A Novel Approach to the Molecular Imprinting of Polychlorinated Aromatic Compounds

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Abstract: The aim of this investigation was to determine whether relatively weak interactions, such as hydrogen bonds to aromatic chlorine atoms and interactions involving aromatic π electrons could be exploited within artificial receptors, constructed using the technique of molecular imprinting. For the purposes of this investigation we chose 2,3,7,8-tetrachlorodibenzodioxin (TCDD) as the model target. Imprinted polymers have been prepared with two new templates designed to create recognition sites for TCDD. The first of these, the bis-*N*-(4-vinylphenyl)urea derivative of 2,8-dichloro-3,7-diaminodibenzodioxin, employed a carbonyl spacer to introduce aromatic amines into the polymer after reductive cleavage of the template. The second, *N*-(2-(3,7,8-trichlorodibenzodioxinyl))-2-methacryloyloxybenzamide, incorporated a salicylic acid spacer and introduced a methacrylic acid residue into the polymer following hydrolysis. Both amine and acid groups were positioned in such a way as to interact with TCDD through the formation of weak hydrogen bonds to aromatic chlorine atoms. A second recognition element was introduced into the binding sites by the inclusion of a polymerizable, electron-rich, aromatic ether capable of forming $\pi-\pi$ interactions with the electron-deficient dioxin molecule. Polymers imprinted with either template showed significantly higher uptake of TCDD than the corresponding nonimprinted controls, even at concentrations as low as 2 nM.

Introduction

Molecular imprinting has emerged as a powerful technique for the creation of recognition elements in highly cross-linked polymeric matrices.[1] Generally this methodology is based on the simple but elegant principle of using the functionality of a target molecule (template) to assemble its own recognition site by forming interactions with "complementary" functional groups of appropriate polymerizable monomers. These interactions are then "frozen in" by polymerization, carried out in the presence of a high concentration of cross-linker. Subsequent removal of the template creates the polymer's binding site with the precise spatial arrangement of functional groups to ensure highly selective recognition of the target molecule. Most of the recent work in this area has focused on the preparation of imprinted polymers for chiral resolutions,[2] but numerous other applications in the design of catalysts[3] and sensors,[4] in solid-phase extraction,[5] removal of undesired components from complex mixtures,[6] and separation or concentration of proteins[7] and whole cells[8] have also been described.

The majority of polymers for these applications have been prepared using the noncovalent imprinting methodology where the template−monomer complex is formed in situ by association of the target molecule with relatively simple functional monomers such as methacrylic acid, acrylamide,[9] vinylpyridines,[10] and others[11] present in the polymerization mixture. However, to achieve efficient complexation before and during polymerization, the compound of interest must contain suitable functionality to provide sufficiently strong interactions with the constituent monomers.[12] As a result highly functionalized molecules, typically containing multiple carboxy, amino, amido, and keto groups, remain the most popular targets for imprinting.

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Clearly the scope of this methodology would be widened significantly if it was possible to exploit other chemical features of the template molecule such as the ability to form very weak hydrogen bonds, which are unlikely to be formed spontaneously by self-association with conventional monomers, and/or to engage in interactions involving aromatic π electrons. To achieve this, novel functional monomers have to be designed and an appropriate method for their introduction into the polymer's recognition sites developed. The main objective of this investigation was to demonstrate the feasibility of the preparation of imprinted polymers, utilizing recognition elements based on such interactions.

Polychlorinated aromatic compounds would be particularly attractive models for the purpose of this investigation on account of two structural features, namely the presence of aromatic chlorine atoms which are known to form exceedingly weak hydrogen bonds in nonpolar solvents[13] and an aromatic π electron system which can interact with that of other aromatic molecules.[14] In addition, many polychlorinated aromatic compounds (PCDDs, PCDFs, PCBs) are presently of great environmental concern, and the development of a generic approach to the preparation of polymers specific to these molecules would be of considerable practical value. In particular, 2,3,7,8-tetrachlorodibenzodioxin (TCDD) was chosen as a representative target compound, because it is the most prominent member of a large family of polychlorinated dibenzodioxins which are currently the subject of extensive monitoring[15] due to their extraordinary toxicity toward mammals.[16]

Results and Discussion

To achieve specific recognition of TCDD both the aromatic π electron system and the chlorine atoms must be engaged in interactions with functional groups forming the binding site of an imprinted polymer. This posed two immediate questions: what functionality would be most suitable for the recognition and how might it be introduced into the binding sites? As hydrogen-bonded complexes between aromatic chlorine atoms of TCDD and conventional monomers acting as hydrogen bond donors in noncovalent imprinting (e.g., methacrylic acid, acrylamide) were probably too weak to enable efficient complexation of the template in the polymerization mixture, we employed a modification of the "sacrificial spacer" methodology recently developed in our laboratory.[17] For this approach to work a TCDD analogue was required, covalently linked to polymerizable monomer(s) such that hydrogen bond donors would be generated in the recognition site by the chemical treatment used to remove the template. To satisfy these requirements, 2,8-diamino-3,7-dichlorodibenzodioxin (**1**) was prepared as described in the Experimental Section and reacted with 2 mol equiv of 4-vinylphenylisocyanate to give the diurea template **2**, where the two urea bridges were designed to act as sacrificial spacers (Scheme 1A). Hydride reduction of the template after its incorporation into the polymer would release **1**, leaving two aromatic amines in the recognition site, positioned in such a way as to form hydrogen bonds with the chlorine atoms of TCDD subsequently bound to the polymer (Scheme 1B).

