500 MHz in chloroform-*d* or acetone-d_6 using residual solvent protons as lock signal. Chemical shifts are reported in δ relative to TMS for both ^{1}H and ^{13}C signals. The [^{1}H,^{13}C] HSQC and HMBC spectra were obtained with use of a 1024 × 256 point data matrix, 8 scans per increment, and processed with 2048 × 1024 points. Spectral widths of 8 000 and 21 367 Hz were used in the F2 (^{1}H) and F1 (^{13}C) domains, respectively. HMBC was optimized for an 8 Hz scalar coupling. ^{13}C Shifts were assigned from HMBC and HSQC experiments.

Mass spectrometry

Mass spectra were acquired on a VG70-250 SEQ hybrid mass spectrometer using an electron impact source. Accurate mass measurements were made using a direct insertion probe with the mass spectrometer tuned to a resolving power of 10 K. Full-scan mass spectra of the biphenyl catechols as bisheptafluorobutyryl imidazole derivatives were acquired by GC/MS analysis on an HP 5890 GC interfaced to an HP5989A mass spectrometer or the VG70-250 SEQ hybrid mass spectrometer operated in the EI mode at a resolving power of 1 K. Chlorine-substituted compounds all exhibited the requisite isotopic patterns; for clarity and convenience, the low resolution mass spectra are presented to include only the ^{35}Cl isotopomer; exact mass measurements were done on the isotopomers indicated. GC separations were done on an EC-5 fused silica column (30 m × 0.32 mm × 1 µm, Alltech, Deerfield, IL); helium head pressure, 10 psi; injection port temperature, 250 °C. Column temperature was held at 75 °C for 2 min, then ramped to 300 °C at 10 °C min^{-1} and held for 3 min.

Methylation of catechols, general procedure

The chlorocatechol (4.2 mmol) and methyl sulfate (6 mL) were added to a 500 mL round bottom flask containing 20 g K_2CO_3 dried at 110 °C for 30 min. Following the addition of acetone (50 mL), the mixture was heated for 36 h at 60 °C. Acetone was removed under a stream of argon, water (50 mL) added to the resulting oily residue and the mixture extracted (2× 50 mL) with ether. The ether extracts were washed with water (50 mL), dried over Na_2SO_4 and the ether evaporated to yield chlorodimethyl ether that could be directly used in the coupling reaction.

Coupling of dimethoxybiphenyls, general procedure

Isoamyl nitrite (29 mmol) was added to a mixture of aniline (or 2,5-dichloroaniline) (14 mmol) and dimethoxybenzene (or chlorinated dimethoxybenzene) (72 mmol) and the mixture heated with stirring under argon at 130 °C for 16 h. Isoamyl alcohol formed during the reaction was evaporated from the hot reaction mixture under a stream of argon. Excess dimethoxybenzene was removed under vacuum and the reaction products were separated by column chromatography on silica gel.

2,3-Dimethoxybiphenyl (1) and 3,4-dimethoxybiphenyl (2). Coupling of aniline with 1,2-dimethoxybenzene, followed by silica gel column chromatography yielded isomers **1** (7%) and **2** (6%). (***1***): ^{1}H-NMR (500 MHz, acetone-d_6): δ_H 7.49 (d, 2H, J = 7.0 Hz), 7.39 (t, 2H, J = 7.0 Hz), 7.32 (t, 1H, J = 7.0 Hz), 7.11 (t, J = 7.4 Hz, 1H), 7.03 (dd, 1H, J = 7.4, 1.6 Hz), 6.91 (dd, 1H, J = 7.4, 1.6 Hz), 3.91 (s, 3H), 3.6 (s, 3H). *m*/*z* (EI-MS, 70 eV) 214 (M^+,100%), 199 (35, $M^+ - CH_3$), 181 (33, $M^+ - C_2H_6$). (***2***): ^{1}H-NMR (500 MHZ, acetone-d_6): δ_H 7.61 (d, 2H, J = 7.7 Hz,), 7.41 (ψt, 2H, J = 7.8 Hz,), 7.28 (ψt, 1H, J = 7.6 Hz,), 7.22 (d, 1H, J = 2.1 Hz,), 7.17 (dd, 1H, J = 8.4, 2.1 Hz,), 7.02 (d, 1H, J = 8.4 Hz,), 3.89(s, 3H), 3.84, (s, 3H). *m*/*z* (EI-MS, 70 eV) 214 (M^+,100%), 199 (37, $M^+ - CH_3$), 181 (23, $M^+ - C_2H_6$).

