Synthesis and Photodecomposition of Two Azido Derivatives of PCB

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Polychlorinated Biphenyls, Photodecomposition, Azido Compounds

Synthesis of the PCB derivatives 4-azido-2,2′,4′,5,5′-pentachlorobiphenyl and 4,4′-diazido-2,2′,5,5′-tetrachlorobiphenyl is described. The compounds are easily photoactivated by UV-C irradiation. Thus these derivatives are valuable photolabels which have been used already in studies of intracellular distribution.

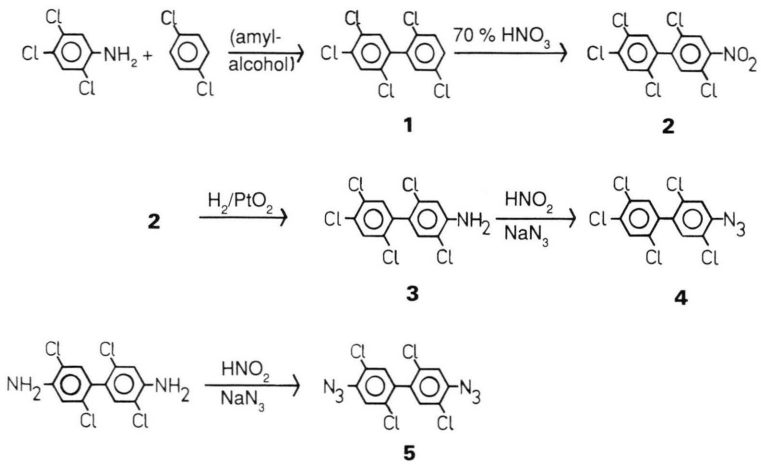
Introduction

Extensive use of polychlorinated biphenyls (PCB) in the past has led to widespread contamination of the environment. In addition to passive accumulation and deposition in fat tissues, an active involvement of PCB in cellular metabolism has been documented [1]. Particular attention has been paid to the role of PCB in carcinogenesis including the tumor-promoting activity of PCB in rat liver [2, 3].

To examine the extent of PCB association with nuclear components we studied the distribution of the PCB congener 2,2′,4,4′,5,5′-hexachlorobiphenyl (6-CB) into the nucleus and the association with nuclear DNA in human liver cells. By formation of photo-induced covalent bonds we were able to stabilize 6-CB interaction with DNA and to analyze 6-CB DNA adducts [4]. Our study showed that 6-CB readily distributed into the nuclei of cultured cells and came into direct contact with DNA bases. This conclusion was supported by experiments with two easily photo-activatable azido analogues of 6-CB. The synthesis of these compounds as well as their photo-lability will be shown in this contribution. The potential biochemical use of these compounds will be referred to.

Experimental

The synthetic routes for the two azido PCB compounds are outlined in the following scheme:



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Synthesis of 4-azido-2,2',4',5,5'-pentachlorobiphenyl

2,2',4',5,5'-Pentachlorobiphenyl (**1**)

The compound was synthesized by coupling 2,4,5-trichloroaniline (1.5 g, Fluka) with 1,4-dichlorobenzene (33 g) as described by G. Sundström [5].

4-Nitro-2,2',4',5,5'-pentachlorobiphenyl (**2**)

To a solution of 440 mg of **1** in 2 ml of acetic acid were added 10 ml of 70% nitric acid. The solution was refluxed for 2 h, cooled and diluted with 10 ml of water. Reaction products were extracted with 3×10 ml of chloroform. The organic phase was dried with calcium chloride, filtrated, and the solvent evaporated. The residue was taken up in a few ml of *n*-hexane/ethyl acetate (9/1) and chromatographed over a silica gel column (Merck Kieselgel 60, bed volume 90 ml). 10 ml fractions were eluted with *n*-hexane/ethyl acetate (9/1); samples were taken to monitor product composition by TLC. Fractions containing compound **2** were combined, the solvent evaporated and the product recrystallized from methanol. Alternatively, purification was completed by TLC. Recovery of pure **2:** 100 mg = 20% of theory. M.p. 97 °C. TLC (*n*-hexane/ethyl acetate = 9/1): R_F = 0.70. IR (KBr): 1565 cm^{-1} and 1535 cm^{-1} (s, NO_2).

