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Synthesis of polychlorinated biphenyls and their metabolites with a modified Suzuki-coupling

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Abstract

A modified procedure for the synthesis of polychlorinated biphenyls (PCBs) utilizing the Suzuki-coupling, a palladium-catalyzed cross-coupling reaction, is described. The coupling of (chlorinated) benzene boronic acids with bromochlorobenzenes, using $Pd(dppf)_2Cl_2$ (dppf = 1,1′-bis(diphenylphosphino)ferrocene) as the catalyst and aqueous sodium carbonate as the base, gave the desired PCB congeners in moderate to good yields. Eleven PCB congeners, including environmentally important PCB congeners and metabolites, were synthesized using this modified procedure. This new catalyst $Pd(dppf)_2Cl_2$ offers the advantage of being less air-sensitive and has a longer shelf life compared to $Pd(PPh_4)_4$. Three new (di-)methoxylated PCB congeners were synthesized using the same procedure by either coupling a chlorinated benzene boronic acid with a bromo (di-)methoxybenzene or by coupling a (di-)methoxy benzene boronic acid with a chlorinated bromobenzene. The dimethoxylated PCB congeners were readily converted into the respective dihydroxylated PCB derivatives using boron tribromide in dichloromethane. This approach offers the advantage of high selectivity and moderate to good yields compared to conventional methods such as the Cadogan reaction and allows the use of less toxic starting materials.

Keywords: PCB; Methoxylated PCB; Synthesis; Palladium; Cross-coupling

1. Introduction

Polychlorinated biphenyls (PCBs) were manufactured for a large number of technical applications including for use in transformers and capacitors (Hansen, 1999; Robertson and Hansen, 2001). Their widespread use and their persistence in the environment have resulted in their worldwide distribution. Physicochemical characteristics, such as lipophilicity and stability towards biological and thermal degradation, have resulted in their accumulation in the food chain, raising concerns about human health effects. Animal and epidemiological studies have implicated PCBs in a number of human disease processes such as carcinogenesis and arteriosclerosis. However, possible mechanisms of PCB toxicity are still poorly understood, partly because technical PCB products contain a complex mixture of the possible 209 PCB derivatives or congeners. Studies of the biological effects of PCBs as well as studies of

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their chemical transport, degradation and remediation greatly benefit from the availability of single congeners. This situation is further complicated by the fact that PCBs are metabolized in the environment as well as in vivo to hydroxy- and sulfur-containing metabolites (Letcher et al., 2000). Many questions remain regarding the biological and toxic effects of PCBs and their metabolites, largely because these compounds are difficult to synthesize in large quantities for biological studies.

We recently published the straightforward synthesis of several PCB congeners (Lehmler and Robertson, 2001b) and various (di-)methoxylated and (di-)hydroxylated PCB metabolites via the Suzuki-coupling (Bauer et al., 1995; McLean et al., 1996; Lehmler and Robertson, 2001a). This cross-coupling reaction between an aryl boronic acid and an aryl bromide (Suzuki, 1999) was conducted in the presence of tetrakis(triphenylphosphine) palladium(0) and aqueous sodium carbonate in toluene/ethanol at 80 °C for 12 h. This approach has significant advantages over conventional methods commonly employed for the synthesis of individual PCB congeners such as the Cadogan (Cadogan et al., 1962) or Ullmann (Fanta, 1974) coupling. Advantages include the formation of a single product in a single step in high yields. At the same time no highly toxic byproducts such as dibenzofurans are formed during the reaction (Moron et al., 1973). One drawback of the conventional Suzuki-coupling is the tetrakis(triphenylphosphine) palladium(0) catalyst itself which, in our hands, appears to be difficult to store over periods of several month and causes the formation of various side products (Lehmler and Robertson, 2001b). We therefore investigated $Pd(dppf)_2Cl_2$ (dppf = 1,1′-bis(diphenylphosphino)ferrocene) as a catalyst for the synthesis of individual PCB congeners using the Suzuki-coupling. This catalyst apparently can be stored without any problems even over extended periods of time. At the same time we streamlined the clean-up step of the reaction with the goal of reducing the potential exposure of laboratory personnel to PCBs and of minimizing the generation of PCB-containing hazardous wastes.

This modified procedure has been utilized to synthesize eleven important PCB congeners such as PCB 77 and PCB 118 as well as selected methoxylated and hydroxylated PCB congeners. Methoxylated PCB congeners are of interest as analytical standards for PCB metabolism studies while hydroxylated PCB congeners were synthesized to be used in various in vitro and in vivo toxicity studies.

2. Materials and methods

All PCB congeners and PCB metabolites were characterized by ^1H and ^{13}C NMR, FT-IR and GC–MS spectroscopy. The IR spectra were obtained using a Nicolet Magna-IR 560 Spectrometer ESP. The ^1H and ^{13}CNMR spectra were recorded on a Varian VXR-400S NMR Spectrometer by using $CDCl_3$ (Cambridge Isotope Laboratories, Andover, MA) as solvent and TMS (tetramethylsilane) as internal standard. Combustion analysis of all new compounds was performed by Atlantic Microlab (Atlanta, GA). If not stated otherwise, a Thermal Analysis 2920 differential scanning instrument equipped with a refrigerated cooling system (RSC) of a Perkin Elmer DSC 7 were used to measure the melting points. Samples were heated at a rate of 10°/min from 20 to 230 °C and the onset of the main phase transition was determined as reported previously (Lehmler et al., 2001). The analytical data of all compounds are in agreement with their proposed structure as well as literature data (Safe and Hutzinger, 1972; Welti and Sissons, 1972; Wilson, 1975; Hutzinger et al., 1983; Yanagisawa et al., 1986, 1987). The purity of all PCB congeners and methoxy PCBs was analyzed with a Hewlett Packard 5890 A Gas Chromatograph equipped with a HP-1 (Methyl Silicone Gum) column (Hewlett Packard, Avondale, PA) and determined based on relative peak area. The following conditions were used for the gas chromatographic analysis: injector: 255 °C, detector (FID): 300 °C, starting temperature: 40 °C, final temperature: 245 °C, heating rate: 10 °C/min. In addition, the GC–MS analysis of all compounds was performed by the Mass Spectrometry Facility of the University of Kentucky (Lexington, KY). *Caution*: PCBs and their metabolites are reasonably anticipated to be human carcinogens and should therefore be handled in an appropriate manner. All phenols and anisoles have a high vapor pressure and should be handled in a fume hood.

