PREDICTIVE IDENTIFICATION OF PENTABROMOCYCLODODECENE (PBCD) ISOMERS WITH HIGH BINDING AFFINITY TO hTTR

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Abstract
The binding affinities of the six main hexabromocyclododecane (HBCD) stereoisomers and all of their possible 48 allylic pentabromocyclododecene (PBCD) metabolites to the endocrinous human transthyretin receptor (hTTR) were investigated and compared to the natural binder thyroxine, and the two brominated diphenyl ethers BDE-47 and 3-hydroxy-BDE-47. The endocrine disrupting potency was approximated by a combination of two methods: a surface matching with the natural binder thyroxine (T_4) followed by approximation of free binding energies for various binding modes within hTTR. The results indicate slightly higher binding affinities for both BDE structures than for T_4 itself and similarly high affinities for two trans-configurated PBCD isomers. For many other PBCD isomers, intermediate values were computed, whereas all HBCD diastereomers yielded significantly lower binding affinities.

Introduction
1,2,5,6,9,10-Hexabromocyclododecane (HBCD) is a major flame retardant additive contained in various plastics and textiles[1] and is increasingly observed in environmental compartments and biota[2,3,4]. HBCD has received increasing awareness from trace analysts and toxicologists since endocrine effects similar to those observed for other flame retardants have been reported[5,6,7,8,9,10]. Consequently, the EU is concerned with a risk assessment and restrictions of its further use as plastic additive might be expected for the future. The situation in case of HBCD is especially complicated as the technical product consists of three diastereomeric pairs of enantiomers, named α, β, and γ-HBCD (Figure 1)[11] dominated by γ-HBCD while in biological systems the α-HBCD is predominating. Moreover, there is evidence that certain pentabromo derivatives of HBCD occur in biota and occupational environment[12,13]. Though HBCD itself is not likely to display major endrocine effects in connection with the thyroid system, their pentabromocyclododecene derivatives (PBCD) might significantly add to the overall observed endocrine effect on the thyroid system in animal models[5,6,7,8,9,10]. Therefore, the estimation of the potential of PBCD to interact with the thyroxine receptor human transthyretin (hTTR) is of interest in the course of a comprehensive risk assessment.
The huge structural options for PBCD isomers (Figure 1) derived from α, β, and γ-HBCD makes it impossible to assign the correct stereoisomerism of the PBCD derivatives observed by mass spectrometry let alone to synthesise them for toxicological tests. Consequently, a purely theoretical study was launched in order to compare HBCD and PBCD isomers with the natural binder thyroxine regarding the respective binding affinity. Therefore, the binding modes of the parent stereoisomers (±)-α-, (±)-β-, and (±)-γ-HBCD and all possible PBCD derivatives to the hTTR protein were simulated by means of surface matching with thyroxine and the approximation of their binding affinities to the hTTR protein. In addition, thyroxine itself was taken into account as well as BDE-47 and 3-hydroxy-BDE-47, due to reports about their endocrinous effects[9] and the obviously high structural similarity.

Materials and Methods
The structures chosen to be investigated with regard to their docking behaviour were the six parent HBCD stereoisomers, all possible PBCD structures derived from the elimination of one mole of HBr from either one of the six parent HBCD stereoisomers and two variants of BDE. All structures were constructed with Chemsketch11 and exported as mol files. After careful verification of the correct stereochemistry the structures were parameterised by the Merck Molecular Force Field (mmff)[14] as implemented in amiraMol[15]. The conformational space of all ligands was sampled with the hybrid Monte-Carlo algorithm using the ZIB software ZIBgridfree of which the output trajectory was used as input for a semi-flexible docking performed with the ZIB software FADO[16]. It computes intermolecular and intramolecular potential energies for an arbitrary number of different orientations and geometries

of the ligands within the binding pocket of hTTR with a subsequent energy optimisation for the entire molecular complex. The crystal structure of hTTR had been retrieved from the RCSB Protein Data Bank (PDB)[17] under the ID 1ICT[18]. The resulting set of highly dockable geometries was taken as input for the pairwise surface alignment tool[19] which had been implemented at ZIB as well. In a first step, structural (geometric shape) and physico-chemical properties (atom types) of a ligand and of the reference molecule thyroxine were defined and projected on their molecular surfaces (solvent excluding surface). In the next step, these modified surfaces were aligned aiming at a maximised matching score. The parameters were set to standard values except of the distances of geometry points of any atom type which were set to 1.3 and the number of iterations which was set to 30, resulting in a higher resolution and improved matching and all in all yielding a proposition of binding modes to be scored in the following according to their affinity to binding site I of the hTTR crystal structure 1ICT. Hence, spatial orientations of those HBCD and PBCD conformations that yielded a significantly high surface alignment score in the previous step were chosen for constructing a 3D energy hyperplane around the binding pocket of hTTR. For this purpose, the ligands were arranged along a 3D grid covering both the pharmasite and the entrance to the binding site at a resolution varying between 0.2 and 0.7 Å depending on sterical properties of the ligand. Each combination of the receptor and a ligand's position in the grid underwent a resilient backpropagation (rProp) energy minimization[20], with the inner parts of hTTR kept flexible together with the ligand. In contrast to the developers recommendation, the user parameters, i. e. maximal/minimal step size ($\gamma_{max}/\gamma_{min}$) and increasing/decreasing factor (η^+/η^-) of the gradient-based algorithm were set to $50/10^{-6}$ and 1.4/0.7, respectively, with the initial step size γ_0 set to 0.005. Afterwards, a knowledge-based scoring approach adapted from a modified Gohlke function[21], taking additionally into account the ligand's energy difference between the bound and the unbound state, was applied. The surface matching score together with the approximated binding affinity give evidence about the endocrine disrupting potency of HBCD and PBCD stereoisomers.