Analysis for $C_{12}H_4Cl_5NO_2$

Calcd	C 38.8	H 1.1	Cl 47.7	N 3.8,
Found	C 38.8	H 1.2	Cl 47.6	N 3.9.

MS: m/z = 369 (90%, M^+), 339 (10%, M^+-NO), 311 (70%, M^+-CNO_2), 288 (95%, M^+-ClNO_2), 253 (30%, $M^+-Cl_2NO_2$), 241 (25%, $M^+-CCl_2NO_2$), 218 (100%, $M^+-Cl_3NO_2$). ^1H NMR (in $CDCl_3$, TMS internal standard): four singlets, δ = 7.37, 7.48, 7.65, and 8.05.

4-Amino-2,2',4',5,5'-pentachlorobiphenyl (**3**)

340 mg of the NO_2-compound **2** was reduced with PtO_2 (80 mg)/H_2 in 5 ml of cyclohexane. Recovery of pure **3:** 265 mg = 85%. M.p. 103–104 °C. TLC (*n*-hexane/ethyl acetate = 9/1): R_F = 0.37 (detected with Merck glucose-phosphoric acid stain for aromatic amines). IR (KBr): 3500 cm^{-1} and 3400 cm^{-1} (s, NH_2).

4-Azido-2,2',4',5,5'-pentachlorobiphenyl (**4**)

Incorporation of the azido group was carried out under dim red light. To 50 mg of **3** in 0.5 ml of 50% sulfuric acid was dropwise added 150 mg of sodium nitrite in 0.5 ml of water. The temperature of the stirred reaction mixture was kept below +5 °C. After completion of the reaction, excess nitrite was destroyed with urea (100 mg in 0.5 ml of water). The substitution reaction was started by slow addition of 150 mg of sodium azide in 0.5 ml of water. The reaction mixture was stirred for additional 30 min during which period the temperature was allowed to rise to room temperature. The solution was extracted with 2×10 ml of ether. The ether phase was dried (sodium sulfate), evaporated, and the residue dissolved in ethanol. The product was purified by TLC (*n*-hexane/ethyl acetate = 9/1): R_F = 0.77. Recovery of pure **4:** 18 mg = 35%. M.p. (decomp.) 55–57 °C. UV (methanol): λ_{max} = 222 nm, λ = 262 nm, shoulders at 290 nm and 305 nm. IR (KBr): 2110 cm^{-1} (s, N_3). MS: m/z = 365 (10%, M^+), 337 (30%, M^+-N_2), 302 (100%, M^+-ClN_2), 275 (5%), 267 (10%), 232 (45%, $M^+-Cl_3N_2$).

Analysis for $C_{12}H_4Cl_5N_3$

Calcd	C 39.3	H 1.1	Cl 48.2	N 11.4,
Found	C 39.6	H 1.4	Cl 47.4	N 11.2.

Synthesis of 4,4'-diazido-2,2',5,5'-tetrachlorobiphenyl (**5**)

Reaction conditions were as described for compound **4;** dim red light was used to prevent photodecomposition. Briefly, 200 mg of 2,2',5,5'-tetrachlorobenzidine (TCI Chemicals) were reacted with 0.4 g of sodium nitrite in 3 ml of 50% sulfuric acid. After treatment with urea (0.3 g), the azido groups were introduced by slowly adding 1 g of sodium azide in 1.5 ml of water. Reaction products were extracted with ether, dried, and the solvent evaporated. Recrystallization from ethanol gave 93 mg of pure **5** = 40%. M.p. 118–120 °C. UV (ethanol): λ_{max} = 224 nm, λ = 267 nm, shoulders at 290 nm and 305 nm. IR (KBr): 2130 cm^{-1} (s, N_3). MS: m/z = 372 (15%, M^+), 344 (20%, M^+-N_2), 316 (10%, M^+-N_4), 289 (5%, M^+-CHN_5), 281 (50%, M^+-ClN_4), 255 (10%), 254 (5%), 246 (100%, $M^+-Cl_2N_4$), 245 (15%), 219 (20%, $M^+-CHCl_2N_5$), 211 (40%), 210 (30%).