2.1. General procedure for the synthesis of PCBs and PCB derivatives

Sodium carbonate (5 ml, 2 M aq.) was added to a solution of a chlorinated bromo- or iodobenzene (5 mmol) and $Pd(dppf)_2Cl_2$ (dppf = 1,1′-bis(diphenylphosphino)ferrocene) (0.18 mg) in 1,4-dioxane (20 ml). A solution of a chlorobenzene boronic acid (5 mmol) in ethanol (10 ml) was added slowly to the solution of the bromo or iodobenzene under a nitrogen atmosphere. The reaction mixture was heated under reflux for 12–16 h. The reaction mixture was allowed to cool to room temperature and Alumina (20 to 40 g, Alumina adsorption 80–200 mesh, Fisher Scientific, Pittsburg, PA) was added. The solvent was removed under reduced pressure and the resulting powder was filtered through an Alumina plug (≈70–100 g) with 150–250 ml *n*-hexanes as eluent to yield the desired PCB congener. Methoxylated PCBs were eluted with 150–250 ml *n*-hexanes-ethyl acetate (9:1 v/v). The crude PCB deriva-

tives were purified either by recrystallization or column chromatography as described previously (Bauer et al., 1995; Lehmler and Robertson, 2001b).

*2.1.1. 2,6-Dichlorobiphenyl (**3a**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.19–7.28 (m, 3H), 7.38–7.49 (m, 5H).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.00 (2×CH), 128.05 (CH), 128.18 (2×CH), 128.97 (CH), 129.47 (2×CH), 134.92, 136.94, 139.48.

EI-MS m/z (relative intensity, %): 222 (97, $C_{12}H_8Cl_2^{\bullet+}$), 186 (11, M-HCl), 152 (100, M–Cl_2).

IR (KBr pellet) [cm^{-1}]: 1557, 1437, 1423, 1191, 788, 777, 759, 697.

Elemental Analysis Calcd. for $C_{12}H_8Cl_2$: C 64.58, H 3.62; found: C 64.79, H 3.74.

*2.1.2. 2,4,4′-Trichlorobiphenyl (**3b**): (Lehmler and Robertson, 2001b)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.23 (d, $J = 8.4$ Hz, 1H, H-6), 7.30 (dd, $J = 8.4$ Hz, $J = 2.0$ Hz, 1H, H-5), 7.33 (AA′XX′ system, 2H, H-3′,5′), 7.40 (AA′XX′ system, 2H, H-2′,6′), 7.48 (d, $J = 2.0$ Hz, 1H, H-3).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 127.26 (CH), 128.41 (2×CH), 129.81 (CH), 130.66 (2×CH), 131.88 (CH), 133.16, 134.06, 136.63, 137.83.

EI-MS m/z (relative intensity, %): 256 (100, $C_{12}H_7Cl_3^{\bullet+}$), 186 (65, M^+–Cl_2), 150 (26, M^+–Cl_3).

IR (KBr pellet) [cm^{-1}]: 1465, 1003, 877, 841, 808, 735, 542, 443.

*2.1.3. 2,4′,5-Trichlorobiphenyl (**3c**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.25 (dd, $J = 2.4$ Hz, $J = 8.4$ Hz, 1H, H-4), 7.30 (d, $J = 2.4$ Hz, 1H, H-6), 7.34 (AA′XX′ system, 2H, H-3′,5′), 7.38 (d, $J = 8.4$ Hz, 1H, H-3), 7.40 (AA′XX′ system, 2H, H-2′,6′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.44 (2×CH), 128.79 (CH), 130.61 (2×CH), 130.73, 130.96 (CH), 131.11 (CH), 132.69, 134.26), 136.49, 140.69.

EI-MS m/z (relative intensity, %): 256 (100, $C_{12}H_7Cl_3^{\bullet+}$), 186 (82, M–Cl_2).

IR (KBr pellet) [cm^{-1}]: 1497, 1456, 1098, 1025, 1012, 828, 818, 586, 427.

Elemental Analysis Calcd. for $C_{12}H_7C_3$: C 55.94, H 2.74; found: C 56.01, H 2.81.

*2.1.4. 2,4,5-Trichlorobiphenyl (**3d**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.38–7.48 (m, 5H), 7.44 (s, 1H, H-6), 7.58 (s, 1H, H-3).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.52 (2×CH), 128.56 (CH), 129.41 (2×CH), 131.26, 131.39 (CH), 131.54, 132.16, 132.57 (CH), 137.49, 140.57.

EI-MS m/z (relative intensity, %): 256 (100, $C_{12}H_7Cl_3^{\bullet+}$), 186 (55, M^+–Cl_2), 150 (41, M^+–Cl_3).

IR (KBr pellet) [cm^{-1}]: 1454, 1442, 1088, 1045, 886, 758, 694, 679, 601, 435.

Elemental Analysis Calcd. for $C_{12}H_7C_3$: C 55.94, H 2.74; found: C 56.21, H 2.74.

