REGIOSPECIFIC SYNTHESIS OF POLYCHLORINATED DIBENZOFURANS WITH CHLORINE-37 EXCESS

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Summary

The synthesis of regiospecifically chlorine-37 labeled di- and trichlorodibenzofurans is described. The strategy for introducing a chlorine-37 label regiospecifically has been to reduce the nitro derivative to the corresponding amine. The amine is converted to the diazonium salt with *t*-butyl nitrite, and this product is converted to the final product via the Sandmeyer reaction with chlorine-37 labeled cuprous chloride.

Key words: Chlorine-37 labeled polychlorinated dibenzofuran

Introduction

Polychlorinated dibenzofurans (PCDBFs) constitute a family of organic chemicals represented by the structural formula and numbering system shown in Figure 1. There is considerable interest in PCDBFs because they are major contaminants in technical grade pentachlorophenol, a biocide used extensively in the wood products industry.[1] PCDBFs also have been detected among the pyrolysis products of polychlorinated biphenyls and chlorinated benzenes.[2] The extraordinary toxicity of some PCDBFs that has been demonstrated through animal experiments[3] and to some extent through accidental exposure of humans to these compounds[4], have prompted efforts to develop convenient analytical and synthetic procedures to study this class of compounds in a more controlled way.

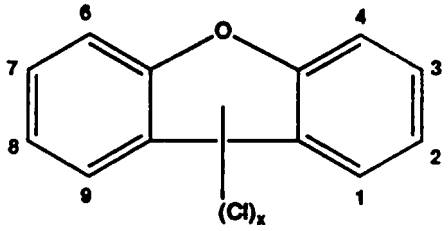


Figure 1. Numbering system of PCDBF

0362-4803/91/010043-20$10.00

Received July 25, 1990
Revised September 14, 1990

Our own efforts have been directed largely towards an understanding of the mechanism of dechlorination in electron capture negative ion mass spectrometry (ECNIMS) and the development of analytical protocols for their analyses that are both sensitive and specific with respect to positional isomers. It has been shown, for example, that a linear correlation exists between the branching ratio, log $[(M\text{-}Cl)^-]/[Cl^-]$ and the energies of low-lying unoccupied molecular orbitals for polychlorinated dibenzo-*p*-dioxins (PCDDs) under the ECNIMS conditions.[5] On this basis it was hypothesized, and later confirmed, that branching ratios can be related to differences in the energy of products formed provided one knows the position from which the chlorine atom or ion is lost during dissociative electron capture. A similar correlation has been observed for PCDBFs.[6] Attempts to identify the labile chlorines of PCDBFs under ECNIMS conditions has led to the synthesis of chlorine-37 labeled regiospecific isomers. General methods for the syntheses of dichloro- and trichloroisomers are described.

Experimental Section

Instrumentation. NMR spectra were obtained on a Bruker AM 400. Analytical GC-MS analyses were performed on a Finnigan 4023 quadrupole mass spectrometer in the EI mode. A 30 m DB-1301 (J & W, 0.25 mm i.d.) capillary column with splitless injection technique was used. Exact mass measurements were made on a Kratos MS50 high resolution mass spectrometer in the EI mode. HPLC analyses and separations were carried out using a Beckman 421 HPLC equipped with a dual pump system and a variable UV detector (254 nm wavelength was used). A preparative reverse-phase column (Sephadex C-18, 250 X 10 mm) was used. Photolyses were carried out in a Rayonet merry-go-round reactor equipped with eight 300 nm Rull lamps.

Materials. Spectral grade solvents were used without further purification. Chlorine gas was purchased from Matheson Co. (99.5%, Newark, CA). Anhydrous $Cu^{37}Cl$ was prepared from $Na^{37}Cl$ by the procedure of Tubandt et al.[7] $Na^{37}Cl$ was purchased from Isotec Inc. (95.6% ^{37}Cl enriched, Miamisburg, OH).

Product Analyses. Large scale (more than 200 mg) chromatographic preparations were carried out on a column (2 x 40 cm) of silica CC-7 special (Mallinckrodt) or silica 60 (EM Reagents) with hexane/dichloromethane (7:3) as eluting solvent. Most small scale products (less than 200 mg) were purified on preparative silica TLC plates (EM science, 2 mm, 20 x 20 cm) with hexane/dichloromethane (6:4) or hexane/chloroform (9:1) as developing solvents. Further separations, if necessary, were achieved by reverse-phase HPLC. A mixture of water (30%) and methanol (70%) was used, and the methanol ratio was increased to 95% in 30 min. gradually. The flow rate was 2.5 mL/min. 1(^{37}Cl),6-dichloro-DBF and 3(^{37}Cl),6-dichloro-DBF were collected by repeated separation of the mixture with collection of the individual fractions on a Gilson Model FC-80 fraction collector. Structural assignments for the intermediate compounds and final chlorine-37 labeled PCDBFs were made by various NMR techniques, including ^{1}H, ^{13}C, ^{1}H-^{1}H, ^{1}H-^{13}C, NOE and relay experiments. When the non-labeled compounds were commercially available or previously reported, comparisons of ^{1}H NMR or GC retention times were used. Exact masses for all final products were determined by peak matching techniques (PFK was used as the reference) on a high resolution mass spectrometer set at a resolving power of 12,000.

Synthesis of Cu^{37}Cl. Equimolar amounts (10 mmol each) of anhydrous cupric sulfate (Aldrich) and Na^{37}Cl (Isotec Inc.) were added in 10 mL of water in a 50 mL three-necked round bottom flask. Sulfur dioxide gas (Atheson Inc.) was bubbled slowly (1 mL/sec) into the stirred solution. A precipitate of Cu^{37}Cl fell out as a white crystalline product, which was washed with SO_2-saturated water (50 mL). After drying by filter suction the crystalline material was put under high vacuum and heated over 110 °C until completely dry. The chlorine-37 isotope enrichment of Cu^{37}Cl was determined by fast atom bombardment in the high resolution mode. For these studies it was 95.6%.

Nitration of Dibenzofuran. A suspension of dibenzofuran (0.17 g, 1.0 mmol) in 20 mL of THF was stirred at room temperature and treated with 4 mL of trifluoroacetic anhydride followed by solid ammonium nitrate (0.8 g, 1.0 mmol). After stirring for 12 h or

longer the mixture was poured into 100 mL water and extracted with chloroform (2 x 100 mL). The product ratios were measured by GCMS analysis using the EI mode. The solution was concentrated and chromatographed on a silica column with hexane/dichloromethane (5:95). By TLC, the first spot gives a product, 2-nitro-DBF which has the following spectral data: EIMS; *m/e* 213(100), 167(25), 155(22), 139(63), ^{1}H NMR; CD_3COCD_3; δ 9.08(d), 8.46(dd), 8.37(dd), 7.88(d), 7.77(dd), 7.68(td), 7.53(td). The second spot, due to 3-nitro-DBF, has the following spectral data: EIMS; *m/e* 213(100), 183(24), 167(25), 139(65), ^{1}H NMR; CD_3COCD_3; δ 8.54(d), 8.39(d), 8.33(dd), 8.30(dd), 7.78(dd), 7.70(td), 7.53(td). The remaining spots included a mixture of 3-, 1-, and 4-nitro-DBFs.