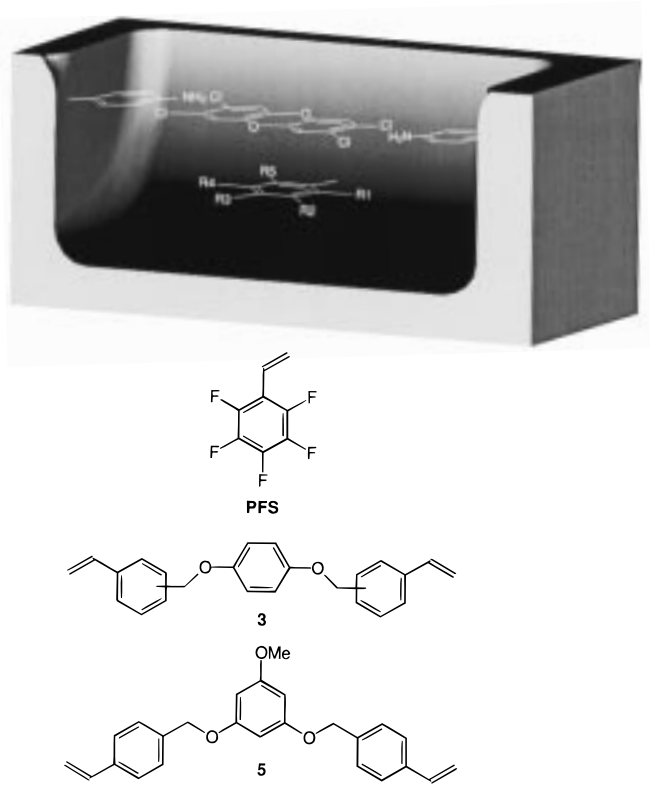

Figure 1. Schematic representation of the proposed binding site of polymers prepared with template **2**. Urea-derived amine groups are shown interacting with the aromatic chlorine atoms of TCDD. The position of the electron-rich or electron-deficient aromatic comonomer in the site is also shown along with the structures of pentafluorostyrene (**PFS**), 1,4-bis(3/4-vinylbenzyloxy)benzene (**3**), and 1-methoxy-3,5-bis-(4-vinylbenzyloxy)benzene (**5**).

The aromatic π electron system of TCDD was targeted through noncovalent interactions. To this end, several aromatic comonomers, capable of forming $\pi-\pi$ interactions with the template at the stage of polymerization, were prepared and tested. The electron-deficient 2,3,4,5,6-pentafluorostyrene (**PFS**) and the electron rich 1,4-bis(3/4-vinylbenzoxy)benzene (**3**) were used in the initial experiments. As a consequence of the template structure and the expected $\pi-\pi$ complex between the diurea template and the monomer, the designed recognition site would be "equipped" with two aromatic amino groups situated on both sides of the cavity, coplanar with the bound TCDD and the charge transfer monomer forming the "floor" of the imprint, as illustrated schematically in Figure 1. Although the individual interactions between these functional groups and TCDD are weak, in combination they should lead to the formation of specific recognition sites.

All polymers were prepared in bulk, using azobisisobutyronitrile (AIBN) as radical initiator and divinylbenzene (DVB) as cross-linking monomer (Table 1). DVB was used in preference to other commonly employed cross-linkers such as ethyleneglycol dimethacrylate because its polymers would be

(12) This is not crucial for covalent imprinting,[1a,b] but the arsenal of suitable monomers is currently limited since relatively few functional groups are capable of forming reversible covalent bonds, e.g., boronate esters, Schiff bases, or ketals.

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(15) A highly sensitive aqueous antibody-based assay (ELISA) for TCDD has recently been developed.See: (a) Sanborn, J. R.; Gee, S. J.; Gilman, S. D.; Sugawara, Y.; Jones, A. D.; Rogers, J.; Szurdoki, F.; Stanker, L. H.; Stoutamire, D. W.; Hammock, B. D. *J. Agric. Food Chem.* **1998**, *46*, 2407−2416. (b) Sugawara, Y.; Gee, S. J.; Sanborn, J. R.; Gilman, S. D.; Hammock, B. D. *Anal. Chem.* **1998**, *70*, 1092−1099.) However, TCDD and its analogues are very poorly soluble in water, hence such an assay may have limited applicability. There is therefore still a need to develop materials specific to dioxins which are capable of working in organic solvents.