*2.1.5. 2,3′,4′,5-Tetrachlorobiphenyl (**3e**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.22–7.28 (m, 3H, H-4,6,6′), 7.38 (m, 1H, H-3), 7.47–7.50 (m, 2H, H-2′,6′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.68 (CH), 129.27 (CH), 130.22 (CH), 130.69, 130.84 (CH), 131.16 (CH), 131.22 (CH), 132.44, 132.52, 132.85, 137.90, 139.48.

EI-MS m/z (relative intensity, %): 290 (78, $C_{12}H_6Cl_4^{\bullet+}$), 220 (55, M–Cl_2), 150 (23, M-4Cl).

IR (KBr pellet) [cm^{-1}]: 1456, 1360, 1139, 1103, 1029, 881, 820, 811, 613.

Elemental Analysis Calcd. for $C_{12}H_6Cl_4$: C 49.34, H 2.07; found: C 49.59, H 2.09.

*2.1.6. 3,3′,4,4′-Tetrachlorobiphenyl (**3f**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.34 (dd, $J = 8.4$ Hz, $J = 2.0$ Hz, 2H, H-6,6′), 7.50 (d, $J = 8.4$ Hz, 2H, H-5,5′), 7.60 (d, $J = 2.0$ Hz, 2H, H-2,2′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 126.14 (CH), 128.78 (CH), 130.94 (CH), 132.44, 133.20, 138.68.

EI-MS m/z (relative intensity, %): 290 (92, $C_{12}H_6Cl_4^{\bullet+}$), 220 (46, M–Cl_2).

IR (KBr pellet) [cm^{-1}]: 1462, 1453, 1134, 818.

Elemental Analysis Calcd. for $C_{12}H_6Cl_4$: C 49.34, H 2.07; found: C 49.37, H 1.98.

*2.1.7. 2,4,4′,5-Tetrachlorobiphenyl (**3g**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.31 (AA′XX′ system, 2H, H-3′,5′), 7.38 (s, 1H, H-6), 7.39 (AA′XX′ system, 2H, H-2′,6′), 7.56 (s, 1H, H-3).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.56 (2×CH), 130.53 (2×CH), 131.21, 131.27 (CH), 132.12 (CH), 132.34, 134.55, 135.52, 139.08.

EI-MS m/z (relative intensity, %): 290 (76, $C_{12}H_6Cl_4^{\bullet+}$), 220 (40, M^+–Cl_2), 150 (11, M^+–Cl_4).

IR (KBr pellet) [cm^{-1}]: 1455, 1142, 1084, 1036, 1014, 891, 869, 835, 736, 681, 622, 558, 491, 454, 423.

Elemental Analysis Calcd. for $C_{12}H_6Cl_4$: C 49.34, H 2.07; found: C 49.19, H 1.99.

*2.1.8. 2,3′,4,6-Tetrachlorobiphenyl (**3h**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.08–7.11 (m, 1H, H-5′), 7.21–7.23 (m, 1H, H-6′), 7.37–7.39 (m, 2H, H-2′,4′), 7.40 (s, 2H, H-3,5).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 127.80 (CH), 128.13 (2×CH), 128.60 (CH), 129.63 (CH), 129.66 (CH), 134.21, 134.39, 135.36, 136.77, 137.51.

EI-MS m/z (relative intensity, %): 290 (76, $C_{12}H_6Cl_4^{\bullet+}$), 220 (61, M–Cl_2), 185 (12, M–Cl_3).

IR (neat) [cm^{-1}]: 1575, 1430, 1367, 1183, 1079, 841, 800, 698, 663, 576, 497.

Elemental Analysis Calcd. for $C_{12}H_6Cl_4$: C 49.34, H 2.07; found: C 49.69, H 2.20.

*2.1.9. 2,4,4′,6-Tetrachlorobiphenyl (**3i**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.17 (AA′XX′ system, 2H, H-3′,5′), 7.43 (s, 2H, H-3,5), 7.44 (AA′XX′ system, 2H, H-2′,6′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.15 (2×CH), 128.69 (2×CH), 130.98 (2×CH), 134.25, 134.50, 135.42, 137.01.

EI-MS m/z (relative intensity, %): 290 (100, $C_{12}H_6Cl_4^{\bullet+}$), 254 (2, M-HCl), 220 (50, M–Cl_2), 150 (17, M–Cl_4).

IR (KBr pellet) [cm^{-1}]: 1542, 1444, 1098, 1020, 1001, 854, 797, 736, 574.

Elemental Analysis Calcd. for $C_{12}H_6Cl_4$: C 49.34, H 2.07; found: C 49.53, H 2.04.

*2.1.10. 2,3,3′,4′,5-Pentachlorobiphenyl (**3j**)*

1H-NMR ($CDCl_3$, 400 MHz) δ 7.20 (dd, $J = 2.4$ Hz, 1H, H-2′), 7.22 (dd, $J = 8.4$ Hz, $J = 2.4$ Hz, 1H, H-6′), 7.47 (d, $J = 2.4$ Hz, 1H, H-6), 7.49 (d, $J = 2.4$ Hz, 1H, H-4), 7.50 (d, $J = 8.4$ Hz, 1H, H-5′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.47 (CH), 129.14 (CH), 129.64, 129.73 (CH), 130.28 (CH), 130.97 (CH), 132.54, 132.71, 132.86, 134.59, 137.70, 141.07.

EI-MS m/z (relative intensity, %): 324 (60, $C_{12}H_5Cl_5^{\bullet+}$), 254 (38, M–Cl_2).

IR (KBr pellet) [cm^{-1}]: 1543, 1474, 1413, 1360, 1136, 1032, 868, 820, 623.