Typical Procedure for Reduction of Nitro Group to Amino Group. Granular tin (Aldrich, 30 mesh, 3 g, 25 mmol) was added to a warm solution of the nitro compound (1 mmol) dissolved in methanol (10-20 mL). Concentrated hydrochloric acid (10-20 mL) was added drop-wise with stirring at a rate so as to maintain a gentle reflux. The mixture was refluxed for 0.5-2 hours after which the solution was allowed to cool to room temperature and poured into 100 mL of water. The aqueous mixture was then adjusted to pH 8 with a concentrated sodium hydroxide solution. Tin hydroxide was filtered off and the solution was extracted with chloroform (2 x 100 mL). The combined solution was concentrated and chromatographed on a preparative TLC plate with dichloromethane.

Conversion of Amine to Chlorine-37 Compound. Anhydrous $Cu^{37}Cl$ (20 mg, 0.2 mmol), *t*-butyl nitrite (0.1 g, 1 mmol) and anhydrous acetonitrile (5 mL) were added to a 25 mL three-necked round-bottom flask which was cooled on an ice bath. The amine (0.1 mmol) in acetonitrile (10 mL) was slowly added under rapid stirring. As the amine was added the reaction solution turned black with evolving nitrogen. After the addition was completed the reaction mixture was slowly warmed to room temperature or, if necessary, to 60 °C. The completion of reaction was checked by TLC or GCMS. The final reaction mixture was initially passed through a small-scale neutral alumina column (disposable pipets were used) and further purified on a preparative silica TLC plate or by reverse phase

HPLC. Typical range of yields of pure chlorine-37 labeled dichloro-DBFs was 42-95%.

General Method for Surface Chlorination. A Kontes #K-420000 chromatographic column with a stainless steel tee was used for chlorination. The column was packed with sodium sulfate (1.5 g) followed by silica gel (3 g). A solution of dibenzofuran was added to the top of the column and dispersed further with a portion of acetone. Another glass wool plug was then placed about 5 cm above the packing. The acetone was completely evaporated with nitrogen gas and chlorine gas was slowly bubbled through the column. (The chlorine gas flow was not measured but maintained at the same rate throughout all the reactions.) Heating tape around the column was used to increase the reaction temperature. The chlorine gas was turned off at the end of the reaction period and the column was purged with nitrogen to remove the residual chlorine. The tee was removed from the column and products were eluted with acetone (100 mL).

2(^{37}Cl),6-Dichloro-DBF. 1,3-Dichloro-2-fluorobenzene (Aldrich, 1 g, 6.1 mmol), 4-aminophenol (Aldrich, 1 g, 9.2 mmol), dried potassium carbonate (EM science, 2 g, 14.5 mmol), 18-crown-6 (Aldrich, 50 mg, 0.19 mmol), and acetonitrile (50 mL) were refluxed for 2 days. The mixture was filtered and the residue washed with hot acetonitrile (50 mL). The combined solution was concentrated and chromatographed on a silica column. 4′-Amino-2,6-dichloro-DPE (280 mg, 1.1 mmol, 18% yield) has the following spectral data: EIMS; *m/e* 255(62), 253(100), 183(15), 108(86), ^{1}H NMR; $CDCl_3$; δ 7.38(d, 2H), 7.09(m, 2H), 6.37(dq), 6.18(m, 2H), 3.54(b, NH_2). The amino group was converted to chlorine to give 2,4′(^{37}Cl),6-trichloro-DPE [EIMS; *m/e* 276(69), 274(100), 239(12), 204(86), 202(56), 113(21), 75(30), ^{1}H NMR; CD_3COCD_3; δ 7.61(d, 2H), 7.39(q, 2H), 7.14(dq), 6.90(t), 6.81 (dq)]. The ether (27 mg, 0.1 mmol) was dissolved in acetone (10^{-3} M) and photolyzed at 300 nm for 3 hours. The product was concentrated and pure dichloro-DBF (95% yield) was collected by reverse-phase HPLC [EIMS; *m/e* 240(32), 238(100), 175(13), 119(10) 87(10), ^{1}H NMR; CD_3COCD_3; δ 8.22(d), 8.15(dd), 7.77(d), 7.63(dd), 7.61(dd), 7.45(t)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

1(^{37}Cl),6-Dichloro-DBF and 3(^{37}Cl),6-Dichloro-DBF. Procedures were the same as those for the synthesis of 2(^{37}Cl),6-dichloro-DBF. The final photoproducts were separated by reverse-phase HPLC from repeated collections. The ratio of two compounds 1.2:1 (98% photolysis yield) and the structure of each compound was confirmed by 2D NMR [3-amino-2′,6′-dichloro-DPE: EIMS; *m/e* 255(31), 253(50), 218(22), 183(100), 92(20), 65(22), ^{1}H NMR; $CDCl_3$; δ 7.38 (d, 2H), 7.09 (m, 2H), 6.37(dq), 6.18(m, 2H), 3.54(b, NH_2)] [2,4′(^{37}Cl),6-trichloro-DPE: EIMS; *m/e* 278(14), 276(77), 274(100), 239(14), 204(78), 202(53), ^{1}H NMR; CD_3COCD_3; δ 7.61(d, 2H), 7.39(q, 2H), 7.14(dq), 6.90(t), 6.81(dq)] [1(^{37}Cl),6-dichloro-DBF: EIMS; *m/e* 240(32), 238(100), 175(13), 119(10), 87(100), ^{1}H NMR; CD_3COCD_3; δ 8.32(dd), 7.76(dd), 7.68(dd), 7.65(t), 7.52(td,2H)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766. [3(^{37}Cl),6-dichloro-DBF: EIMS; *m/e* 240(30), 238(100), 175(13), 119(10), 87(10), ^{1}H NMR; CD_3COCD_3; δ 8.18(d), 8.12(dd), 7.88(d), 7.63(dd), 7.51(dd), 7.46(td)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

2,7(^{37}Cl)-Dichloro-DBF. 3-Nitro-DBF (43 mg, 0.2 mmol) was chlorinated by chlorine gas in a silica column at room temperature for 1.5 h. After rinsing the column with acetone (100 mL), the solution was concentrated and chromatographed on a preparative silica TLC plate. The major compound isolated was 2-chloro-7-nitro-DBF (41 mg, 83% yield) [EIMS; *m/e* 249(30), 247(100), 217(22), 201(20), 173(55), 138(20), ^{1}H NMR; CD_3COCD_3; δ 8.57(d), 8.45(d), 8.38(s), 8.36(dd), 7.85(d), 7.71(dd)]. The nitro compound was reduced by Sn/HCl in methanol for 1 h and the crude product was diazotized and chlorinated with $Cu^{37}Cl$. 2,7(^{37}Cl)-dichloro-DBF (74% yield) was isolated from a preparative TLC plate [EIMS; *m/e* 240(38), 238(100), 175(20), 137(15), 87(17), ^{1}H NMR; CD_3COCD_3; δ 8.21(d), 8.18(d), 7.80(d), 7.70(d), 7.58(dd), 7.49(dd)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

3(^{37}Cl),4-Dichloro-DBF. 3-Nitro-DBF (120 mg, 0.6 mmol) was reduced by Sn/HCl in methanol for 2 h. The crude mixture was chromatographed on a preparative TLC plate.