Results and Discussion

The human thyroid receptor hTTR was chosen for this study since respective endocrinous effects from various brominated substances including brominated diphenyl ethers (BDE) have been reported[9]. In contrast to some BDE and tetrabromo bisphenol A (TBBPA), HBCD does not display any obvious close structural similarity to the natural binder thyroxine. Therefore, the question arose if any metabolisation products might be responsible for the effects sometimes observed for HBCD.

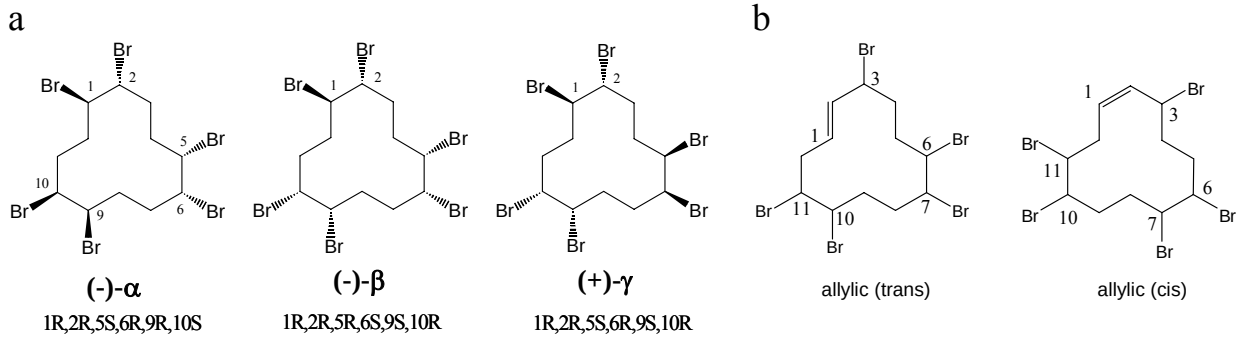

Figure 1: a - (+)-*α*-HBCD, (+)-*β*-HBCD, (+)-*γ*-HBCD; **b** - PBCD structures investigated.

Hence, the PBCD stereoisomers as formed by elimination of a mole of hydrogen bromide from HBCD was considered as candidates, in particular, since the production of pure PBCD for experimental investigations shapes up as a challenging task. Meanwhile, also tetrabromocyclododecaenes have been observed as degradation product of HBCD in household dust[13]. Figure 1 shows the parent HBCD stereoisomers and the general structures of the

investigated PBCD. It should be noted that the absolute configurations of the enantiomers of α-, β-, and γ-HBCD were only recently correlated with the order of chromatographic elution and sense of optical rotation[22]. In this study all possible allylic PBCD structures derived from both respective enantiomers of these three diastereomers α-, β-, and γ-HBCD were included. The formation of allyl bromide is proposed in[12] and considered more likely than that of vinylic structures, which were therefore not included. Due to the existence of a C_2 symmetry in case of α- and γ-HBCD the double bond formations on C_1-C_{12} and C_2-C_3, C_4-C_5 and C_6-C_7, C_8-C_9 and C_{10}-C_{11}, respectively, lead to the same PBCD isomer. The hTTR protein was investigated with regard to binding site I, originally occupied with the thyroxine residue het128 of the respective crystal structure 1ICT. The second thyroxine binding site was omitted in this work due to sterical reasons since this site was strongly buried by side chains of hTTR. Figure 2 displays the consecutive steps taken in order to compare the binding affinity of the PBCD, HBCD, andBDE structures with that of the natural binder thyroxine.