Elemental Analysis Calcd. for $C_{12}H_5Cl_5$: C 44.13, H 1.54; found: C 44.01, H 1.61.

*2.1.11. 2,3′,4,4′,5-Pentachlorobiphenyl (**3k**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.26 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H, H-6′), 7.42 (s, 1H, H-6), 7.51 (d, $J = 2.0$ Hz, 1H, H-2′), 7.52 (d, $J = 8.0$ Hz, 1H, H-5′), 7.61 (s, 1H, H-3).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 128.60 (CH), 130.34 (CH), 131.09 (CH), 131.16, 131.40 (CH), 132.03 (CH), 132.60, 132.84, 132.93, 136.94, 137.86.

EI-MS m/z (relative intensity, %): 322 (62, $C_{12}H_5Cl_5^{\bullet+}$), 254 (29, M–HCl), 218 (5, M–HCl–Cl_2), 184 (8, M–Cl_4).

IR (KBr pellet) [cm^{-1}]: 3084, 1453, 1145, 1091, 925, 877.

Elemental Analysis Calcd. for $C_{12}H_5Cl_5$: C 44.13, H 1.54; found: C 44.31, H 1.50.

*2.1.12. 2′,5′-Dichloro-3,4-dimethoxybiphenyl (**5**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.91 (s, 3H, –OCH_3), 3.93 (s, 3H, –OCH_3), 6.92 (d, $J = 8$ Hz, 1H, H-5), 6.96 (dd, $J = 2$ Hz, $J = 8$ Hz, 1H, H-6), 6.98 (d, $J = 2$ Hz, 1H, H-2), 7.22 (dd, $J = 2.4$ Hz, $J = 8.4$ Hz, 1H, H-4′), 7.34 (d, $J = 2.4$ Hz, 1H, H-6′), 7.38 (d, $J = 8.4$ Hz, 1H, H-3′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 55.85 (–OCH_3), 55.92 (–OCH_3), 110.72, 112.54, 121.72, 128.12, 130.74, 130.85, 130.98, 131.10, 132.45, 141.65, 148.43, 148.89.

EI-MS m/z (relative intensity, %): 282 (100, $C_{14}H_{12}Cl_2O_2^{\bullet+}$), 267 (39, M–$CH_3$), 239 (60), 204 (98).

IR (KBr pellet) [cm^{-1}]: 3449, 1605, 1502, 1466, 1242, 1141, 1030, 807, 647.

Elemental Analysis Calcd. for $C_{14}H_{12}O_2Cl_2$: C 59.36, H 4.27; found C 59.16, H 4.14.

*2.1.13. 2′,5′-Dichloro-3,4-dihydroxybiphenyl (**6**)*

m.p. = 132 °C.

^{1}H-NMR (CD_3COCD_3, 400 MHz) δ 6.79 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H, H-6), 6.92 (d, $J = 8.0$ Hz, 1H, H-5), 6.96 (d, $J = 2.4$ Hz, 1H, H-2), 7.34 (dd, $J = 2.4$ Hz, $J = 8.4$ Hz, 1H, H-4′), 7.36 (dd, $J = 0.4$ Hz, $J = 2.4$ Hz, 1H, H-6′), 7.49 (dd, $J = 0.8$ Hz, $J = 8.4$ Hz, 1H, H-3′).

^{13}C-NMR (CD_3COCD_3, 100 MHz) δ 116.01 (CH), 117.32 (CH), 121.94 (CH), 128.97 (CH), 130.68, 131.61, 131.87 (CH), 132.21 (CH), 133.13, 143.15, 145.65, 146.32.

EI-MS m/z (relative intensity, %): 398 (21, $C_{18}H_{24}Cl_2O_2Si_2^{\bullet+}$), 383 (6, M^+–CH_3), 310 (11, M^+–$Si(CH_3)$), 73 (100).

IR (KBr pellet) [cm^{-1}]: 3499, 1621, 1560, 1584, 1523, 1513, 1442, 1386, 1329, 1300, 1208, 1097, 1028, 923, 874, 815, 799, 774, 642, 550.

*2.1.14. 3′,4′,-Dichloro-2,3-dimethoxybiphenyl (**8**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.61 (s, 1H, –OCH_3), 3.91 (s, 1H, –OCH_3), 6.90 (dd, $J = 2.0$ Hz, $J = 8.0$ Hz, 1H, H-6), 6.96 (dd, $J = 2.0$ Hz, $J = 8.4$ Hz, 1H, H-4′), 7.11 (dd, $J = 8.0$ Hz, $J = 8.0$ Hz, 1H, H-5′), 7.41 (dd, $J = 2.0$ Hz, $J = 8.8$ Hz, 1H, H-6), 7.47 (d, $J = 8.8$ Hz, 1H, H-5), 7.65 (d, $J = 2.4$ Hz, 1H, H-6).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 55.93 (–OCH_3), 60.68 (–OCH_3), 112.26 (CH), 122.05 (CH), 124.30 (CH), 128.70 (CH), 129.98 (CH), 131.07 (CH), 131.18, 132.04, 133.44, 138.16, 146.41, 153.18.

EI-MS m/z (relative intensity, %): 282 (83, $C_{14}H_{12}Cl_2O_2^{\bullet+}$), 267 (32, M–$CH_3$), 239 (100, M–$C_2H_3O$).

IR (KBr pellet) [cm^{-1}]: 1578, 1454, 1265, 1117, 1034, 1004, 878, 793, 676.

Elemental Analysis Calcd. for $C_{14}H_{12}O_2Cl_2$: C 59.36, H 4.27; found: C 59.09, H 4.36.