(16) Safe, S. H. *Annu. Rev. Pharmacol. Toxicol.* **1986**, *26*, 371−399.

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Scheme 1. (A) Synthesis of the Diurea Template **2** and (B) Schematic Representation of the Preparation of Polymers Imprinted with Template **2** Showing the Positioning of Aromatic Amine Groups in the Recognition Site via the Urea Functionality (incorporating a carbonyl spacer)

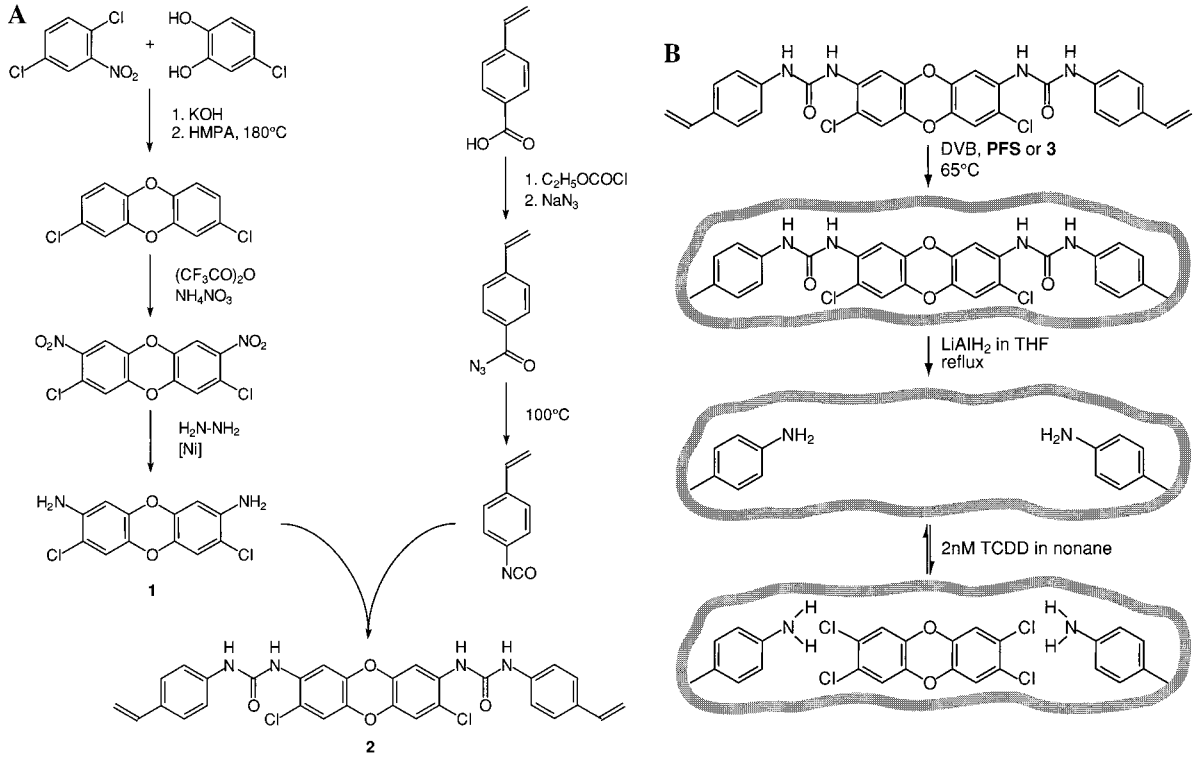


Table 1. Binding of TCDD (2 nM in Nonane) to Polymers Imprinted with Template **2** and Their Nonimprinted Counterparts[a]

polymer	cross-linker, DVB [mol %]	template (or functional monomer) [mol %]	co-monomer [mol %]	template removal [%]	specific surface area [m^2/g]	bound TCDD [pmol/g]
P1	99.5	**AS**, 0.5	none		149	5.72 ± 0.38
P2	94.5	**AS**, 0.5	**PFS**, 5		106	5.22 ± 0.48
P3	94.5	**AS**, 0.5	**3**, 5		242	7.50 ± 0.95
P4	99.5	**2**, 0.5	none	44	149	6.55 ± 0.24
P5	94.5	**2**, 0.5	**PFS**, 5	26	143	6.37 ± 0.46
P6	94.5	**2**, 0.5	**3**, 5	43	278	9.30 ± 0.82

[a] All polymers were prepared with DMSO as the porogenic solvent.