2,5-Dichloro-2′,3′-dimethoxybiphenyl (3) and 2,5-dichloro-3′,4′-dimethoxybiphenyl (4). Coupling 2,5-dichloroaniline with 1,2-dimethoxybenzene yielded, after column chromatography **3**, (11%) and **4**, (9%). (***3***): ^{1}H-NMR (500 MHz, acetone-d_6): δ_H 7.56 (d, 1H, J = 8.6 Hz), 7.41 (dd, 1H, J = 8.6, 2.7 Hz), 7.34 (d, 1H, J = 2.7 Hz,), 7.09–7.15 (m, 2 H), 6.79–6.75 (m, 1H), 3.90 (s, 3H), 3.62 (s, 3H). *m*/*z* (EI-MS, 70 eV) 282 (M^+,100%), 267(25, $M^+ - CH_3$), 232(62, $M^+ - CH_3Cl$). (***4***):^{1}H-NMR (500 MHz, acetone-d_6) δ_H 7.51 (d, 1 H, J = 8.9 Hz,), 7.42 (d, 1H, J = 2.4 Hz), 7.37 (dd, 1H, J = 8.9, 2.4 Hz,), 7.05 (d, 1H, J = 2.2 Hz,), 7.03 (d, 1H, J = 7.7 Hz,), 6.98 (dd, 1H, J = 7.7, 2.2 Hz,), 3.86 (s, 3H), 3.85 (s, 3H). *m*/*z* (EI-MS, 70 eV) 282 (M^+, 100%), 267(30, $M^+ - CH_3$), 239(39, $M^+ - C_2H_3O$), 204(29, $M^+ - C_2H_3OCl$).

2,3-Dimethoxy-2′,4,5′-trichlorobiphenyl (5), 3,4-dimethoxy-2′,5,5′-trichlorobiphenyl (6), and 3,4-dimethoxy-2,2′,5′-trichlorobiphenyl (7). Coupling of 3-chloro-1,2-dimethoxybenzene and 1,4-dichloroaniline yielded a mixture inseparable by column chromatography, which was subjected directly to demethylation.

3,4-Dimethoxy-2,2′,5,5′-tetrachlorobiphenyl (8). Coupling of 1,4-dichloro-2,3-dimethoxybenzene and 1,4-dichloroaniline yielded **8** (20%). ^{1}H-NMR (500 MHz, acetone-d_6): δ_H 7.57 (d, 1H, J = 8.7 Hz,), 7.50 (dd, 1H, J = 8.7, 2.6 Hz,), 7.41 (d, 1H, J = 2.6 Hz,), 7.23 (s, 1H), 3.97 (s, 3H), 3.94 (s, 3H). *m*/*z* (EI-MS, 70 eV) 350 (M^+, 100%), 335 (12, $M^+ - CH_3$), 307 (8, $M^+ - C_2H_3O$), 294 (20, $M^+ - C_3H_6O$), 272 (10, $M^+ - C_2H_3OCl$).

General procedure for demethylation to biphenyl catechols

Demethylation was accomplished by treatment of the dimethoxy biphenyls with boron tribromide as described by Bauer *et al.*[11] To a stirred solution of boron tribromide in anhydrous CH_2Cl_2, a CH_2Cl_2 solution of the dimethoxy biphenyl was added drop-wise and the reaction continued for 1 h at −40 °C and an additional 2 h at 4 °C. Following the addition of ice cold water, the organic layer was separated and the aqueous layer washed with ether. The combined organic layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure. The catechols were further purified by thin-layer chromatography on silica developed in 5 : 95 methanol/chloroform or by HPLC.