*2.1.15. 3′,4′-Dichloro-2,3-dihydroxybiphenyl (**9**)*

m.p. = 123 °C.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.86 (dd, $J = 8.0$ Hz, $J = 2.4$ Hz, 1H, H-6), 6.90 (“t”, $J = 8.0$ Hz, 1H, H-5), 6.93 (dd, $J = 8.0$ Hz, $J = 2.4$ Hz, 1H, H-3), 7.41 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 1H, H-6′), 7.55 (d, $J = 8.0$ Hz, 1H, H-5′), 7.67 (d, $J = 2.0$ Hz, 1H, H-2′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 115.40 (CH), 121.18 (CH), 122.17 (CH), 126.47, 128.54 (CH), 130.91 (CH), 131.15 (CH), 132.02, 133.09, 137.31, 140.88, 143.97.

EI-MS m/z (relative intensity, %): 398 (15, $C_{18}H_{24}Cl_2O_2Si_2^{\bullet+}$), 383 (8, M^+–CH_3), 310 (8, M^+–$Si(CH_3)_4$), 73 (100).

IR (KBr pellet) [cm^{-1}]: 3464, 1552, 1384, 1255, 1164, 1085, 678, 509.

*2.1.16. 3,4′-Dichloro-4-methoxybiphenyl (**11**)*

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.98 (s, 3H, –OCH_3), 6.99 (d, $J = 8.4$ Hz, 1H, H-5), 7.39 (AA′XX′ system, 2H, H-3′,5′), 7.41 (dd, $J = 8.4$ Hz, $J = 2.4$ Hz, 1H, H-6), 7.45 (AA′XX′ system, 2H, H-2′,6′), 7.57 (d, $J = 2.4$ Hz, 1H, H-2).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 56.25 (–OCH_3), 112.29 (CH), 122.91, 126.13 (CH), 127.92 (2×CH), 128.69 (CH), 128.98 (2×CH), 133.29, 133.40, 137.99, 154.62.

EI-MS m/z (relative intensity, %): 252 (100, $C_{13}H_{11}OCl_2^{\bullet+}$), 237 (96, M–$CH_3$), 209 (47, M–$C_2H_3O$).

IR (KBr pellet) [cm^{-1}]: 3434, 1600, 1486, 1291, 1063, 808.

Elemental Analysis Calcd. for $C_{13}H_{10}OCl_2$: C 61.66, H 3.98; found C 61.91, H 3.93.

*2.1.17. 2,2′,5,5′-Tetramethoxybiphenyl (**12**)*

m.p. = 96–97 °C.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.73 (s, –OCH_3, 6H), 3.78 (s, –OCH_3, 6H), 6.83–6.88 (m, 4H), 6.91 (d, $J = 8.4$ Hz, d, $J = 0.8$ Hz, 2H, H-6, H-6′).

^{13}C-NMR ($CDCl_3$, 100 MHz) δ 55.67 (2×–OCH_3), 56.44 (2×–OCH_3), 112.40 (2×CH), 113.34 (2×CH), 117.08 (2×CH), 128.56, 151.24, 153.26.

EI-MS m/z (relative intensity, %): 274 (100, $C_{16}H_{18}O_4^{\bullet+}$).

IR (KBr pellet) [cm^{-1}]: 2829, 1498, 1469, 1463, 1224, 1037.

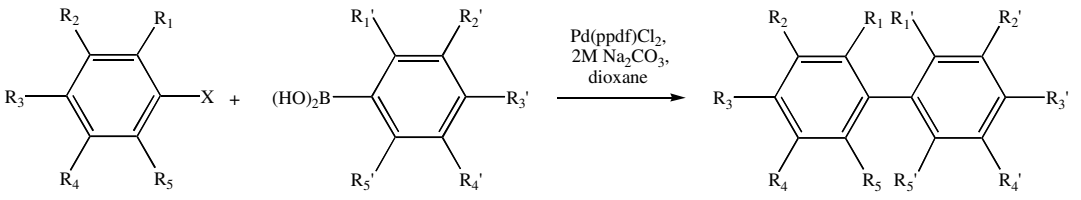
Elemental Analysis Calcd. for $C_{16}H_{18}O_4$: C 70.04, H 6.62; found: C 70.02, H 6.59.

2.2. Crystal structure analysis

X-ray diffraction data of 2,2′,5,5′-tetramethoxybiphenyl (**12**) were collected at 173 K on a Nonius kappaCCD diffractometer from an irregular block-shaped crystal. Raw data were integrated, scaled, merged and corrected for Lorentz-polarization effects using the HKL-SMN package (Otwinowski and Minor, 1997). The structure was solved by direct methods and difference Fourier (Sheldrick, 1997). Refinement was carried out against F^2 by weighted full-matrix least-squares (Sheldrick, 1997). Hydrogen atoms were found in difference maps but subsequently placed at calculated positions and refined using a riding model. Non-hydrogen atoms were refined with anisotropic displacement parameters. Atomic scattering factors were taken from the International Tables for Crystallography (Wilson, 1992). Further details of the crystal structure analysis are available from the Cambridge Structural Database.

3. Results and discussion

The effects of different reaction solvents, reaction temperatures, bases, and reaction times were investigated to optimize the reaction conditions for the Suzuki-coupling using $Pd(dppf)_2Cl_2$ as a catalyst (Scheme 1).