more likely to withstand the drastic conditions necessary for template removal. DMSO was used as the porogen since it was the only solvent among those tested to dissolve **2** sufficiently well. Nonimprinted polymers **P1**−**P3** were prepared in exactly the same way as **2**-imprinted polymers **P4**−**P6**, except that the template was replaced by 4-aminostyrene (**AS**). The amount of **AS** was chosen such that the density of amino groups incorporated into the polymer would be similar to that of the imprinted polymers **P4**−**P6** after removal of the template. Thus, the chemical compositions of the hydrolyzed imprinted and control polymers were essentially the same, the only difference being the distribution of the recognition elements in the polymer matrix (preassembled versus random, respectively). Hence an increase in the binding of TCDD, if observed, would be due to imprinting, as opposed to nonspecific adsorption to the polymer surface. Both sets of polymers were subjected to the same template removal procedure, which involved treatment with $LiAlH_4$ in refluxing THF.[18] Nonimprinted polymers were included to account for any effects the template removal procedure might have on the polymer matrix itself. The template removal in polymers imprinted with **2** was quantified by determination of the chlorine content of the polymers before and after the reduction and was typically about 50%.[19]

The binding of TCDD to polymers was assessed in batch experiments, where 10 mg of polymer were incubated for 24 h with 0.5 mL of anhydrous nonane containing 1 pmol (10^{-12} mol) of [1,6-$^3H_2$]-TCDD,[20] after which time the remaining activity in the filtrate was measured by scintillation counting. All uptake measurements were done in 5 replicates. The amounts of TCDD bound per gram of polymer for **P1**−**P6** are presented in Table 1, along with the specific surface areas of each polymer. Entries for polymers **P1**−**P3** show the extent of nonspecific binding, i.e., binding that occurs "outside" an imprinted recognition site. Binding to **P2** was considerably lower than that to **P3**, which reflects the higher electron-donating strength of **3** over **PFS**. **P1**, prepared in the absence of comonomer, shows similar binding to **P2**. These results are suggestive of the formation of $\pi-\pi$ interactions between TCDD and elements of the polymer surface, with TCDD acting as an electron

(18) Hydride reduction has previously been used to cleave the template from DVB-based polymers imprinted with sterol methacrylate esters. See: Byström, S. E.; Börje, A.; Akermark, B. *J. Am. Chem. Soc.* **1993**, *115*, 2081−2083.

(19) As the degree of cleavage was around 50% for both templates, nonimprinted polymers were prepared with either 0.5 mol % 4-aminostyrene for comparison with template **2** or 0.25 mol % methacrylic acid in the case of **4** (see Tables 1 and 2).

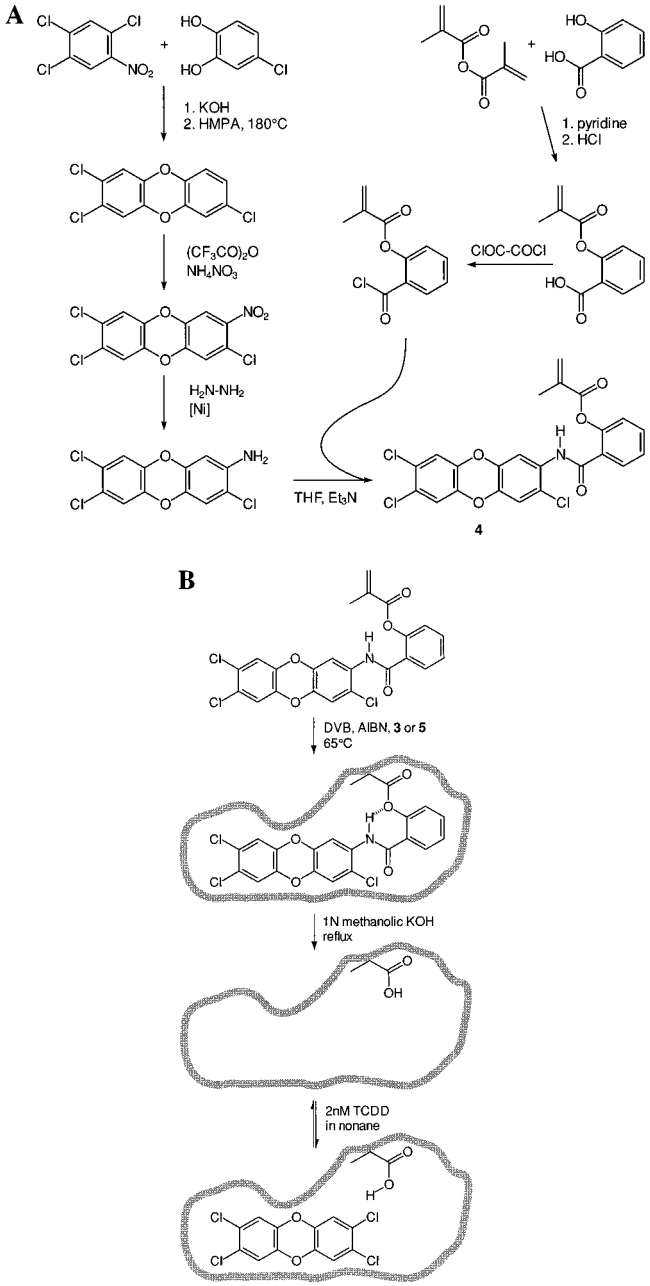
(20) The concentration of TCDD used in uptake experiments (2 nM) was chosen to represent that typically encountered in the analysis of extracts from food and environmental samples.

