SYNTHESIS AND IDENTIFICATION OF BR_7-BR_9 OH/MEO-PBDES IN HUMAN SERUM

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Abstract

Synthetic methods were developed for the preparation of authentic highly brominated (Br_7-Br_9) OH-PBDE standards and the corresponding methoxylated PBDEs (MeO-PBDEs), and 25 Br_7-Br_9 *o*-, *m*-, and *p*-OH/MeO-PBDEs were synthesized with the methods. Using the synthesized compounds as standards, we successfully identified two OH-octaBDEs and one OH-nonaBDE predominant metabolites in human serum from electronic waste dismantling workers.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants widely used in a variety of consumer products including textiles, furniture, appliances, and computers. Although the pentaBDE and octaBDE formulations were banned in Europe and voluntarily phased out in the United States in 2004, decaBDE (BDE-209) is still used commercially in large amounts. In recent years, it has been found that PBDEs could be biotransformed to hydroxyated PBDEs (OH-PBDEs) (*1*). OH-PBDEs elicit multiple toxic effects on organisms, such as neurotoxic effects and disruption on thyroid hormone homeostasis, oxidative phosphorylation, and estradiol synthesis. Thus, there is a growing concern over OH-PBDEs.

Multiple highly brominated OH-PBDEs can be produced from BDE-209. BDE-209 can be converted to hexa- to nona-BDEs by UV and/or sunlight (*2*), which can be biotransformed into various OH-PBDEs. Alternatively, BDE-209 can also be converted to highly brominated OH-PBDEs through debromination in vivo and subsequent biotransformation. The hydroxy groups in the OH- PBDEs could be at any of *ortho*-, *meta*-, and *para*-positions relative to the diphenyl ether bond. Hence, to identify these metabolites in biological samples, various highly brominated OH-PBDE reference standards are needed. However, so far synthesis of these compounds has mostly been focused on lowly brominated (Br_2-Br_6) OH-PBDEs (*3*-*4*), synthesis of highly brominated OH-PBDEs (Br_7-Br_9) has been extremely limited.

In the present work, we developed synthetic methods for the preparation of authentic highly brominated OH-PBDE standards and the corresponding methoxylated PBDEs (MeO-PBDEs) and synthesized these compounds with the methods. Using the synthesized compounds as standards, we successfully identified three predominant OH-PBDEs metabolites in human serum from electronic waste dismantling workers.

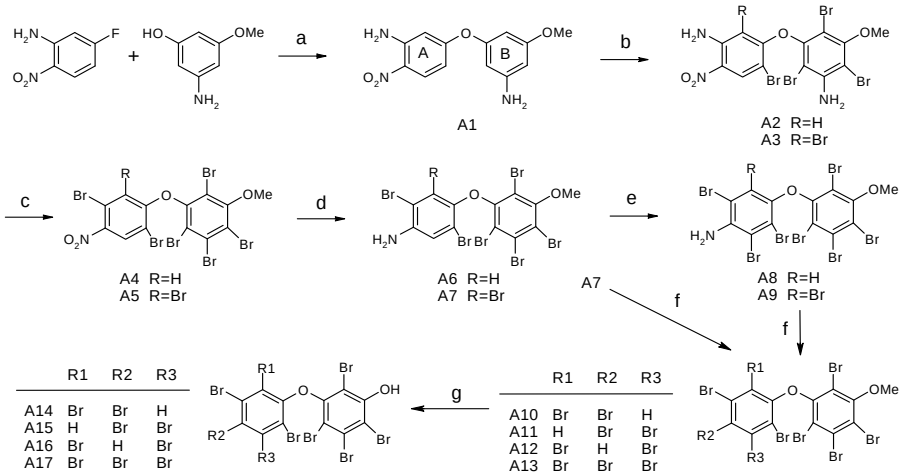
Materials and methods

All chemicals were analytical reagents. The OH/MeO-PBDEs used as reference standards were purified on HPLC. The ^{1}H (500 MHz) and ^{13}C NMR (125MHz) spectra were recorded on a Bruker Avance 500 spectrometer, and electron ionization mass spectra (EI-MS) were obtained using an Agilent 6890N gas chromatograph. A total of 27 mL serum samples were collected from six e-waste dismantling workers in Guiyu town, Shantou City, Guangdong Province. The methods used for sample extraction and cleanup have been described previously (*5*).

Results and discussion

Synthesis Procedure. In the present study, we developed a systematic approach to prepare Br_7-Br_9 OH-PBDEs with various structures. As an example, the procedure to synthesize four Br_8-Br_9 *m*-OH/MeO-PBDEs was briefly described here (Scheme 1). The approach included a coupling reaction between 5-fluoro-2-nitroaniline and methoxyphenol or aminomethoxyphenol to produce diphenyl ethers, and subsequent bromination of the diphenyl ethers, removal of amino/nitro groups, and/or conversion of amino/nitro groups to bromine atoms. Amino group was the key to the approach; it facilitated regioselective bromination on the phenyl rings and was able to be removed or be converted to bromine atom readily.

SCHEME 1

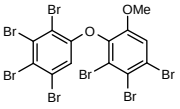


Reagents and conditions: (a) 5-fluoro-2-nitroaniline, Na_2CO_3, DMAC, △; (b) Br_2, AcOH; (c) HBr, $NaNO_2$, H_2O; then CuBr, HBr, △; (d) Fe, AcOH, △; (e) Br_2, CH_2Cl_2, △; (f) identical to (c) (g) BBr_3, CH_2Cl_2, △

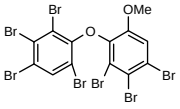
Totally, 25 Br_7-Br_9 *o*-, *m*-, and *p*-OH-PBDEs and the corresponding methoxylated PBDEs (MeO-PBDEs) were successfully synthesized (Scheme 2), demonstrating the feasibility of the approach.