1a-h **2a-c** **3a-k**

1a X = Br; $R_1 = R_5 = Cl$
1b X = Br; $R_1 = R_3 = Cl$
1c X = Br; $R_1 = R_4 = Cl$
1d X = I; $R_1 = R_3 = R_4 = Cl$
1e X = I; $R_2 = R_3 = Cl$
1f X = I; $R_1 = R_3 = R_4 = Cl$
1g X = I; $R_1 = R_3 = R_5 = Cl$
1h X = I; $R_1 = R_2 = R_4 = Cl$

2a $R_1' = R_2' = R_3' = R_4' = R_5' = H$
2b $R_3' = Cl$
2c $R_2' = R_3' = Cl$
2d $R_2' = Cl$

3a $R_1 = R_5 = Cl$
3b $R_1 = R_3 = R_3' = Cl$
3c $R_1 = R_4 = R_3' = Cl$
3d $R_1 = R_3 = R_4 = Cl$
3e $R_1 = R_4 = R_2' = R_3' = Cl$
3f $R_2 = R_3 = R_2' = R_3' = Cl$
3g $R_1 = R_3 = R_4 = R_3' = Cl$
3h $R_1 = R_3 = R_5 = R_2' = Cl$
3i $R_1 = R_3 = R_5 = R_3' = Cl$
3h $R_1 = R_3 = R_5 = R_3' = Cl$

Scheme 1. Synthesis of PCB congeners using the modified Suzuki-coupling reaction. See Table 1 for the respective boronic acids and chloro bromo- or chloro iodobenzenes. R_n and $R_n' = H$ if not stated otherwise.

The effect of different reaction conditions is illustrated using the synthesis of 2,4,5-trichlorobiphenyl (**3d**) as an example in Table 1. The choice of solvent is of great importance and apparently influences the purity and yield of the (crude) product significantly. In contrast to the classical catalyst used for Suzuki-coupling reactions, $Pd(PPh_3)_4$, $Pd(dppf)_2Cl_2$ is not soluble in nonpolar solvents such as toluene or benzene. However, it is soluble in polar solvents such as 1,4-dioxane, THF and DMF. Reactions performed in DMF and THF, especially when CsF was employed as a base (Wright et al., 1994), resulted in a high percentage of impurities (usually about 20% based on peak area) and, in all cases, significant amounts of starting material were present, even after reaction times of 2 days. The purification of product obtained in DMF and THF was therefore tedious and several recrystallizations were required to obtain purities above 98%. Moderate to good yields (36–77%, see Tables 1 and 2) were obtained when the reaction was performed in 1,4-dioxane using aqueous sodium carbonate as base. Only low yields of 2,4,5-trichlorobiphenyl (**3d**) were obtained with CsF (3–15%, see Table 1), which is frequently used as a base in Suzuki reactions (Wright et al., 1994).

In addition to using a different catalyst, we also modified the clean-up step of the Suzuki-coupling with the goal of minimizing exposure of laboratory personnel and of reducing the generation of highly contaminated, PCB hazardous wastes. Our previously published procedure for the Suzuki-coupling (Bauer et al., 1995; Lehmler and Robertson, 2001a,b) calls for hydrogen peroxide to destroy excess benzene boronic acid. With some boronic acids this deboronation step offers the advantage that a volatile benzene derivative is formed which can be removed with the solvent under reduced pressure, while inorganic boronic acids salts are removed from the reaction mixture by extraction with sodium hydroxide. Unfortunately most chlorobenzenes are not volatile and can not easily be removed under reduced pressure. Because the properties, i.e. polarity, of chlorobenzenes are similar to the properties of PCBs, it is also difficult to remove these side products of the deboronation by column chromatography, thus requiring (several) recrystallization(s) of the product. Instead, excess benzene boronic acids can be easily removed by coating the crude reaction mixture on Alumina and, subsequently, eluting the product from the dry adsorbent using hexanes as eluent. This workup also avoids

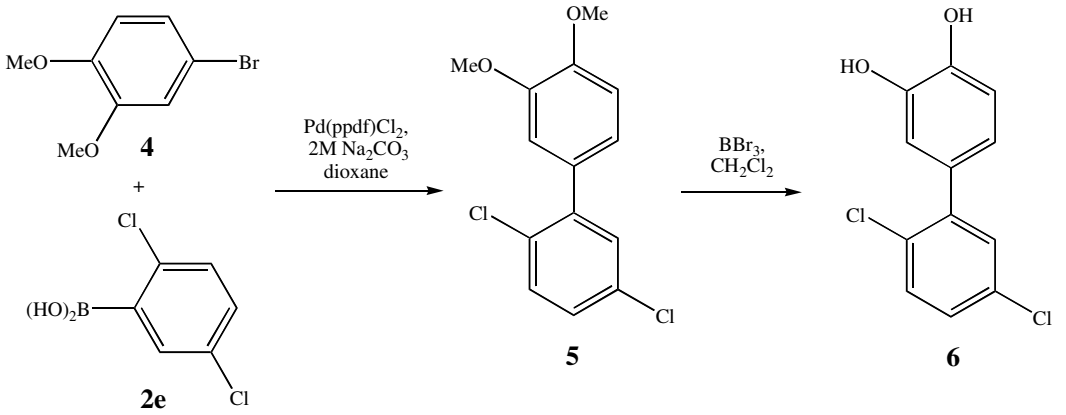
Table 1
Effect of different reaction conditions on the yield of 2,4,5-trichlorobiphenyl (**3d**)

Solvent	Temperature [°C]	Base	Time [h]	Yield [%]
THF	80	CsF	49	15
DMF	65	Na_2CO_3	18	9
DMF	65	CsF	17	15
DMF	100	CsF	24	3
Dioxane	100	CsF	49	3
Dioxane	100	Na_2CO_3	40	52

Table 2
Synthesis of PCBs and methoxylated PCBs using modified Suzuki-coupling

Bromo- or iodo-benzene	Boronic acid	Product	Melting point [°C]	Melting point [°C] (Bolgar et al., 1995)	Yield [%]
1a	**2a**	**3a**	Oil	35–36	36[a]
1b	**2b**	**3b**	56	58–59	42
1c	**2b**	**3c**	64	64.5–65	61
1d	**2a**	**3d**	80	77–78	52
1c	**2c**	**3e**	101	104–105	68
1e	**2c**	**3f**	179	180–181	69
1d	**2b**	**3g**	130	127–129	16
1f	**2d**	**3h**	Oil	52–54	11
1f	**2b**	**3i**	68	62–63	64
1g	**2c**	**3j**	99	70–71	77
1d	**2c**	**3k**	111	111–113	56
4	**2e**	**5**	80	–	69
1i	**7a**	**8**	88	–	77
10	**2b**	**11**	105	–	41

[a] Purified by two column chromatography steps, first on deactivated Alumina and then on Alumina activated at 400 °C.