acceptor.[21] Increased binding on imprinting, and thus specific interactions, was observed when comparing the imprinted polymers **P4**−**P6** with their nonimprinted counterparts **P1**−**P3**. The ratio of binding between imprinted and nonimprinted polymer was 1.15 in the case of the polymers **P4**/**P1**, prepared without comonomer, 1.22 for the polymers which contained the electron acceptor **PFS** (**P5**/**P2**) and 1.24 for **P6**/**P3** (which incorporated **3**). This suggests that the effect of the hydrogen-bonding structures in the recognition sites is similar irrespective of the nature of the comonomer. The overall binding was highest in the case of **P6**.

To assess further the contribution of weak hydrogen bonding to the recognition of TCDD, we designed and prepared another template, amide **4**, by reacting 2-amino-3,7,8-trichlorodibenzo-dioxin[22] with 2-methacryloyloxybenzoyl chloride (Scheme 2A). In this template, salicylic acid fulfils the role of "sacrificial spacer", maintaining the spatial relationship between the meth-acrylate oxygen atom and the amide template's −NH− group by means of highly favored intramolecular hydrogen bonding. Evidence for this conformation comes from NMR and infrared studies on a model compound.[23] The 2-methacryloyloxyben-zamide group is the first example of a "smart sacrificial spacer" which, in effect, incorporates a captive noncovalent imprint. This approach should be especially suited to the imprinting of templates which contain primary amino groups, which will be discussed in a later paper.[24] After hydrolysis of the template, the methacrylate ester-derived carboxylic acid in the recognition site should be well situated to interact with a chlorine atom of a bound TCDD molecule (Scheme 2B). The imprinted site produced by using this template should differ in a number of key aspects from those arising from template **2**: The sites contain one carboxyl rather than two amino groups; there will be an additional void associated with the site due to the presence of a larger spacer group. In addition, the trichloro-template **4**, being more electron deficient than the dichloro-derivative **2**, should form stronger $\pi-\pi$ interactions which should increase the likelihood of incorporating monomers such as **3** into the recognition site.

As well as preparing polymers imprinted with template **4**, we also investigated what effect the inclusion of a new comonomer with stronger electron-donating properties would have on TCDD binding. As increasing the number of alkoxy groups on the central benzene ring from two to three was expected to yield a better electron donor than **3**, 1-methoxy-3,5-bis(4-vinylbenzyloxy)benzene (**5**) was synthesized and compared to the other comonomers. The results for nonimprinted polymers (Table 2) again showed that the incorporation of **PFS** gave the lowest TCDD uptake, lower even than that for polymers prepared without comonomer. On the other hand, copolymerization with **3**, and especially **5**, significantly increased TCDD binding from 7.62 to 8.70 and 10.78 pmol/g, respectively (**P7**−**P11**). These results confirmed that TCDD binding is indeed positively correlated with the electron-donating property of the comonomer. Consequently, **5** was used in preference to **3** for the preparation of polymers imprinted with template **4**. Template removal was accomplished by refluxing the polymers in methanolic KOH, and the resulting polymer-bound potassium carboxylate functions were converted into the protonated from by washing with dilute sulfuric acid. The extent of template removal in the imprinted polymers **P12**−**P15** was again determined by chlorine microanalysis and was found to

Scheme 2. (A) Synthesis of the Amide Template **4** and (B) Schematic Representation of the Preparation of Polymers Imprinted with Template **4**, Incorporating a Salicylic Acid "Smart Spacer". Intramolecular Hydrogen Bonding Assists in the Prearrangement of Functional Groups Which Will Interact on Rebinding



(21) It was not possible to predict the strength of association between the dioxin templates and the charge-transfer monomers due to the lack of published data on such systems. However, ref 14a attributes retention of TCDD on a 1-pyreneethyl bonded silica stationary phase in HPLC to the formation of charge-transfer complexes but no such retention occurred with electron-deficient bonded phases. This reflects the relative binding strengths of polymers incorporating **PFS** or aromatic ether comonomers and confirms that TCDD is acting as a π-electron acceptor. In principle it could have been possible to calculate K_a for the pyrene−TCDD couple from the published capacity factors if some additional parameters (column void volume and pyrene content) were provided by the authors.