The major spot was (22 mg, 0.1 mmol) 3-amino-4-chloro-DBF [EIMS; *m/e* 219(28), 217(100), 126(15), 109(20), ^{1}H NMR; $CDCl_3$; δ 7.75(dd), 7.57(dd), 7.51(dd), 7.31(td), 7.25(td), 6.57(d), 3.58(b,NH_2)]. The lower spot was (72 mg, 0.4 mmol) 3-amino-DBF [EIMS; *m/e* 183(100), 154(15), 127(15), 92(20), 77(16), ^{1}H NMR; $CDCl_3$; δ 7.79(dd), 7.69(d), 7.47(dd), 7.32(td), 7.27(td), 6.85(d), 6.69(dd), 3.97(b, NH_2)]. 3-Amino-4-chloro-DBF was diazotized and chlorinated with $Cu^{37}Cl$ to yield 3(^{37}Cl),4-dichloro-DBF (81% yield) [EIMS; *m/e* 240(32), 238(100), 175(15), 137(15), 119(18), ^{1}H NMR; CD_3COCD_3; δ 7.93(dd), 7.78(d), 7.66(dd), 7.52(td), 7.45(d), 7.39(td)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

1,2(^{37}Cl)-Dichloro-DBF. A solution of 4-nitro-DPE (Ultra Scientific Co., 150 mg, 0.7 mmol) and 2 equivalents of palladium acetate in acetic acid (50 mL) was heated to reflux for 24 h. The solvent was evaporated and the residue redissolved in chloroform (100 mL). After filtration and concentration, 2-nitro-DBF was isolated from a preparative TLC plate (133 mg, 89% yield) [EIMS; *m/e* 213(100), 183(14), 167(28), 155(12), 139(70), ^{1}H NMR; CD_3COCD_3; δ 9.08(d), 8.46(dd), 8.37(dd), 7.88(d), 7.77(dd), 7.68(td), 7.53(td)]. 2-Nitro-DBF (105 mg, 0.5 mmol) was reduced by Sn/HCl in methanol for 2 h. 2-Amino-DBF was isolated as the major product from a preparative TLC plate (67 mg, 0.4 mmol) [EIMS; *m/e* 184(12), 183(100), 154(10), 127(12), 91(15), ^{1}H NMR; $CDCl_3$; δ 7.79(dd), 7.69(d), 7.48(dd), 7.34(td), 7.25(td), 6.85(d), 6.69(dd), 3.85(b,NH_2)]. 2-Amino-1-chloro-DBF, upper spot from TLC plate (13 mg, 0.06 mmol), has the following spectral characteristics: EIMS; *m/e* 219(31), 217(100), 154(12), 126(10), ^{1}H NMR; $CDCl_3$; δ 8.36(dd), 7.53-7.45(m,2H), 7.36(td), 7.33(d), 6.92(d), 4.04(b, NH_2). 2-Amino-1-chloro-DBF was diazotized and chlorinated with $Cu^{37}Cl$ to give 1,2(^{37}Cl)-dichloro-DBF (42% yield) [EIMS; *m/e* 240(32), 238(100), 175(18), 119(15), 87(22), ^{1}H NMR; CD_3COCD_3; δ 8.41(dd), 7.72(dd), 7.70(d, 2H), 7.66(td), 7.51(td)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

2,3(^{37}Cl)-Dichloro-DBF. 3-Amino-2-methoxy-DBF (Aldrich, 107 mg, 0.5 mmol) was diazotized and chlorinated with $Cu^{37}Cl$. 3(^{37}Cl)chloro-2-methoxy-DBF (94 mg, 75% yield)

was isolated from a preparative TLC plate [EIMS; *m/e* 234(100), 219(75), 191(34), 126(25), ^{1}H NMR; CD_3COCD_3, δ 8.10(dd), 7.85(s), 7.73(s), 7.61(dd), 7.52(td), 7.38(td), 4.00(s, CH_3)]. 3(^{37}Cl)Chloro-2-methoxy-DBF (94 mg), boron tribromide (1 mL), and dichloromethane (15 mL) were refluxed for 2 hours. Dichloromethane (20 mL) and water (20 mL) were slowly added. The layers were separated in a separatory funnel and the water layer extracted with 20 mL of dichloromethane. The dichloromethane phase was dried over anhydrous sodium sulfate, filtered, and concentrated. The product was chromatographed on a preparative silica column to give 3(^{37}Cl)-chloro-2-hydroxy-DBF (72 mg, 84% yield). This compound was dissolved in acetone (10 mL) and potassium carbonate (0.2 g, 1.5 mmol) was added. The mixed solution was stirred with a magnetic bar at room temperature and trifluoromethanesulfonyl chloride solution in acetone (1 M, 10 mL) was slowly added. After completion of the reaction, 3(^{37}Cl)-chloro-2-triflate-DBF was isolated by preparative TLC (105 mg, 88% yield) [EIMS; *m/e* 352(35), 219(100), 191(36), 126(30), ^{1}H NMR; $CDCl_3$; δ 8.38(s), 8.23(dd), 8.03(s), 7.70(dd), 7.63(td), 7.47(td)]. The triflate compound was redissolved in carbon tetrachloride saturated with chlorine gas (10^{-2} M) and photolyzed in a pyrex tube at 300 nm for 1 hour. The crude solution was initially passed through a small alumina column to remove impurities. Final 2,3(^{37}Cl)-dichloro-DBF was isolated from a preparative TLC plate (91% yield) [EIMS; *m/e* 240(32), 238(100), 236(42), 175(20), 173(27), 137(25), 87(34), ^{1}H NMR; $CDCl_3$; δ 8.01(s), 7.89(dd), 7.67(s), 7.55(dd), 7.49(td), 7.36(td)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

2(^{37}Cl),8-Dichloro-DBF. 4-Amino-4′-chloro-DPE (Western Chemical, 220 mg, 1.0 mmol) was diazotized and chlorinated with $Cu^{37}Cl$, and 4(^{37}Cl),4′-dichloro-DPE was isolated from a preparative TLC plate (198 mg, 83% yield) [EIMS; *m/e* 242(33), 240(100), 177(24), 175(18), 168(15), 75(18), ^{1}H NMR; CD_3COCD_3; δ 8.68(dd, 4H), 8.31(dd, 4H)]. The solution of the ether and 2 equivalents of palladium acetate in acetic acid (30 mL) was refluxed for 12 h. The solvent was evaporated and the residue redissolved in chloroform. After filtration and concentration 2(^{37}Cl),8-dichloro-DBF was isolated from a preparative TLC plate (52%). The product was further purified by reverse-phase HPLC and purity was

checked by GCMS, and no impurities were detected [EIMS; *m/z* 240(33), 238(100), 175(13), 119(12). ^{1}H NMR; CD_3COCD_3; δ 8.22(d,2H), 7.69(d,2H), 7.56(dd,2H)]. Exact mass calculated for $C_{12}H_6OCl^{37}Cl$: 237.9766; HRMS: 237.9766.