2,3-Dihydroxybiphenyl (9) (12%). ^{1}H NMR (500 MHz, acetone-d_6) δ_H 7.59 (d, 2H, $J_{2',3'}$ (= $J_{5',6'}$) = 7.4 Hz, H 2′ and H 6′), 7.38 (ψt, 2H, $J_{3',4'}$ (= $J_{5',4'}$) ≈ $J_{2',3'}$ (= $J_{5',6'}$) = 7.6 Hz, H 3′ and H 5′), 7.25 (bt, 1H, $J_{4',3'}$ = 7.4 Hz, H 4′), 6.85 (dd, 1H, $J_{4\text{ or }6,5}$ = 7.8 Hz, $J_{4,6}$ = 1.5 Hz, H 4 or H 6), 6.81 (dd, 1H, $J_{4\text{ or }6,5}$ = 7.8 Hz, $J_{4,6}$ = 1.5 Hz, H 4 or H 6), 6.75 (ψt, 1H, $J_{4,5} \approx J_{5,6}$ = 7.7 Hz, H5). *m/z* (EI-MS, 70 eV) 186 (M^+,100%), 168 (10, $M^+ - H_2O$), 157 (21, $M^+ - CHO$), 139 (33, $M^+ - CH_3O_2$). Exact mass: *m/z* 186.0681. $C_{12}H_{10}O_2$ requires 186.0681. *m/z* (GC/EI-MS as di-HFB derivative) 578 (M^+,68%), 409 (100, $M^+ - C_3F_7$), 381 (13, $M^+ - C_4OF_7$), 365 (20, $M^+ - C_4O_2F_7$).

3,4-Dihydroxybiphenyl (10) (61%). ^{1}H NMR (500 MHz, acetone-d_6) δ_H 7.98 (br s, 1H, OH), 7.96 (br s, 1H, OH), 7.54 (d, 2H, $J_{6',5'} = J_{2',3'}$ = 7.3 Hz, H2′ and H6′), 7.38 (ψt, 2H, $J_{2',3'}$ (= $J_{6',5'}$) ≈ $J_{3',4'}$ (= $J_{5',4'}$) = 7.6 Hz, H3′ and H5′), 7.25 (bt, 1H, $J_{4',3' \text{ and } 5'}$ = 7.4 Hz, H4′), 7.12 (d, 1H, $J_{2,6}$ = 2.0 Hz, H2), 6.99 (dd, 1H, $J_{5,6}$ = 8.2, $J_{5,1}$ = 2.0 Hz, H6), 6.89 (d, 1H, $J_{5,6}$ = 8.2 Hz, H5). *m/z* (EI-MS,70 eV) 186 (M^+,100%), 157 (10, $M^+ - CHO$), 139 (35, $M^+ - CH_3O_2$). Exact mass: 186.0681. $C_{12}H_{10}O_2$ requires186.0681. *m/z* (GC/EI/-MS as di-HFB) 578 (M^+,100%), 381 (43, $M^+ - C_4OF_7$), 365 (11, $M^+ - C_4O_2F_7$).