(22) Kennel, S. J.; Jason, C.; Albro, P. W.; Mason, G.; Safe, S. H. *Toxicol. Appl. Pharmacol.* **1986**, *82*, 256−263.

(23) Infrared data on *N*-(4-hexadecylphenyl)-2-methacryloyloxybenza-mide in cyclohexane solution shows no shift for the N−H band at 3434 cm^{-1} in a dilution series from 10 to 0.3 mM, suggesting the absence of intermolecular hydrogen bonding. The ^1H NMR signal for the amide proton (10 mM solution in d_{12}-cyclohexane) shows a temperature-dependent shift from 8.03 (20 °C) to 7.87 ppm (60 °C), resulting from increasing dissociation of the intramolecular hydrogen bond.

(24) Klein, J.-U.; Whitcombe, M. J.; Mulholland, F.; Vulfson, E. N. In preparation.

Table 2. Binding of TCDD (2 nM in Nonane) to Polymers Imprinted with Template **4** and Their Nonimprinted Counterparts

polymer	porogen	cross-linker, DVB [mol %]	template (or functional monomer) [mol %]	comonomer [mol %]	template removal [%]	specific surface area [m^2/g]	bound TCDD [pmol/g]
P7	THF	99.75	**MAA**, 0.25	none		556	7.62 ± 0.63
P8	DMSO	99.75	**MAA**, 0.25	none		144	5.23 ± 0.35
P9	THF	94.75	**MAA**, 0.25	**PFS**, 5		478	6.59 ± 0.49
P10	THF	94.75	**MAA**, 0.25	**3**, 5		439	8.70 ± 0.36
P11	THF	94.75	**MAA**, 0.25	**5**, 5		400	10.78 ± 0.57
P12	THF	99.5	**4**, 0.5	none	81	497	9.17 ± 0.46
P13	DMSO	99.5	**4**, 0.5	none	40	149	6.40 ± 0.56
P14	THF	94.5	**4**, 0.5	**PFS**, 5	ND	326	7.61 ± 0.42
P15	THF	94.5	**4**, 0.5	**5**, 5	62	404	11.98 ± 0.32

Table 3. Effect of Solvent on the Binding of TCDD to Polymers Imprinted with Template **4**

	bound TCDD [pmol/g]		
polymer	nonane	acetonitrile	methanol
P7	7.62 ± 0.63	23.15 ± 0.31	64.76 ± 0.39
P12	9.17 ± 0.46	25.81 ± 0.62	68.84 ± 0.38

be typically around 50%. As before, nonimprinted polymers (identical but for the inclusion of methacrylic acid (**MAA**) in place of the template[19]) were hydrolyzed under the same conditions as the imprinted polymers. Binding of TCDD to polymers **P7**−**P15** was assessed as described above.

The increased solubility of **4** over **2** allowed us to compare the properties of polymers prepared in two polymerization solvents, namely THF and DMSO. The nature of the porogen has a marked influence on the morphology of the polymer. The use of THF resulted in very high surface area polymers (326−556 $m^2\ g^{-1}$, Table 2), which showed increased nonspecific binding over the same polymers prepared in DMSO (106−278 $m^2\ g^{-1}$, Tables 1 and 2). Specific binding for this particular template and cross-linker seems to be largely independent of the porogen used. The ratio of binding to imprinted over nonimprinted polymers is virtually identical for **P12**/**P7** (prepared in THF) and **P13**/**P8** (prepared in DMSO). Similar results were obtained for polymers prepared in the presence of π-electron-donating comonomers.

As with template **2**, polymers imprinted with **4** showed significantly higher uptake of TCDD compared to their nonimprinted counterparts. Binding increased by a factor of 1.20, 1.15, and 1.11 for **P12**/**P7**, **P14**/**P9**, and **P15**/**P11**, respectively. It is interesting to note that the increase in TCDD binding achieved with template **4** was slightly lower than that observed with analogous polymers imprinted with **2**. This is probably due to the formation of only one weak hydrogen bond on binding of TCDD into the recognition site as compared with two in the latter case. However, the difference is clearly too small to draw any definite conclusions. In addition, the two sites were equipped with different functional groups, carboxylic acid and aromatic amine, respectively. To gain further insight into the contribution of hydrogen bonding to the overall binding, the uptake of TCDD by **2**-imprinted polymer **P12** and the corresponding control polymer **P7** was tested in a number of solvents. It was expected that replacement of nonane with solvents capable of competing for hydrogen bonding with TCDD should suppress the specific component of binding. This indeed proved to be the case with the **P12**/**P7** binding ratio dropping from 1.20 in nonane to 1.06 in methanol. A significant reduction, in relative terms, was also observed in acetonitrile. In both polar solvents, uptake of the imprinted polymer remained higher by a small, yet significant amount, compared to the nonimprinted control. The overall binding of TCDD is much higher in acetonitrile and methanol than in nonane, presumably due to the decreased solubility of TCDD, and thus a shift in the partition coefficient between solvent and polymer matrix in favor of the latter.