2,3(^{37}Cl),4-Trichloro-DBF. 3-Amino-DBF (45 mg, 0.3 mmol) in a 50 mL round bottom flask was added by Cl_2/CCl_4 (10 mL) and stirred for 3 minutes. At the end of the reaction, aqueous sodium bisulfite was added to quench the reaction. Chloroform (50 mL) and water (50 mL) were added to the mixture. After shaking, the chloroform layer was separated, washed with water (50 mL), and dried with anhydrous sodium sulfate. The solution was concentrated and chromatographed by preparative TLC with chloroform/ hexane (9:1). 3-Amino-2,4-dichloro-DBF was isolated as the major product (62 mg, 82% yield) [EIMS; *m/e* 253(73), 251(100), 151(13), 126(23), 125(23), 76(14), ^{1}H NMR; $CDCl_3$; δ 7.71(dd), 7.70(s), 7.49(dd), 7.33(td), 7.26(td), 4.67(b, NH_2)]. 3-Amino-2,4-dichloro-DBF was diazotized and chlorinated with $Cu^{37}Cl$. 2,3(^{37}Cl),4-Trichloro-DBF was separated from 2,4-dichloro-DBF by reverse-phase HPLC (49% yield) [2,4-Dichloro-DBF: EIMS; *m/e* 238(65), 236(100), 173(34), 137(23), 86(25), ^{1}H NMR; $CDCl_3$; δ 7.91(dd), 7.83(d), 7.65(dd), 7.54(td), 7.47(dd), 7.39(td)] [2,3(^{37}Cl),4-Trichloro-DBF: EIMS; *m/e* 274(72), 272(100), 209(28), 137(30), 136(15), 104(15), ^{1}H NMR; CD_3COCD_3; δ 7.97(s), 7.91(dd), 7.65(dd), 7.55(td), 7.41(td)]. Exact mass calculated for $C_{12}H_5OCl_2{}^{37}Cl$: 271.9376; HRMS: 271.9376.

1,2(^{37}Cl),3-Trichloro-DBF. Chlorination of 2-amino-DBF (165 mg, 0.9 mmol) was performed as described for 3-amino-DBF. A reaction time of 5 minutes gave mostly 2-amino-1,3-dichloro-DBF (176 mg, 78% yield) [EIMS; *m/e* 253(63), 251(100), 188(12), 126(12), ^{1}H NMR; $CDCl_3$; δ 8.31(dd), 7.53-7.44 (m, 2H), 7.46(s), 7.34(td), 4.39(b, NH_2)]. 2-Amino-1,3-dichloro-DBF was diazotized and chlorinated with $Cu^{37}Cl$. 1,2(^{37}Cl),3-trichloro-DBF was further purified by reverse-phase HPLC (56% yield) [EIMS; *m/e* 274(62), 272(100), 209(25), 137(20), ^{1}H NMR; CD_3COCD_3; δ 8.35(dd), 7.76(s), 7.75(dd), 7.69(td), 7.53(td)]. Exact mass calculated for $C_{12}H_5OCl_2{}^{37}Cl$: 271.9376; HRMS: 271.9377.

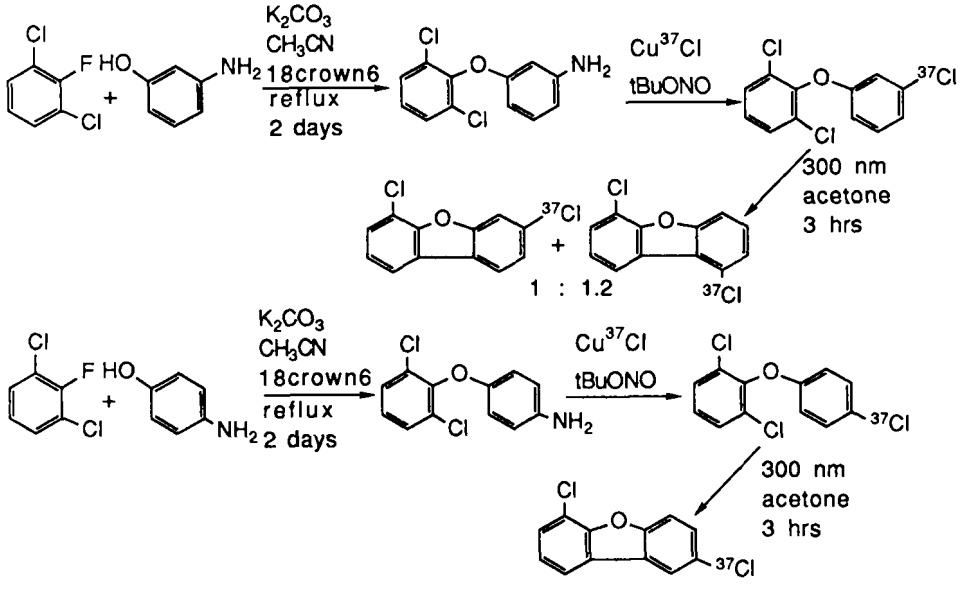
2,3,7(^{37}Cl)-Trichloro-DBF. 3-Nitro-DBF (215 mg, 1 mmol) was chlorinated in a silica column with chlorine gas at 60 °C for 1.5 h. The acetone-eluted solution was concentrated

and 2,3-dichloro-7-nitro-DBF was collected by reverse-phase HPLC. This compound was then reduced with Sn/HCl in methanol for 30 minutes. The crude mixture was diazotized and chlorinated with $Cu^{37}Cl$ (30% overall yield) [EIMS; *m/e* 274(70), 272(100), 209(15), 137(14), ^1H NMR; CD_3COCO_3; δ 8.40(s), 8.19(d), 7.98(s), 7.81(d), 7.49(dd)]. Exact mass calculated for $C_{12}H_5OCl_2{}^{37}Cl$: 271.9376; HRMS: 271.9376.

2,3(^{37}Cl),8-Trichloro-DBF. 3(^{37}Cl)-Chloro-DBF was prepared from the 3-nitro-DBF through reduction, diazotization and chlorination with $Cu^{37}Cl$ by the same methods described above to give 69% overall yield [EIMS; *m/e* 204(100), 139(20), 102(15), ^1H NMR; CD_3COCD_3; δ 8.12(d, 2H), 7.74(d), 7.68(dd), 7.58(td), 7.43(dd), 7.42(td)]. 3(^{37}Cl)-chloro-DBF (42 mg, 0.2 mmol) was chlorinated in a silica column with chlorine gas at room temperature for 0.5 h. The acetone solution was concentrated and chromatographed by reverse-phase HPLC to collect 2,3(^{37}Cl),8-trichloro-DBF (22% yield) [EIMS; m/e 274(72), 272(100), 209(16), 137(15), ^1H NMR; CD_3COCD_3; δ 8.44(s), 8.26(d), 7.99(s), 7.72(d), 7.61(dd)]. Exact mass calculated for $C_{12}H_5OCl_2{}^{37}Cl$: 271.9376; HRMS: 271.9376 Extreme caution should be taken throughout the reaction since the major product is the very toxic 2,3(^{37}Cl),7,8-tetrachloro-DBF.

Results and Discussion

Strategy of Chlorine-37 Labeling on the Dibenzofuran Molecule. Sandmeyer-type chlorination of amino aromatics is well known and a very efficient process.[8] We have used this method to introduce a chlorine-37 label regiospecifically into the dibenzofuran molecule. The amine is converted to the diazonium salt by t-butyl nitrite, and conversion of this product to the chloride is accomplished by chlorine-37 labeled cuprous chloride by the method of Doyle et al.[9] This is a modification of the Sandmeyer reaction. Excess amino compounds and *t*-butyl nitrite were used relative to $Cu^{37}Cl$ (45.6% enriched) to assure maximum incorporation of chlorine-37. In general, no loss of isotopic enrichment was found during the Sandmeyer reaction. Equimolar amounts of substrate, *t*-butyl nitrite and nonlabeled cuprous chloride gave yields in the range 42-95%.