2,5-Dichloro-2′,3′-dihydroxybiphenyl (11) (37%). ^{1}H-NMR (500 MHz, chloroform-*d*) δ_H 7.42 (d, 1H, $J_{3,4}$ = 8.5 Hz, H3), 7.34 (d, 1H, $J_{4,5}$ = 2.5 Hz, H6), 7.31 (dd, 1H, $J_{4,3}$ = 8.5 Hz, $J_{4,6}$ = 2.5 Hz, H4), 6.94 (dd, 1H, $J_{4',5'}$ = 8.0 Hz, $J_{4',6'}$ = 1.4 Hz, H4′), 6.88 (ψt, $J_{5',6'} \approx J_{4',5'}$ = 7.8 Hz, 1H, H5′), 6.71 (dd, 1H, $J_{6',5'}$ = 7.6 Hz, $J_{4',6'}$ = 1.4 Hz, H6′), 5.44 (bs, 1H, O*H*3), 4.98 (bs, 1H, O*H*2). ^{13}C NMR (chloroform-*d*): δ_C 144.0 (C3′), 140.2 (C2′), 137.1 (C1), 132.8 (C2 and/or C5), 131.8 (C6), 130.9 (C3), 129.1 (C4), 125.2 (C1′), 122.1 (C6′), 120.9 (C5′), 115.4 (C4′). *m/z* (EI-MS, 70 eV) 254 (M^+,100%), 219 (45, $M^+ - Cl$), 184 (90, $M^+ - Cl_2$), 173 (22, $M^+ - CH_2O_2Cl$). Exact mass: 253.9881. Required for $C_{12}H_8{}^{35}Cl_2O_2$: 253.9901. *m/z* (GC/EI-MS as di-HFB) (M^+,100%), 477 (7, $M^+ - C_3F_7$), 449 (23, $M^+ - C_4OF_7$), 414 (34, $M^+ - C_4OClF_7$).

2,5-Dichloro-3′,4′-dihydroxybiphenyl (12) (58%). ^{1}H-NMR (500 MHz, acetone-d_6): δ_H 7.49 (d, 1H, $J_{3,4}$ = 8.6 Hz, H3), 7.38 (d, 1H, $J_{4,6}$ = 2.6 Hz H2), 7.36 (dd, 1H, $J_{4,3}$ = 8.6, $J_{4,6}$ = 2.6 Hz), 6.96 (d, 1 H, $J_{2',6'}$ = 2.1 Hz, H2′), 6.92 (d, 1H, $J_{5',6'}$ = 8.2 Hz, H5′), 6.80 (dd, 1H, $J_{6',5'}$ = 8.2 Hz, $J_{6',2'}$ = 2.1 Hz, H6′). ^{13}C NMR (acetone-d_6) δ_C 149.3 (C4′), 148.6 (C3′), 146.2 (C1), 136.0 (C2 or C5), 135.2 (C3), 134.9 (C6), 134.5 (C5 or C2), 133.6 (C1′), 131.9 (C4), 124.9 (C5′), 120.3 (C2′), 118.9 (C6′). *m/z* (EI-MS, 70 eV) 254 (M^+,100%), 225 (10, $M^+ - CHO$), 184 (15, $M^+ - Cl_2$), 173 (52, $M^+ - CH_2O_2Cl$). Exact mass: 253.9869. Required for $C_{12}H_8{}^{35}Cl_2O_2$: 253.9901. *m/z* (GC/EI-MS as di-HFB) 646 (M^+,100%), 449 (33, $M^+ - C_4OF_7$), 433 (20, $M^+ - C_4O_2F_7$), 393 (55, $M^+ - C_4O_2F_7$).

2,3-Dihydroxy-2′,4,5′-trichlorobiphenyl (13). A solution of **5** (5 mg, 0.016 mmol) in CH_2Cl_2 (25 mL) was treated with 1 M of BBr_3 (0.1 mL) to yield 3,4-dihydroxy-2′,5,5′-trichlorobiphenyl (**13**) 4 mg, (88%). ^{1}H-NMR (500 MHz, chloroform-*d*) δ_H 7.42 (d, 1H, $J_{3',4'}$ = 8.4 Hz, H3′), 7.40 (d, 1H, $J_{4',6'}$ = 2.5 Hz, H6′), 7.33 (dd, 1H, $J_{3',4'}$ = 8.4 Hz, $J_{4',6'}$ = 2.5 Hz, H4′), 6.97 (d, $J_{5,6}$ = 8.4 Hz, 1H, H5), 6.74 (d, 1H, $J_{5,6}$ = 8.4 Hz, H6), 5.63 (bs, 1H, O*H*2 or O*H*3), 5.45 (bs, 1H, O*H*3 or O*H*2). ^{13}C (chloroform-*d*) δ_C 144.6 (C2), 142.1 (C3), 139.6 (C1′), 135.1 (C5′ or C2′), 134.4 (C2′ or C5′), 134.0 (C6′), 133.3 (C3′), 131.8 (C4′), 126.9 (C1), 125.0 (C6), 122.4 (C5), 122.4 (C4). (EI-MS, 70 eV) 288 (M^+,100%), 253 (10, $M^+ - Cl$), 239 (10, $M^+ - CH_2Cl$), 218 (7, $M^+ - Cl_2$). Exact mass: 287.9504. Required for $C_{12}H_7{}^{35}Cl_3O_2$: 287.9512.