Conclusions

We have demonstrated that even very weak molecular interactions, such as hydrogen bonds involving aromatic chlorine atoms, can be successfully exploited in the binding of ligands to imprinted polymers. To introduce suitable hydrogen bond donating groups into the polymer recognition sites, two new "sacrificial spacer" strategies (which use covalent imprinting techniques to assemble sites which bind the target molecules in a noncovalent fashion) have been employed in the preparation of imprinted polymers. The first of these exploited the *N*-(4-vinyl)phenyl urea group and used a carbonyl spacer (previously used in the form of a carbonate ester to imprint cholesterol[17]). The second utilized a 2-methacryloyloxybenzamide in which salicylic acid acted as the spacer. This is the first example of a new class of "smart spacer" in which the relative positions of template and polymer functional groups are fixed by the presence of intramolecular hydrogen bonding in the template structure. Template removal of around 50% was achieved, either by reduction in the case of the urea or hydrolysis in the case of the amide, to produce imprinted sites bearing aromatic amine or carboxylic acid groups, respectively. Polymers imprinted with either template showed significantly higher uptake of TCDD than the corresponding nonimprinted controls, even at concentrations as low as 2 nM.[25] In addition, the inclusion of electron-rich aromatic monomers, designed to interact via $\pi-\pi$ interactions with electron-deficient aromatic templates, was also shown to enhance the binding of TCDD to highly cross-linked polymers. 1-Methoxy-3,5-bis(4-vinylbenzyloxy)benzene (**5**) was the most effective of the monomers investigated.

Experimental Section

Materials and Methods. 2,3,4,5,6-Pentafluorostyrene, vinylbenzyl chloride (mixture of 3- and 4-isomers), 4-vinylbenzyl chloride, 3,5-dihydroxyanisole, ethyl chloroformate, 2,5-dichloronitrobenzene, 2,4,5-trichloronitrobenzene, hydrazine hydrate, methacrylic anhydride, oxalyl chloride, 4-aminostyrene, methacrylic acid, divinylbenzene (technical grade, mixture of meta and para isomers, 55%), and anhydrous nonane were obtained from Aldrich. 4-Chlorocatechol was obtained from Fluorochem Ltd. AIBN, which was recrystallized from methanol, and anhydrous acetonitrile were purchased from Fluka. Anhydrous methanol was prepared by drying over 3 Å molecular sieve. [1,6-$^3H_2$]-2,3,7,8-Tetrachlorodibenzodioxin (specific activity 24.4 Ci/mmol) was obtained as a solution of 0.52 mCi/mL in toluene/methanol (96:4) from Greyhound Chromatography. 4-Vinylbenzoic acid was prepared from 4-bromostyrene according to a standard textbook procedure via the Grignard reagent. All other solvents and reagents were laboratory

(25) Unfortunately, it proved to be impossible to perform Scatchard analysis on these polymers with any reasonable degree of accuracy due to the relatively high nonspecific adsorption of the control materials.

reagent grade or better. FT-NMR spectra were obtained on a JEOL EX-270. GC-MS was performed on a Trio 1-S, direct inlet EI-MS on a Kratos MS890, and MALDI-TOF-MS on a Kratos Kompact Maldi 2, the sample being dispersed in 5-chloro-2-mercaptobenzothiazole. FT-IR spectra were recorded on a Perkin-Elmer Series 1600 by diffuse reflectance from samples dispersed in KBr, or from a liquid film between KBr disks. Polymers were ground in a Fritsch Pulverizette grinding mill, equipped with an agate mortar. Polymer suspensions were filtered through Whatman syringe filters with 0.2 μm PTFE membranes prior to scintillation counting. Scintillation counting was done on a Packard Tri-Carb 2700TR, using Zinsser Quicksafe A scintillation cocktail.