Scheme 1

Synthesis of Dichlorodibenzofurans. A series of 1,6-, 2,6-, and 3,6-dichloro-DBF with chlorine-37 on positions 1, 2 or 3 (Scheme 1) were synthesized. A strategy developed previously[10] for synthesis of chlorinated phenoxy phenols was adapted. This method yields products with chlorines in one of the rings at the ortho positions where they are required for cyclization to the dibenzofuran system. The amino group, like the methoxy group in the synthesis of phenoxyphenols should increase the nucleophilicity of the phenoxide ion during the reaction.

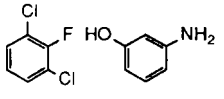
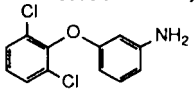
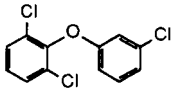
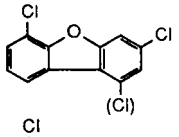
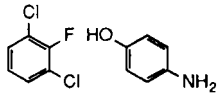
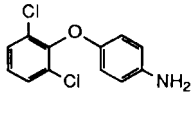
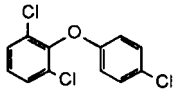
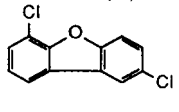
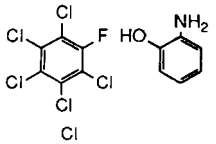
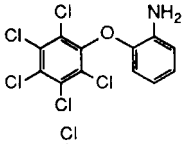
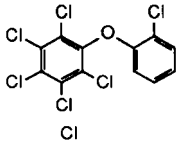
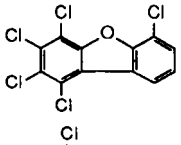
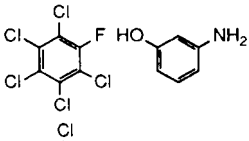
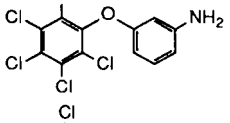
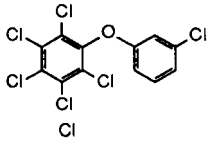
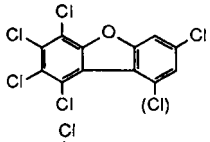
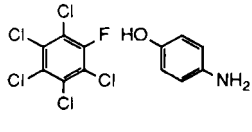
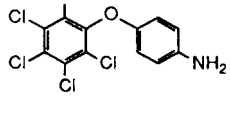
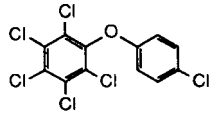
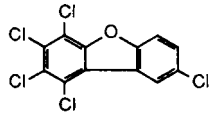
An aminophenol, a chlorinated fluorobenzene in the presence of potassium carbonate, and 18-crown-6 was refluxed in acetonitrile for two days. Chlorines on the activated aromatic substrate were required to be symmetrically placed around the fluorine for reaction to take place. For example, 2,6-dichloro-, 2,3,5,6-tetrachloro-, and 2,3,4,5,6-pentachlorofluorobenzene reacted to form the diphenyl ether (DPE). Unsymmetrically substituted chlorofluorobenzenes failed to react. The higher chlorinated fluorobenzene gave higher yield of product (Table 1) because of its greater electrophilicity. Conversion of the chlorinated aminodiphenyl ether to the diazonium salt, with *t*-butyl nitrite and introduction of chlorine-37 by displacement of the diazonium group with $Cu^{37}Cl$ was carried out in one step.

The photochemical conversion of the chlorine-37 labeled trichloro-DPE was carried out at 300 nm in acetone, which acts as a triplet sensitizer.[11] The triplet-sensitized cyclization reaction gave almost 100% product but in the absence of acetone or other triplet sensitizer the yields of dibenzofuran were poor. No loss of label was encountered in the synthesis, and the use of $Cu^{37}Cl$ with 95% atom excess of chlorine-37 yielded a product with the same atom excess of isotope. Two products from the cyclization of 2′,3(^{37}Cl),6′- by trichloro-DPE were successfully separated by reverse phase HPLC and their structures were confirmed by 2-D NMR COZY spectroscopy (Figure 2).

The introduction of a nitro group at the desired position of the dibenzofuran gave the starting material for the synthesis of several chlorinated dibenzofurans with chlorine-37 at key positions. Among the known nitrating agents, ammonium nitrate in the presence of trifluoroacetic anhydride[12] was found to be most effective for synthesis of mononitrodibenzofuran. The major products were 3-nitro-DBF (72%) and 2-nitro-DBF (16%). The 2-nitro-DBF can also be obtained from the commercially available 4-nitro-DPE (Ultra Scientific Co.) by palladium acetate-promoted cyclization.[13]

With 3-nitro-DBF as starting material and a silica surface as catalyst[14], chlorination proceeded to give 7-chloro-3-nitro-DBF (Scheme 2). Reduction of the nitro group with tin