Analysis for $C_{12}H_4Cl_4N_6$

Calcd	C 38.5	H 1.1	Cl 38.0	N 22.4,
Found	C 38.9	H 1.0	Cl 37.9	N 22.7.

Photodecomposition

Solutions of the azido compounds **4** and **5** (15 μM in methanol) were filled in glass culture dishes of 6 cm diameter. The dishes were placed under a Philips HPK 125-W/L lamp at a distance of 2.5 cm [6]. After various times of exposure irradiation was paused to record the UV absorption

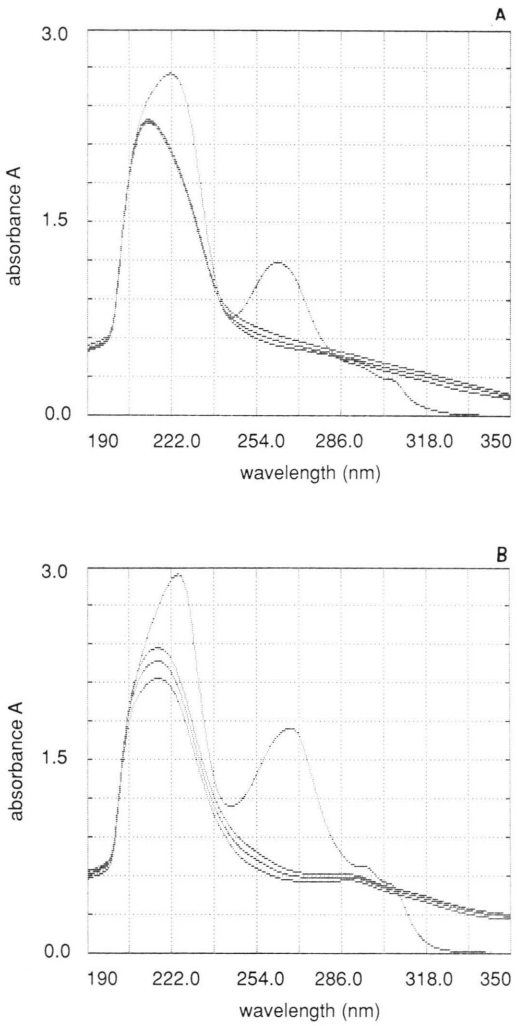

Fig. 1. Photodecomposition of the azido compounds **4** (panel A) and **5** (panel B). Solutions of **4** and **5** (about 15 μM in methanol) were irradiated and the UV spectra recorded (Beckman spectrophotometer DU 7) after 0 (top spectrum), 5, 10 and 15 sec of irradiation.

spectra of the solutions (Beckman DU-7 spectrophotometer). At these experimental conditions, photodecomposition is rapid and actually completed within a few seconds (Fig. 1).

Biochemical implications

As mentioned above, the azido compounds **4** and **5** were included in experiments of uptake and distribution of 6-CB into nuclei of cultured human cells [4]. Photo-induced binding to DNA and subsequent DNA adduct analysis revealed different adduct levels of **4** and **5.** The mono-azido compound **4** formed approximately 25 times more purine nucleotide adducts than the diazido compound **5** [4]. Radioactive labeling of **4** and **5** with tritium or iodine to high specific radioactivity may even more extend the applicability of these compounds. The radioactive compounds would represent valuable PCB photoaffinity reagents. In addition to kinetic studies such as uptake and intracellular binding, the radioactive derivatives could be used in directly identifying specific cellular PCB binding proteins.

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