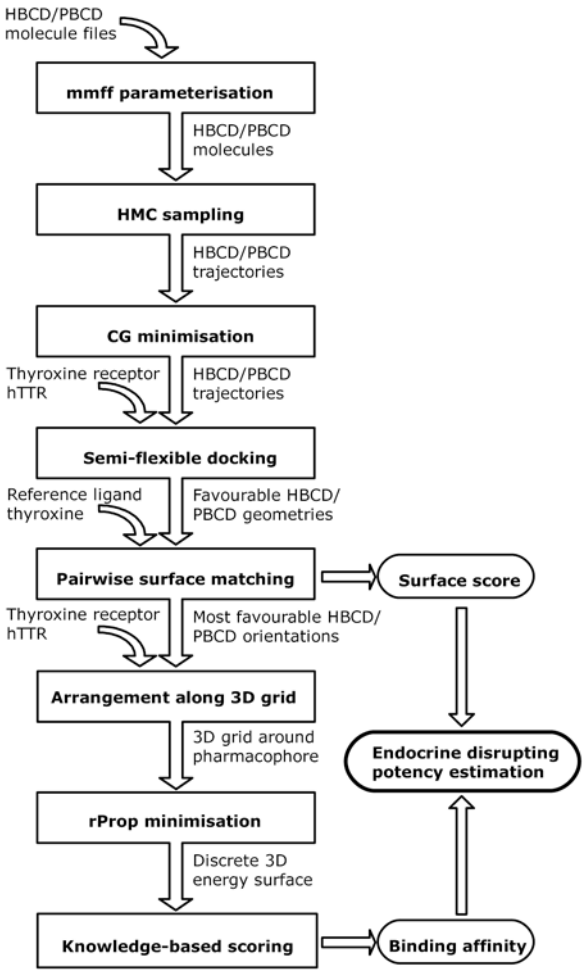

Figure 2: Flow diagram of the steps taken in this work in order to estimate the binding affinity of HBCDs, PBCDs and BDEs to hTTR.

Surface alignment scores as shown in table 1 (PBCD) and table 2 (all other ligands) and were scaled between 0 for the lowest score and 1 in case of thyroxin itself resulting in the values listed in the columns labelled "relative score". With 1.12 and 1.26, only BDE-47 and 3-hydroxy-BDE-47, respectively, yielded higher surface scores than the natural binder thyroxine itself. See figure 3 for the alignment of BDE-OH with thyroxine. Most iodine atoms of 3-hydroxy-BDE-47 are well mapped on bromine atoms, oxygens and aromatic structures are well aligned as well.

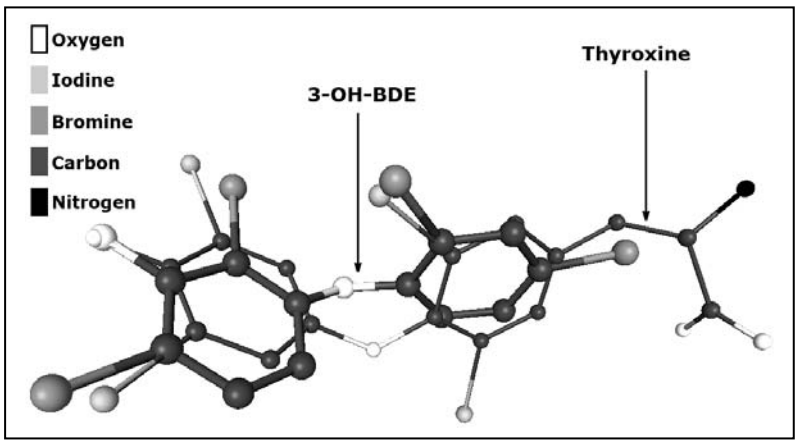

Figure 3: Surface alignment of 3-hydroxy-BDE-47 with thyroxine yielding the highest score of all ligands.

The considerably lowest scores ranging from 0.0 to 0.32 were computed for the HBCD diastereomers which might presumably be caused by the number of six bromine atoms that cannot all be matched. PBCDs were scored within a range from 0.3 to 0.88 and among the 12 highest surface matching scores are nine trans-configurations. The PBCDs with scores greater than the arbitrarily chosen cut off at 0.7 were selected for the estimation of free binding energies. They are presented in the last two columns of tables 1 and 2 together with their rms distances to the natural binding mode. Low binding scores correlate with low energy differences and with high binding affinities. Again, with 61.7 and -67.6, respectively, BDE-47 and 3-hydroxy-BDE-47 achieved slightly better scores than thyroxine, supporting results from the surface matching and from other simulations[23]. Similarly high affinities were computed for the PBCD isomers mta_89 (-63.4) and mtg_89 (-66.8).