Table 1. PCDPEs and PCDBFs prepared from chlorofluorobenzenes and aminophenols

Reactants	Products	yield(%)	PCDPEs	PCDBFs
		15		
		18		
		71		
		65		
		68		

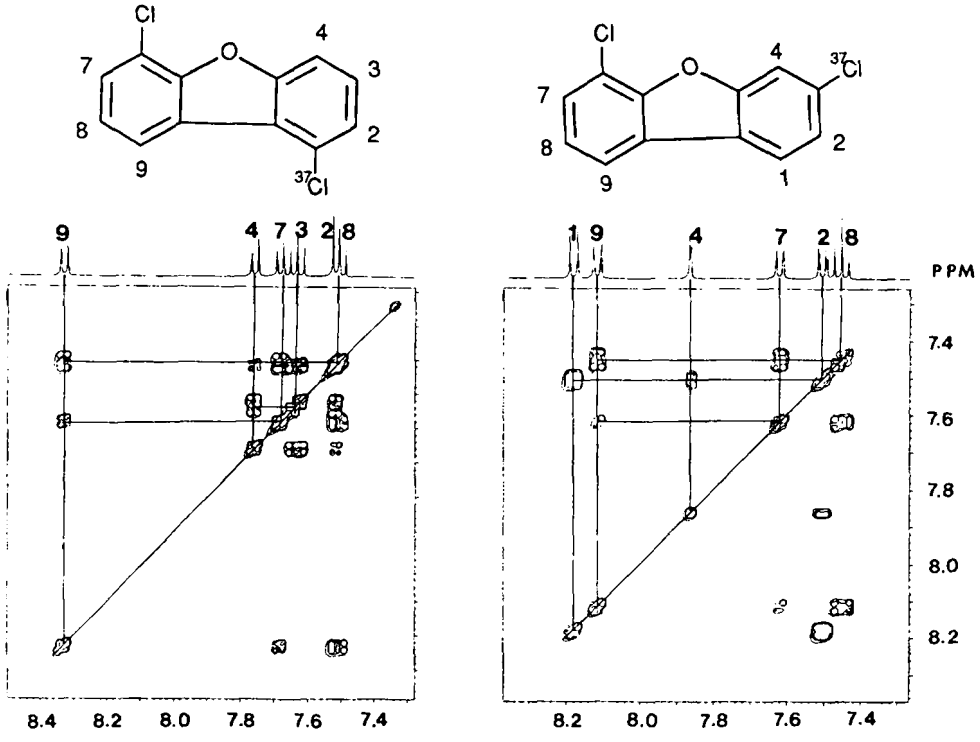
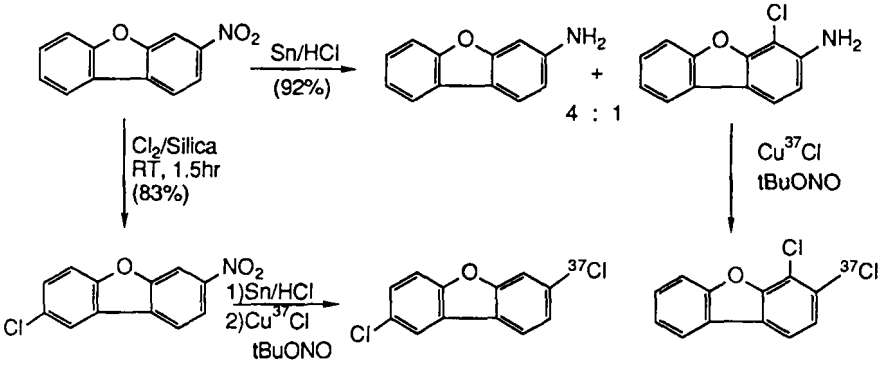
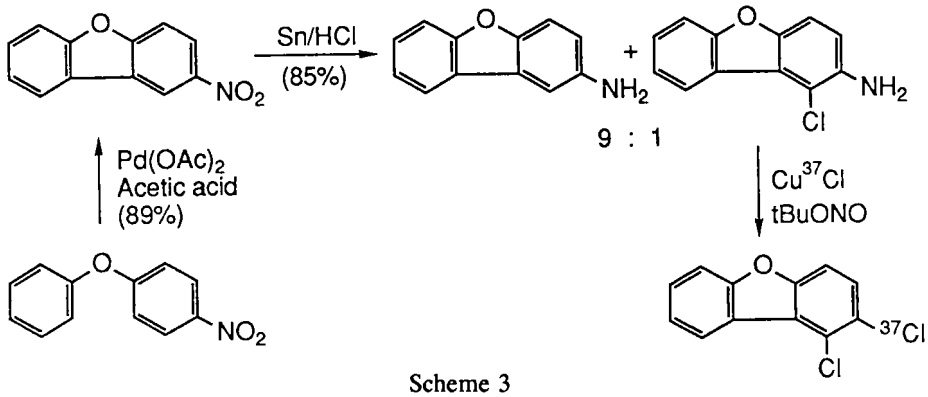


Figure 2. 2-D NMR COZY spectrum of 1(^{37}Cl),6-DCDBF and 3(^{37}CL),6-DCDBF



Scheme 2

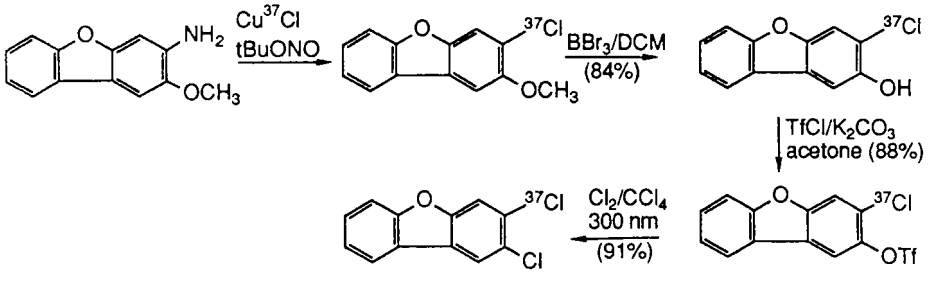
in hydrochloric acid[15] followed by diazotization and chlorination with $Cu^{37}Cl$ gave 3(^{37}Cl),7-dichloro-DBF. In the reduction step some 3-amino-4,7-dichloro-DBF was formed as well. This was observed to be a general phenomenon when nitrodibenzofurans were reduced with tin in the presence of large amounts of concentrated hydrochloric acid[16] and it turned out to be a useful reaction for the preparation of isomers which were otherwise very difficult to obtain.



Scheme 3

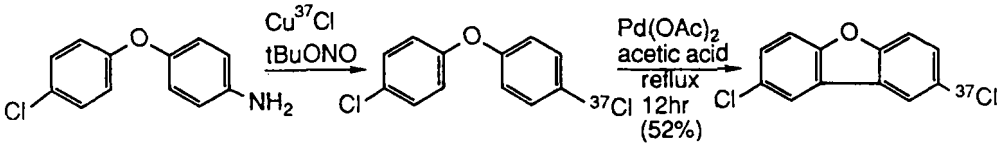
Approximately twenty percent of the reduction product obtained from 3-nitro-DBF was 3-amino-4-chloro-DBF (Scheme 2). Diazotization and chlorination of this compound gave 3,4(^{37}Cl)-dichloro-DBF. In the same way 1,2(^{37}Cl)-dichloro-DBF was obtained from 2-nitro-DBF (Scheme 3).

Chlorine-37 labeled 2,3-dichloro-DBF was prepared from commercially available 3-amino-2-methoxy-DBF (Scheme 4). After conversion of the amino group to chlorine-37 this reaction was demethylated with boron tribromide and the resultant phenolic compound converted to the triflate. Chlorination of this compound with chlorine gas in carbon tetrachloride under photolytic conditions at 300 nm gave a good yield of 2,3(^{37}Cl)-dichloro-DBF.[17] The photolytic conversion involving the singlet state proceeded smoothly to give the product in high yield. However, loss of chlorine-37 enrichment was observed (from 95% to 65%) after photolysis.



Scheme 4

The commercially available 4-amino-4′-chloro-DPE was easily converted to 4,4′(^{37}Cl)-dichloro-DPE (Scheme 5), which in turn could be converted to the dibenzofuran by the method of Åkermark et al.[13], that involved cyclization in acetic acid with the aid of palladium acetate.

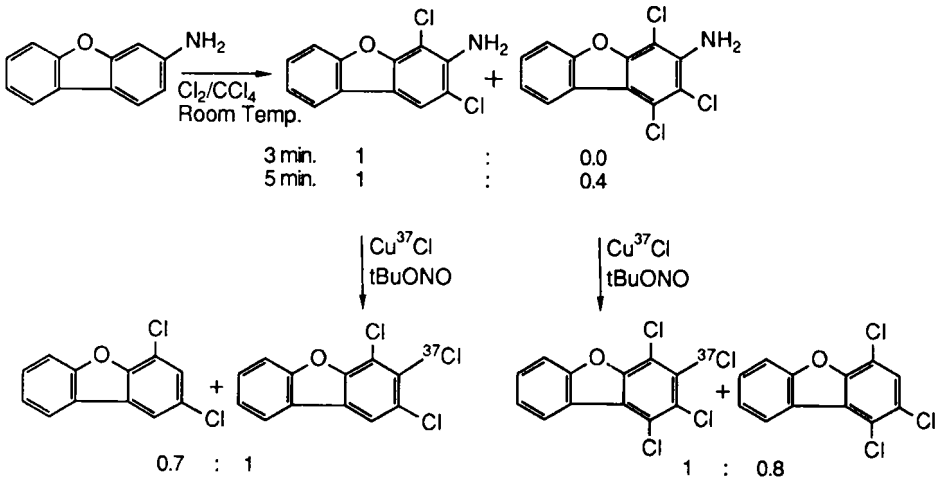


Scheme 5

Synthesis of Trichlorodibenzofurans. Among the possible trichlorodibenzofurans the 1,2,3- and 2,3,4-trichloro-DBFs are particularly difficult to synthesize because the conventional chlorination of dibenzofuran always gives chlorinated products which have an equal distribution of chlorines on the two rings, e.g., 2,8-, 2,3,8-, 2,3,7,8-, etc.[18] The 2,3,4-isomer has been synthesized by Safe[19] by the condensation of 2,3,4,5-tetrachloroaniline and anisole, followed by demethylation and base-catalyzed cyclization. The overall yield after the three reaction steps was approximately 1%.