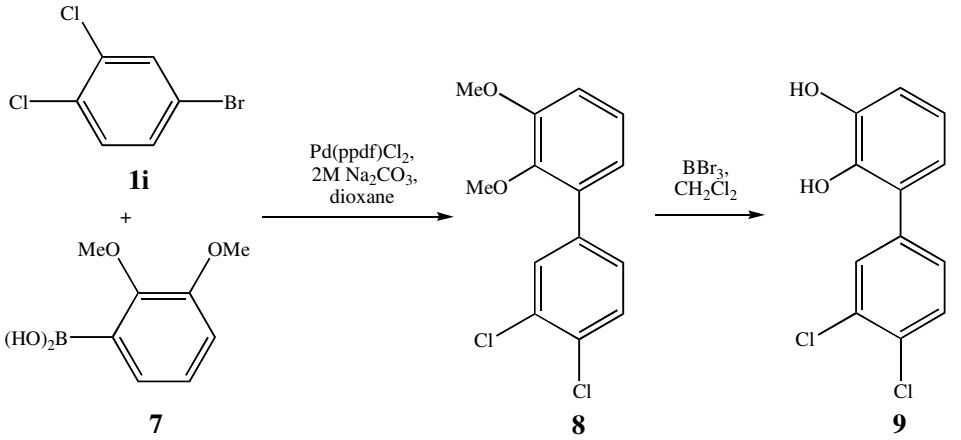


Scheme 2. Synthesis of a dihydroxylated PCB metabolite by the coupling of a chlorobenzene boronic acid with a bromo dimethoxybenzene, followed by demethylation.

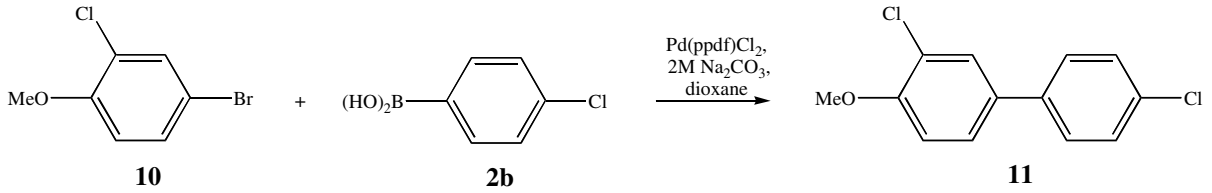
the aqueous extraction step employed to remove inorganic salts. The product is obtained in moderate to good yields and in good purities (>95%) with this modified clean-up procedure (Table 2). The final purification was, in most cases, a single recrystallization step yielding the desired PCB in >99% purity as determined by GC. A variety of PCB congeners, including environmentally important congeners, such as PCB 28, PCB 77 and PCB 118 (Hansen, 1999; Robertson and Hansen, 2001), were synthesized using $Pd(dppf)_2Cl_2$ in 1,4-dioxane and sodium carbonate as base (Table 2). These reaction conditions can be easily used to synthesize large quantities (e.g., >10 g) of a desired PCB congener.

PCB congeners with multiple (i.e., 3 or 4) ortho chlorine substituents have mechanisms of toxicity distinctively different from dioxin-like congeners (Hansen, 1999). There is increasing interest in these compounds from a toxicological as well as from a regulatory point of view. We are therefore particularly interested in improving the availability of PCB congeners with multiple ortho substituents. Using this modified Suzuki-coupling we were able to synthesize PCBs with two ortho chlorine substituents in one phenyl ring (i.e., a 2,6-dichloro substitution pattern) in good yields, for example **3g** and **3h** (Scheme 1). However, we were unable to extend the Suzuki-coupling to the synthesis of congeners with ortho chlorine substituents in both phenyl rings, i.e. a 2,2′-dichloro substitution pattern.

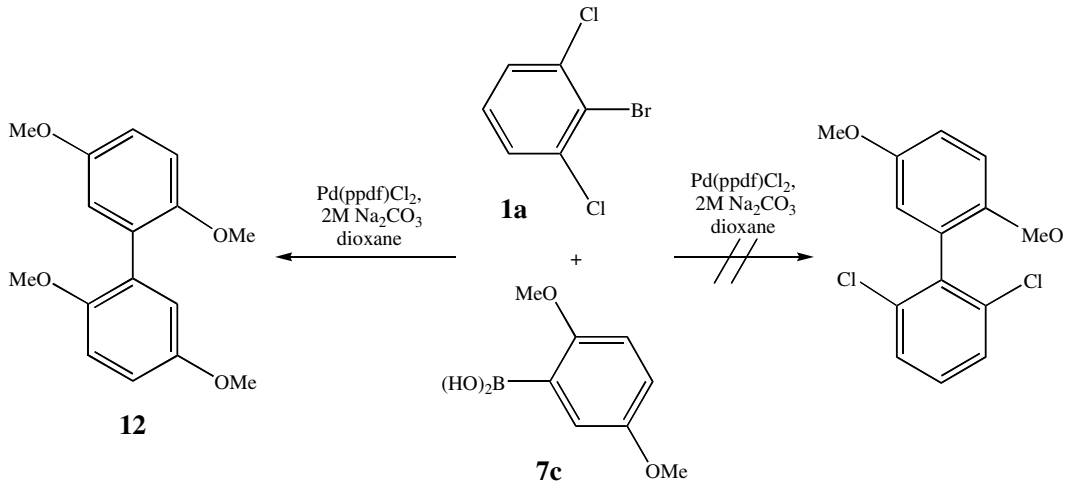
Methoxylated PCBs are frequently employed as GC standards for PCB metabolism studies (Bergman et al., 1994), while hydroxylated PCBs are needed for various in situ and in vitro toxicity studies (Srinivasan et al., 2001; Tampal et al., 2002; van den Hurk et al., 2002). The modified Suzuki-coupling procedure was therefore