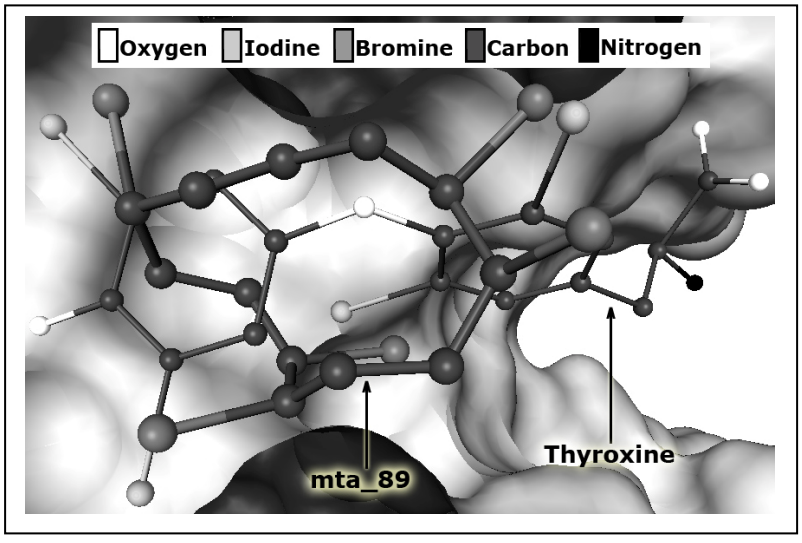

Figure 4: Binding mode of PBCD mta_89 proposed by the combination of surface matching and estimation of binding affinity shown together with the original thyroxine residue within binding site I of the hTTR crystal structure 1ICT. (visualised with the software amira.)

Table 1: List of investigated 3,6,7,10,11-pentabromocyclododeca-(1)-ene stereoisomers (PBCD) with exact stereochemistry, surface matching, and binding affinity parameters.

Original HBCD[a]		Resulting PBCD		Surface matching	Binding affinity[e]	
Isomer	Double bond at	CIP stereochemistry[b]	Acronym[c]	Relative score[d]	Score	rmsd
(-)-α	C_1–C_{12} / C_2–C_3	1Z,3R,6S,7R,10R,11S	mca_23	0.75	-28.4	5.2
(-)-β	C_6–C_7 / C_8–C_9	1E,3S,6R,7R,10S,11R	mta_89	0.88	-63.4	0.68
(-)-γ	C_{10}–C_{11}	1E,3S,6R,7S,10R,11R	mtb_1011	0.77	-13.6	1.46
(-)-α	C_{10}–C_{11}	1Z,3S,6R,7S,10R,11R	mcb_1011	0.71	-21.6	0.71
(-)-β	C_6–C_7 / C_8–C_9	1E,3R,6R,7R,10R,11S	mtg_89	0.78	-66.8	0.72
(+)-γ	C_1–C_{12} / C_2–C_3	1E,3S,6R,7S,10S,11R	pta_23	0.81	-44.0	1.0

[a] Parent HBCD stereoisomer and position of HBr elimination
[b] Denomination of absolute stereochemistry according to the Cahn-Ingold-Prelog rules
[c] Short version of stereoisomer assignment
[d] Surface matching relative to thyroxine
[e] Binding affinity (free binding energy/Gibbs energy) to binding site I of hTTR

Table 2: List of investigated 1,2,5,6,9,10-hexabromocyclododecaene stereoisomers (HBCD), two BDE variants and thyroxine itself with surface matching and binding affinity parameters.

Ligand	Surface Matching	Binding affinity	
	Relative score	Score	rmsd
(-)-α-HBCD	0.07	-	-
(-)-β-HBCD	0.32	-	-
(-)-γ-HBCD	0.00	-34.7	0.87
(+)-α-HBCD	0.26	-	-
(+)-β-HBCD	0.11	-38.5	0.6
(+)-γ-HBCD	0.12	-	-
BDE-47	1.12	-61.7	1.79
3-OH-BDE-47	1.26	-67.6	0.88
Thyroxine	1.00	-61.1	0.5

Hence, together with BDE-47 and 3-hydroxy-BDE-47, both PBCD candidates raise strong evidence of endocrine disrupting potency. It should be noted that the two trans-structures are respectively derived from γ-HBCD, which predominates the technical mixture, and from α-HBCD, which was observed to be by far the predominant HBCD diastereomer in the biotic environment[2,3,4,24] and the dominating one after thermal isomeridation[24,25,26,27]. The binding mode of mta_89, which yields the highest score within hTTR, is depicted in figure 4 together with the natural binder thyroxine in its original mode retrieved from the crystal structure. Bromine atoms are well mapped on iodine atoms. Also sterically, mta_89 fits well fit into the binding pocket which was visualised by applying a cutting plane to the molecular surface of hTTR.

According to the results presented here, the PBCD isomers display substantially higher affinity to hTTR than HBCD stereoisomers. We suppose that this is due to the lower number of bromine atoms in case of PBCD. Further investigations in this field should explicitly include the tetrabromocyclododecenes since they lack another bromine atom and are therefore likely have a higher binding affinity to thyroidal binding site.

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