3,4-Dihydroxy-2′,5,5′-trichlorobiphenyl (14) (30%). ^{1}H-NMR (500 MHz, acetone-d_6) δ_H 7.42 (d, 1H, $J_{3',4'}$ = 7.8 Hz, H3′), 7.33 (d, 1H, $J_{6',4'}$ = 2.5 Hz, H6′), 7.30 (dd, 1H, $J_{4',3'}$ = 7.9 Hz, $J_{4',6'}$ = 2.5 Hz, H4′), 6.94 (d, 1H, $J_{5,2}$ = 2.0 Hz, H5), 6.93 (d, 1H, $J_{5,2}$ = 2.0 Hz, H2). ^{13}C (chloroform-*d*) δ_C 115.5(C2), 119.5 (C5), 121.2 (C6), 128.8 (C4′), 130.9 (C3′ + C6′), 132.0 (C2′), 132.6 (C5′), 139.3 (C4), 139.8 (C1), 140.3 (C1′), 144.5 (C3). *m/z* (EI-MS, 70 eV) 186 (M^+,30%), 118 (10, $M^+ - C_4H_{12}$), 70 (100). Exact mass: 287.9535. Required for $C_{12}H_7{}^{35}Cl_3O_2$: 287.9512. *m/z* (GC/EI-MS as di-HFB) 680 (M^+, 75%), 645 (5, $M^+ - Cl$), 483 (100, $M^+ - C_4OF_7$), 455 (43, $M^+ - C_5O_2F_7$), 427 (75, $M^+ - C_6O_3F_7$).

3,4-Dihydroxy-2,2′,5′-trichlorobiphenyl (15) (46%). ^{1}H-NMR (500 MHz, chloroform-*d*) δ_H 7.38 (d, 1H, $J_{3,4}$ = 8.4 Hz, H3′), 7.28 (dd, 1H, $J_{4',3'}$ = 8.4 Hz, $J_{4',6'}$ = 2.1 Hz, H4′), 7.24 (d, 1H, $J_{6',4'}$ = 2.1 Hz, H6′), 6.91 (d, 1H, $J_{6,5}$ = 8.4 Hz, H6), 6.73 (d, 1H, $J_{5,6}$ = 8.4 Hz, H5), 5.81 (bs, 1H, OH3), 5.79 (bs, 1H, OH4). ^{13}C (chloroform-*d*) δ_C 147.4 (C4), 141.8 (C3), 141.6 (C1′), 135.1 (C1), 134.8 (C5′), 134.3 (C6′), 134.1 (C2′), 133.3 (C3′), 131.9 (C4′), 125.0 (C6), 122.0 (C2), 116.3 (C5). (EI-MS, 70 eV) 288 (M^+,57%), 253 (22, $M^+ - Cl$), 239 (12, $M^+ - CH_2Cl$), 218 (10, $[M^+ - Cl_2]$). Exact mass: 287.9535. Required for $C_{12}H_7{}^{35}Cl_3O_2$: 287.9512. *m/z* (GC/EI-MS as di-HFB) 680 (M^+,100%), 645 (7, $M^+ - Cl$), 483 (21, $M^+ - C_4OF_7$), 455 (26, $M^+ - C_5O_2F_7$), 427 (50, $M^+ - C_6O_3F_7$).