Scheme 3. Synthesis of a dihydroxylated PCB metabolite by the coupling of a dimethoxy benzene boronic acid with a chlorobromobenzene, followed by demethylation.



Scheme 4. Synthesis of 4-methoxylated PCB congeners.



Scheme 5. Coupling of di-*ortho* chlorinated bromobenzenes with mono-*ortho* methoxy benzene boronic acids.

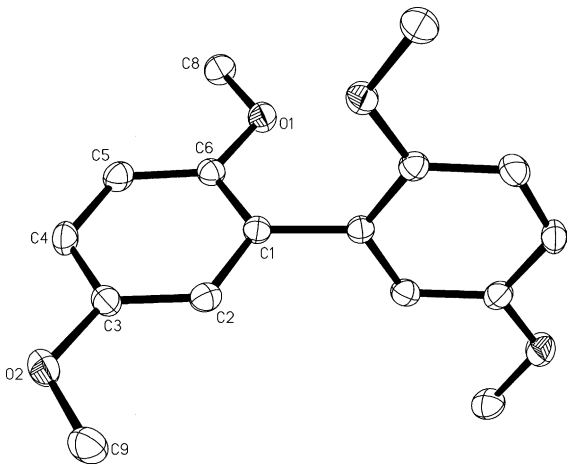


Fig. 1. Molecular structure of 2,2′,5,5′-tetramethoxybiphenyl (**12**).

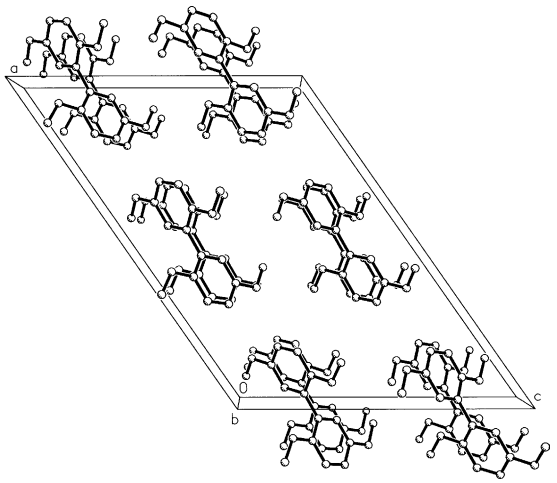


Fig. 2. Packing of 2,2′,5,5′-tetramethoxybiphenyl (**12**) molecules.

applied to the synthesis of methoxylated and hydroxylated PCB derivatives. Dimethoxylated PCBs were synthesized by coupling of a bromo dimethoxybenzene (e.g., **4**) with a chlorinated benzene boronic acid **2** (Scheme 2) or by coupling of a (chlorinated) bromobenzene **1** with a dimethoxybenzene boronic acid such as **7** (Scheme 3). In both approaches the desired dimethoxy PCB was eluted from the Alumina with hexanes-ethylacetate (9:1 v/v) instead of hexanes. The modified Suzuki-coupling can also be employed to synthesize PCB metabolites similar to the ones found in human plasma, as illustrated for one methoxylated standard (**11**) in Scheme 4.

We investigated the coupling of 2,5-dimethoxybenzene boronic acid **7c** with bromo-2,6-dichlorobenzene **1a** (Scheme 5). We were unable to obtain the desired tri-ortho substituted 2′,6′-dichloro-2,5-dimethoxybiphenyl, which is in agreement with our previous observations that the reaction conditions employed are not suitable for the synthesis of PCBs with one ortho-chlorine substituent in each phenyl ring (i.e., a 2,2′-dichloro substitution pattern). Instead, small quantities of 2,2′, 5,5′-tetramethoxybiphenyl **12,** the homocoupling coupling product of 2,5-dimethoxybenzene boronic acid **7c,** were isolated. The structure of this product was confirmed by NMR, IR and GC–MS as well as a crystal structure analysis. A thermal ellipsoid plot and the molecular packing of the tetramethoxy biphenyl **12** are shown in Figs. 1 and 2, respectively. In contrast, we were never able to detect or isolate the corresponding homocoupling product of an ortho substituted chlorobenzene boronic acid. At this point we can not explain this observation but most likely steric and/or electronic effects prevent the homocoupling of the chlorinated boronic acids.

In summary, the modified Suzuki reaction described herein allows the straightforward synthesis of a large variety of PCB derivatives in good to moderate yields. The $Pd(dppf)_2Cl_2$ catalyst, although more expensive, apparently offers the advantage of an improved stability during storage and lowers the occurrence of side reactions. The improved clean-up procedure eliminates the deboronation as well as the aqueous extraction step without significantly lowering the reaction yield or affecting the purity of the product, thus reducing exposure of laboratory personnel during work-up and minimizing generation of PCB contaminated hazardous wastes.

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