Identification of OH-PBDEs in human serum. We analyzed a pooled serum sample collected from six e-waste dismantling workers in Guiyu town using GC/ECNI-MS. More than 20 unknown structure brominated phenols were observed in the phenolic fraction with three compounds appearing as predominant metabolites.

SCHEME 2



6'-MeO-BDE170 (**C12**)



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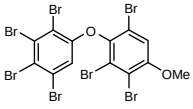
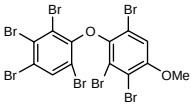


4'-MeO-BDE174 (**E10**)



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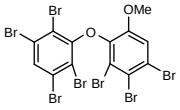

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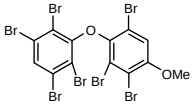

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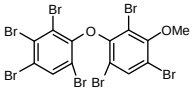

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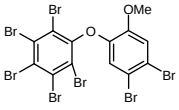

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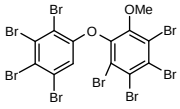
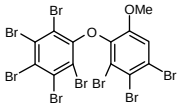


6-MeO-BDE194 (**C22**)



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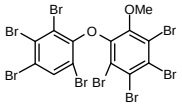
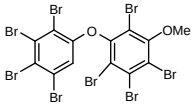
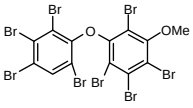


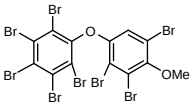
6-MeO-BDE196 (**C24**)



5'-MeO-BDE196 (**A11**)



4-MeO-BDE197 (**A10**)



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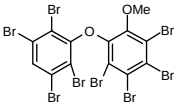

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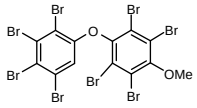
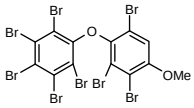
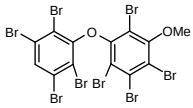


4'-MeO-BDE199 (**E22**)



4'-MeO-BDE200 (**E12**)



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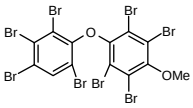
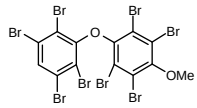
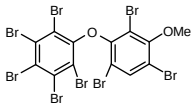


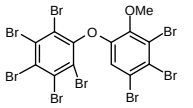
4'-MeO-BDE201 (**E21**)



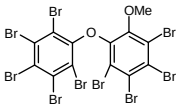
4-MeO-BDE202 (**E23**)



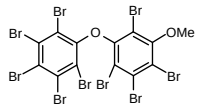
3'-MeO-BDE204 (**B10**)



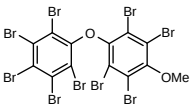
2'-MeO-BDE205 (**C25**)



6'-MeO-BDE206 (**C26**)



5'-MeO-BDE207 (**A13**)



4'-MeO-BDE208 (**E24**)

The three compounds was identified as 6-OH-BDE199, 6-OHBDE196, and 6'-OH-BDE206 by coelution on three gas chromatographic columns with different polarities (DB-5 nonpolar, DB-17 medium polar, and SP-2331 high polar) (Figure 1). Identification of the remained metabolites was still in progress. The two OH-octaBDEs and one

OH-nonaBDE could be oxidatively metabolized from highly brominated diphenyl ethers in human serum, following continuous long-term exposure to high concentrations of BDE-209. The hydroxy groups in the three compounds were all located at the *ortho*-position. However, the metabolites in mice were found to be predominantly *meta*- and *para*-OH-PBDEs (*1*). Qiu et al. (*6*) suggested that the differences could be explained by differences in cytochrome P450 enzyme expression between humans and mice.

In summary, we developed methods for synthesis of highly brominated OH/MeO-PBDEs and successfully synthesized 25 Br_7-Br_9 *o*-, *m*-, and *p*-OH/MeO-PBDEs. Using the compounds as standards, three predominant metabolites in human serum were identified. Due to complication of metabolites in biological samples, more highly brominated OH/MeO-PBDEs need to be synthesized. Besides being used as the reference standards to identify the metabolites in biological samples, the synthesized OH/MeO-PBDEs can also be used in toxicological studies, and ecological and human health risk assessment.

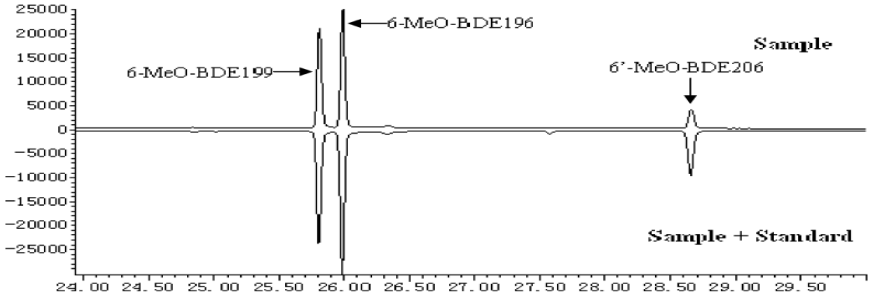


Fig. 1 GC/MS chromatogram of coelution of synthetic standards and pooled sera on DB-5 column in ECNI mode.

Acknowledgements

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6. Qiu X, Bigsby RM, Hites RA. (2009); *Environ Health Perspect*. 117: 93-408.