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# SYNTHESIS OF CARBON-13 ($^{13}C_{12}$) AND DEUTERATED ($D_{18}$) GAMMA-HEXABROMOCYCLODODECANE AND THEIR USE AS SURROGATE STANDARDS IN LC/MS AND GC/MS ANALYSIS

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## Introduction

The hexabromocyclododecanes (HBCDs) constitute one of the most important groups of flame retardants[1, 2]. They are used primarily in the building industry as additives in foam insulation and they are also found in products such as vehicle seat cushions, furniture and textiles[3, 4].

HBCD is an alicyclic compound containing 75% by weight of bromine (see Figure 1). HBCD is synthesized industrially by the addition of bromine to cis-trans-trans-1,5,9-cyclododecatriene[5,6] to give a mixture of three diastereomers (α, β, γ, Figure 1) with the gamma (γ) isomer usually predominating (70% to 90%).

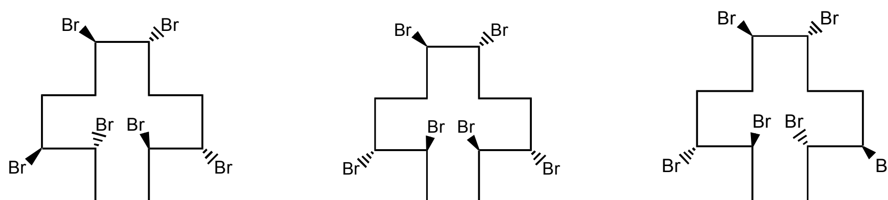

**Figure 1**. Structures of the α-(left), β-(middle) and γ-(right) HBCD isomers

Recently, a considerable amount of attention has been devoted to the analysis of brominated flame retardants primarily because of the dramatic growth in their industrial production and their increasing levels found in the environment[1,7]. Various reports have appeared on techniques to analyze environmental samples for the presence of HBCD[1,7,8]. However, no mass-labeled HBCD compounds were available for use as surrogate standards.

This study reports the synthesis of the fully deuterated γ-hexabromocyclododecane (d-gHBCD) and the carbon-13 mass labeled ($^{13}C_{12}$) γ-hexabromocyclododecane (c-gHBCD). The use of these compounds as surrogate standards, with ultimate analysis by GC/MS or LC/MS, is evaluated.

## Methods and materials

***Chemicals***. The α, β and γ-HBCD congeners were purified from a commercial technical mixture as described in the literature[6,9]. HPLC grade acetonitrile and methanol were obtained from Fisher Scientific. All other chemicals were reagent grade.

***Liquid Chromatography***. A Waters Prep LC4000 HPLC system equipped with a Nova-Pak C18 column (3.9 cm x 150 mm i.d.) and a Waters 2487 Dual Wavelength Absorbance detector set at 220nm and 254nm was used for all samples. The mobile phase consisted of 80% acetonitrile, 19% water and 1% methanol. The flow rate was set at 0.8mL per minute and the chromatography performed at ambient temperature.

***Gas Chromatography / Mass Spectrometry***. The analysis was carried out using a HP5890 gas chromatograph (GC) connected to a HP5970 series mass selective detector (MSD). A 15m DB-5HT capillary column (0.25mm id x 0.1um film thickness) was installed in the GC. The temperature program was as follows: initial temperature of 140°C, 1 minute isothermal, 40°C/min. to 200°C and hold for 5.5 minutes, 10°C/min. to 325°C and hold for 20 minutes; Splitless injection (215°C) was used with the detector (MSD) set at 250°C. Mass spectra were obtained by scanning from m/z 50 to 800 at a scan rate of 1.09 scans/second.

***Liquid Chromatography / Mass Spectrometry***. The detection of HBCD was based on a method recently described by Budakowski and Tomy[8]. Separations were performed on C18 analytical column (5.0 cm x 2.1 mm i.d., 4 μm particle size) at a rate of 300μL/minute. MS/MS detection for HBCD and dHBCD used the following respective MRM transitions: m/z 640.6 ([M-H]-) to m/z 79 (Br-) and m/z 659 ([M-D]-) to m/z 79 (Br).

**Results and Discussions**

***Bromination of d-CDT***. Deuterated $d_{18}$-cis-trans-trans-1,5,9-cyclododecatriene and $^{13}C_{12}$-cis-trans-trans-1,5,9-cyclododecatriene were brominated as described in reference 5. The γ-isomers were purified by repeated crystallization[9]. The chemical purity of the γ-isomers were verified by HPLC and GC/MS to be >98%. The melting point (m.p.) for d-gHBCD and c-gHBCD were determined to be 195-198°C and 195.5-197°C, respectively. This was similar to the m.p. of the native γ-isomer purified in our laboratory with a m.p. of 198-202°C and compares very well to other reported literature values (196-198°C[6] and 206°C[9]).

***HPLC and NMR of d-HBCD***. HPLC analysis of the d-gHBCD and c-gHBCD each show a clean signal with a retention time of approximately 5.4 minutes, similar to the retention time of native gHBCD. No impurities, including α and β isomers, were detected.