Electrophilic chlorination of aminodibenzofuran followed by substitutive deamination reaction is one method to introduce chlorines in the ring bearing the amino group.[20] This reaction is efficient because of the electron-releasing effect of the amino group. Generally, amines are very reactive towards electrophilic halogenation. The reaction may be carried out without catalysts and most often cannot be controlled to provide monochlorinated products.[21] For reactions in the absence of a catalyst, the attacking entity is chlorine molecule which has been polarized by the ring. Evidence for molecular chlorine as the attacking species is provided by the fact that acids, bases, and other ions, especially chloride ion, accelerate the rate about equally.[22]

The chlorination of 3-amino-DBF was carried out in Cl_2/CCl_4 solution at room temperature without catalyst. The reactions proceeded very rapidly. In 3 minutes a dichloro- compound was formed exclusively, and in 5 minutes a mixture of dichloro- and trichloro- compound in a ratio of 1:0.4 was obtained (Scheme 6).

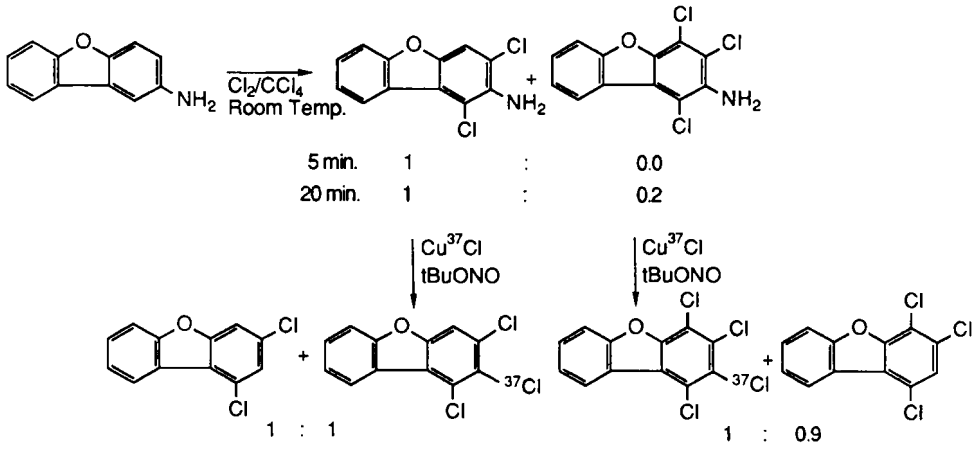


Scheme 6

The trichloro compound was identified as 3-amino-1,2,4-trichloro-DBF. 3-Amino-2,4-dichloro-DBF was then diazotized and chlorinated with $Cu^{37}Cl$. The reaction gave a dichloro-DBF and a trichloro-DBF in the ratio of 0.7:1. The mass spectrum of the dichloro-compound showed that the compound had only natural isotopic abundance of chlorine, indicating that deamination had occurred. This was observed to be a general phenomenon when the amino group had neighboring chlorines. Lowering the initial temperature decreased the amounts of deamination products considerably. The solvent, acetonitrile, could be a hydrogen source in the deamination reaction; this was confirmed by the deuterium incorporation when acetonitrile-d_3 was used as solvent.

By the above method 2-amino-DBF was chlorinated and 1,2(^{37}Cl),3-trichloro-DBF and 1,2(^{37}Cl),3,4-tetrachloro-DBF were obtained (Scheme 7). The rate of chlorination of this compound was somewhat slower than that of the 3-amino-DBF.

The structure of the 2,3,4-trichloro-DBF was determined by 2-D NMR with the help of the NOE technique. The proton NMR spectrum showed four coupled hydrogens and one singlet. Four hydrogens coupled to one another are present in one ring and the single



Scheme 7

proton is in the ring with the three chlorines. AM1 calculation of the neutral molecule indicates that protons 1 and 9 should be 2.88 Å apart. This distance is sufficiently close to be able to observe an NOE if these protons are both present.[23] A strong NOE was

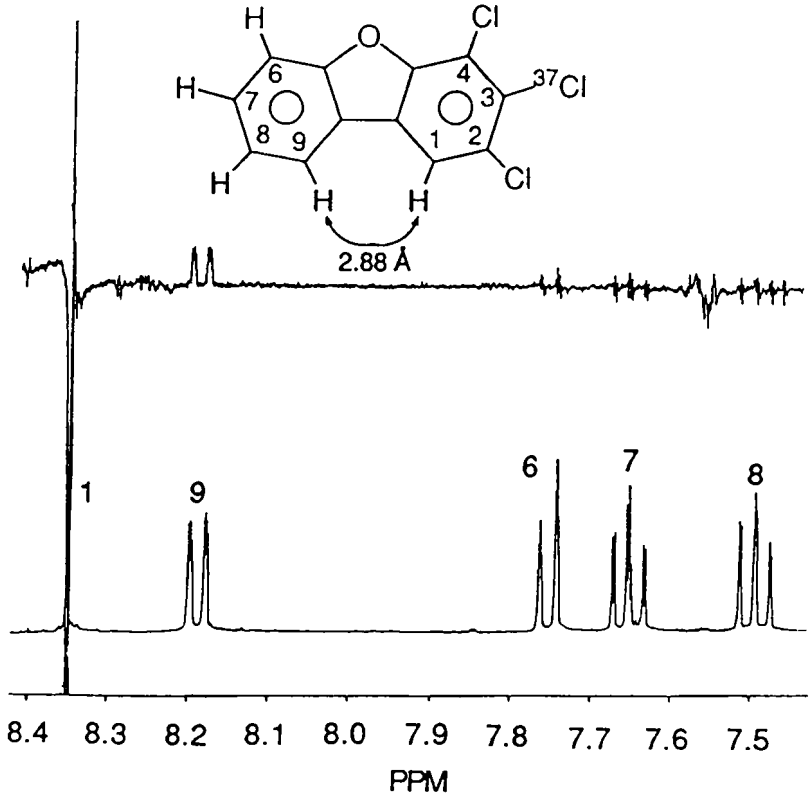


Figure 3. NOE experiment of 2,3(^{37}Cl),4-TrCDBF with irradiation of H_9

observed by irradiation of the singlet (Figure 3). Thus, this proton must be within 3.0 Å of another proton, which is H_9. This was confirmed by irradiation of the H_9 doublet which shows an NOE for H_1 (Figure 4). The structure of the product must, therefore, be 2,3,4-trichloro-DBF. The remaining protons were assigned by noting that H_9 showed a slight NOE upon irradiation of H_1, and H_6 and H_7 were assigned by ^1H-^1H homonuclear and ^1H-^{13}C heteronuclear correlations. A further experiment was performed to confirm the location of chlorine-37 on the 3-position. Photolytic dechlorination with triethylamine gave 2,4-dichloro-DBF exclusively, which was shown by mass spectrometry to have only two natural chlorines. Therefore, there was no regiochemical scrambling of the chlorine-37.