3,4-Dihydroxy-2,2′,5,5′-tetrachlorobiphenyl (16) (76%). ^{1}H-NMR (500 MHz, acetone-d_6) δ_H 7.53 (d, 1H, $J_{3',4'}$ = 8.7 Hz, H3′), 7.45 (dd, 1 H, $J_{4',3'}$ = 8.6 Hz, $J_{4',6'}$ = 2.8 Hz, H4′), 7.39 (d, 1H, $J_{6',4'}$ = 2.8 Hz, H6′), 6.88 (s, 1H, H6). *m/z* (EI-MS, 70 eV) 322 (M^+ of $^{35}Cl_4$ isotopomer, 30), 287 (15, $M^+ - Cl$), 252 (12, $M^+ - Cl_2$), 223 (13, $M^+ - CHOCl_2$). Exact mass major isotopomer: 323.9117. Required for $C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$: 323.9092. *m/z* (GC/EI-MS as di-HFB) 714 (M^+, 67%), 517 (18, $M^+ - C_4OF_7$), 505 (5, $M^+ - C_5OF_7$), 489 (21, $M^+ - C_5O_2F_7$).

[3,4,6-$^2H_3$]-2,5-Dichloronitrobenzene (18). To [2,3,5,6-$^2H_4$]-1,4-dichlorobenzene (**17**), 1.02 g (0.68 mmol), 3.33 mL fuming nitric acid was added drop-wise with cooling in a water bath. After stirring for 15 min at ambient temperature, the reaction mixture was added to 50 mL water cooled to 0 °C in an ice bath, and the precipitate collected by filtration to give [3,4,6-$^2H_3$]-2,5-dichloronitrobenzene (**18**), 1.31 g (99%).

[3,4,6-$^2H_3$]-2,5-Dichloroaniline (19). A solution of **18** (50 mg, 0.26 mmol) in methanol (10 mL) and 10 N HCl (0.2 mL) containing 5%Pd/C (5 mg) was hydrogenated at atmospheric pressure and ambient temperature until the theoretical amount of hydrogen (6.4 mL) was absorbed. Filtration followed by evaporation of methanol yielded [3,4,6-$^2H_3$]-2,5-dichloroaniline as the hydrochloride. The salt was dissolved in water (20 mL), neutralized with K_2CO_3 and extracted into diethyl ether. Drying over anhydrous Na_2SO_4 followed by evaporation of ether yielded [3,4,6-$^2H_3$]-2,5-dichloroaniline (**19**) 36 mg, (84%). *m/z* (EI-MS, 70 eV) 164 (M^+, 100%), 129 (35, $M^+ - Cl$), 102 (25, $M^+ - HCNCl$), 93 (17, $M^+ - HCl_2$).

[3,4,6-$^2H_3$]-3′,4′-Dimethoxy-2,2′5,5′-tetrachlorobiphenyl (20). Coupling of **19** (36 mg, 0.219 mmol) and 3,6-dichloro-1,2-dimethoxybenzene (300 mg, 1.4 mmol) yielded **20**, 10 mg (27%). ^{1}H-NMR (400 MHZ, acetone-d_6) δ_H 7.23 (s, 1H), 3.85 (s, 3H), 3.78 (s, 3H). *m/z* (EI-MS, 70 eV) 353(M^+, 100), 338 (12, $M^+ - CH_3$), 310 (8, $M^+ - C_2H_3O$), 297 (20, $M^+ - C_3H_6O$), 275 (10, $M^+ - C_2H_3OCl$).

Synthesis of [3,4,6-$^2H_3$]-3′,4′-dihydroxy-2,2′5,5′-tetrachlorobiphenyl (21). A solution of **20** (10 mg, 0.028 mmol) was treated with boron tribromide (BBr_3) (2 mL) yielding [3,4,6-$^2H_3$]-3′,4′-dihydroxy-2,2′5,5′-tetrachlorobiphenyl (**21**) 5 mg, (54%). ^{1}H-NMR (400 MHz, acetone-d_6) δ_H 6.88 (s, 1H, 5′H). *m/z* (EI-MS, 70 eV) 325 (M^+,25%), 286 (20, $M^+ - C_4H_{12}$), 70 (100). Exact mass major isotopomer: 326.9316. Required for $C_{12}H_3{}^{35}Cl_3{}^{37}Cl\ {}^2H_3O_2$: 326.9281.

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