The $^{2}H$ NMR of d-gHBCD at 75°C in methylene bromide shows eight distinct singlets (see Figure 2) representing nine deuteriums, which is consistent with the $C_2$ axis of symmetry for the gamma structure. Native gHBCD gives essentially identical shifts although the spectrum is much more complicated due to proton-proton coupling.

**Figure 2**. $^{2}H$ NMR of d-gHBCD and $^{1}H$ NMR of gHBCD

***GC/MS of d-HBCD***. GC/MS analyses showed no decomposition of HBCD when the temperature of the injector port was maintained at 215°C. The GC/MS of the three native HBCD isomers (α, β, γ) each gave one, reasonably shaped peak. All had the same retention time. The d-gHBCD also gave a single peak with a slightly shorter retention time relative to the native compound (see Figure 3). This phenomenon of a deuterated analogue having a shorter retention time has been observed previously[11,12]. As expected, c-gHBCD co-eluted with gHBCD.

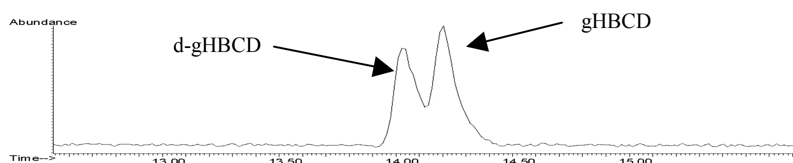


**Figure 3**. GC/MS TIC Chromatogram of d-gHBCD/gHBCD mixture

The molecular ion for either the d-gHBCD or c-gHBCD could not be observed but the mass fragment m/z 579 (MI [$C_{12}D_{18}Br_6$] – Br) for d-gHBCD or the mass fragment m/z 573 (MI [$^{13}C_{12}H_{18}Br_6$] – Br) for c-gHBCD were clearly evident with both patterns being consistent for an ion containing five bromine atoms (see Figure 4). The fragmentation pattern for the native gHBCD was similar to the mass labeled compounds except for the expected lower masses.

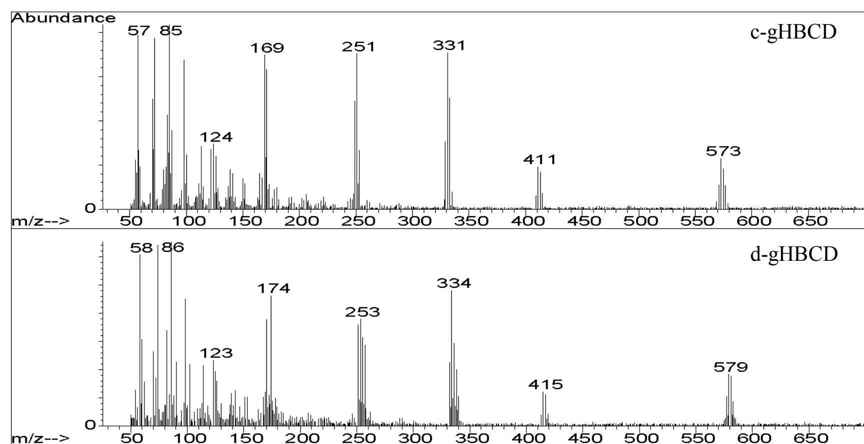


**Figure 4**. Mass spectrum of d-gHBCD and c-gHBCD

***LC/MS of d-HBCD***. LC/MS analyses showed no decomposition of HBCD. The LC/MS of the three native HBCD isomers (α, β, γ) each gave one, reasonably shaped peak with retention times of 6.15, 6.42 and 6.52 minutes, respectively. The d-gHBCD also gave a single peak with a slightly shorter retention time[11] of 6.47 minutes relative to the native compound (see Figure 6). This separation of approximately 3 seconds between gHBCD and d-gHBCD can produce a partial separation on an instrument with excellent resolution.

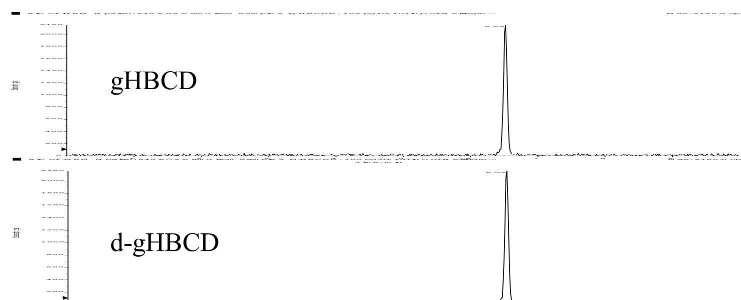


**Figure 6**. Elution of gHBCD ($r_t$: 6.52 min) and d-gHBCD ($r_t$: 6.47 min) off a $C_{18}$ analytical column

The molecular ion for d-gHBCD was observed and the mass fragment m/z 657.6 (MI [$C_{12}D_{18}Br_6$] – D) was clearly evident with the pattern being consistent for an ion containing six bromine atoms.

No time was available to complete the LC/MS analysis of c-gHBCD before the submission of this short paper but data will be presented at the Dioxin'2003 conference.

**Conclusions**

This work has provided pure deuterated and $^{13}C_{12}$ gamma-hexabromocyclododecane, which should prove useful as surrogate standards for the mass spectral analysis and quantification of HBCD.

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