By use of the silica based chlorination, 3-nitro-DBF and 3(^{37}Cl)-chloro-DBF were chlorinated. The chlorination of 3-nitro-DBF occurred at positions 7 and 8, while the

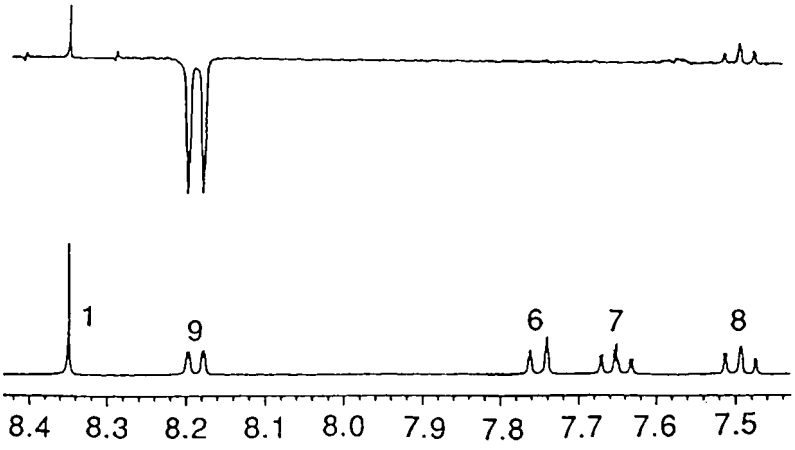
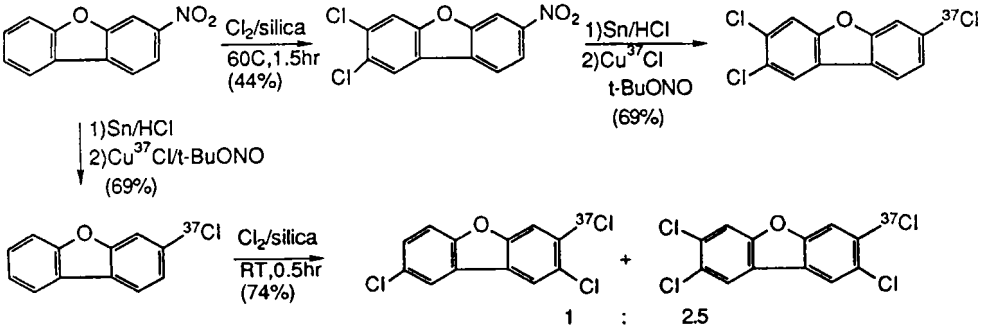


Figure 4. NOE experiment of 2,3(^{37}Cl),4-TrCDBF with irradiation of H_1



Scheme 8

chlorination of 3(^{37}Cl)-chloro-DBF gave chlorine incorporation at positions 2 and 8 (Scheme 8). These two different regiochemistries yielded 2,3,7(^{37}Cl)- and 2,3(^{37}Cl),8-trichloro-DBFs respectively and allowed a comparison to be made of the reactivity of the C_3 position with and without the effects of a neighboring chlorine in the ECNI mass spectrometry. In both cases, however, extreme caution was exercised since the highly toxic 2,3(^{37}Cl),7,8-tetrachloro-DBF can be produced. The two structures could be identified easily by ^{1}H NMR because of the ortho and meta coupling, respectively, of protons H_7 or H_8 to H_9 (Figure 5). The most down-field protons are H_1.

Acknowledgement. We gratefully acknowledge support from the National Institutes of Health, NIEHS ES00040 and NIEHS ES00210. We also wish to thank Rodger Kohnert

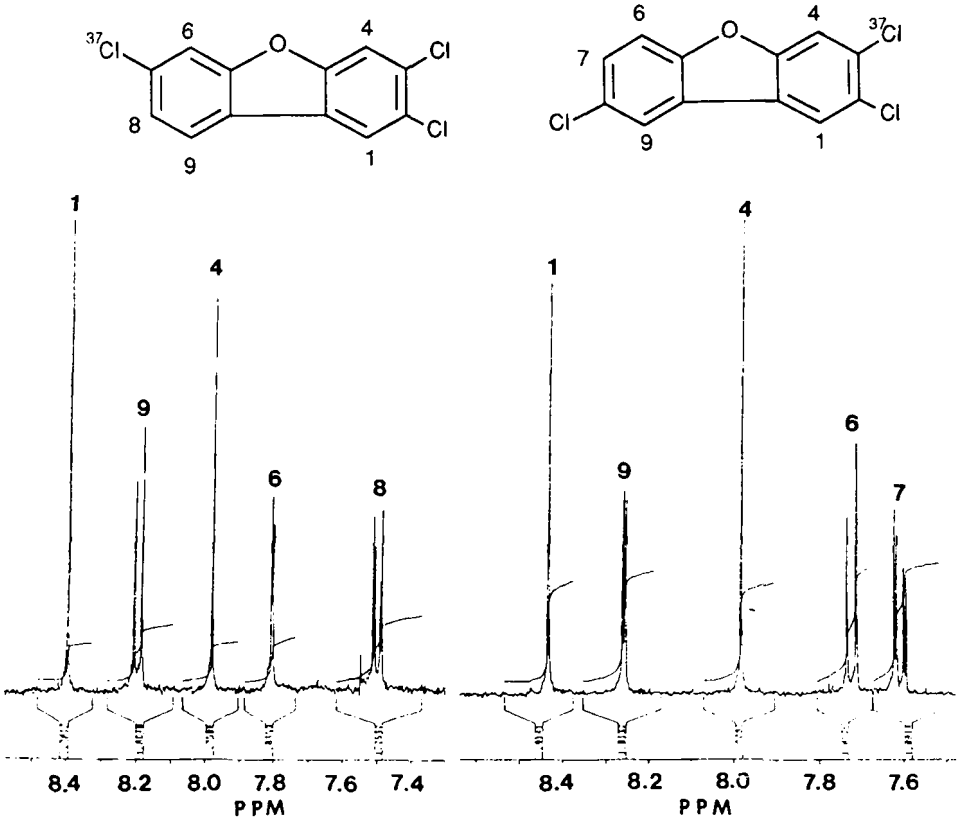


Figure 5. NMR spectrum of 2,3,7(^{37}Cl)-TrCDBF and 2,3(^{37}Cl),8-TrCDBF

for NMR analysis and Jung-Suk Jang for the photolysis work. The bruker AM 400 NMR spectrometer was purchased in part through grants from the National Science Foundation (CHE-8216190) and the M.J. Murdock Charitable Trust to Oregon State University. This is Oregon Agricultural Experiment Station Publication No. 9276.

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