Synthesis of Templates and Monomers. 4-Vinylphenyl isocyanate was prepared by the Curtius rearrangement from the corresponding acyl azide. 4-Vinylbenzoic acid (0.085 mmol) was dissolved in 500 mL of acetone and cooled to 0 °C. Triethylamine (0.1 mol) in 40 mL of acetone was added, followed by the dropwise addition of a solution of 0.11 mol of ethyl chloroformate in 40 mL of acetone. After the solution was stirred for 30 min at 0 °C, 0.13 mol of sodium azide in 30 mL of water was added. After further stirring for 1 h, the mixture was poured into 400 mL of ice-cold water and extracted with 3 × 70 mL of cold toluene. The toluene extracts were dried in the freezer, first over $MgSO_4$, then over P_2O_5. The dried solution was dropped slowly into a flask suspended in a boiling water bath. After being heated for 1 h, the solvent was removed and the product obtained by distillation under reduced pressure as a colorless oil (52%), making sure to leave a small residue in the distillation flask. IR (cm^{-1}) 2270 (−NCO); ^{1}H NMR ($CDCl_3$) δ (ppm) 5.24 (dd, 1H, ^2J = 1 Hz, ^3J = 11 Hz, *cis*-C*H*$_2$=CH), 5.70 (dd, 1H, ^2J = 1 Hz, ^3J = 18 Hz, *trans*-C*H*$_2$=CH), 6.65 (dd, 2H, $J(Z)$ = 11 Hz, $J(E)$ = 18 Hz, *cis*-C*H*$_2$=CH), 7.03 (d, 2H, J = 9 Hz, arom CH), 7.34 (d, 2H, J = 9 Hz, arom CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 114.2 (H$_2$*C*=CH), 124.8, 127.3 (2×t), 132.6, 135.2 (2×q), 135.6 (H$_2$C=*C*H); GC-MS m/z (M^+) 145.

2,8-Dichloro-3,7-dinitrodibenzodioxin was obtained according to a modified protocol from Kennel et al.[22] 4-Chlorocatechol (20 mmol) was dissolved in 20 mL of 1 N methanolic KOH, the solvent was evaporated, and the residue dried under reduced pressure. 2,5-Dichloronitrobenzene (20 mmol), 1 g of K_2CO_3, and 30 mL of HMPA were added and the mixture was heated to 180 °C for 24 h. After the mixture was cooled, 50 mL of water was added. The resultant precipitate was filtered off and extracted with boiling dichloromethane. The extract was filtered and evaporated to dryness. The residue of evaporation was chromatographed on silica with petroleum ether. The first fraction was collected and yielded 1.23 g (24%) of a mixture of 2,7- and 2,8-dichlorodibenzodioxin. Recrystallization from carbon tetrachloride/petroleum ether yielded predominantly the 2,7-isomer. Recrystallization from methanol of the evaporated filtrate from the first recrystallization yielded 443 mg (9%) of pure 2,8-dichlorodibenzodioxin. ^{1}H NMR ($CDCl_3$) δ (ppm) 6.74 (dd, 1H, ^2J = 1 Hz, ^3J = 8 Hz), 6.9 (m, 2H); ^{13}C NMR[26] ($CDCl_3$) δ (ppm) 116.8, 117.2, 124.0 (3×t); GC-MS m/z (M^+) 252. The intermediate 2,8-dichlorodibenzodioxin (1.75 mmol), 18 mL of nitromethane, and 6 mL of trifluoroacetic anhydride were cooled in an ice bath. Ammonium nitrate (4.3 mmol) was added slowly while the mixture was stirred. On completion of the reaction, 6 mL of methanol was added and the product was filtered off. Recrystallization from chloroform yielded 443 mg (74%) of mustard yellow shiny plates, mp 245 °C (sublimation). IR (cm^{-1}) 3096 (C−H), 1530 (NO_2), 1478 (dioxin skeleton), 1341 (NO_2); ^{1}H NMR (d_8-THF) δ (ppm) 7.35, (s, 2H), 7.75 (s, 2H); ^{13}C NMR (d_8-THF) δ (ppm) 115.2, 120.1 (2×t), 123.7, 140.9, 145.0, 145.2 (4×q); EI-HRMS calcd for $C_{12}H_4N_2O_6Cl_2^+$ 341.9446, found 341.9451; isotope distribution calcd (found) 342 = 100 (100), 343 = 14.0 (14.6), 344 = 66.9 (64.6), 345 = 9.3 (8.7), 346 = 11.9 (11.1), 347 = 1.6 (1.6).

2,8-Dichloro-3,7-bis(*N*′-(4-vinylphenyl)ureido)dibenzodioxin (2). Ethanol (15 mL), 23 mL of THF, 1.5 mL of hydrazine hydrate, and 1.5 g of Raney nickel were heated to reflux and 0.61 mmol of 2,8-dichloro-3,7-dinitrodibenzodioxin, dissolved in 17 mL of THF, was added dropwise. Another 1.5 mL of hydrazine hydrate was added, and on completion of the reaction the mixture was cooled and filtered. The filtrate was evaporated to yield crude 2,8-diamino-3,7-dichlorodibenzodioxin (**1**) as a white, readily oxidized solid. Under an atmosphere of nitrogen, 0.6 mmol of **1** was dissolved in 20 mL of boiling THF. 4-Vinylphenyl isocyanate (1.22 mmol) was added dropwise and refluxing was continued for 30 min. The mixture was allowed to cool and filtered. The filtrate was poured into water, and the precipitate was filtered off to yield 90 mg (26%) of an off-white solid, mp >300 °C dec. IR (cm^{-1}) 3308 (N−H), 1643, 1593, 1556 (3×NH−CO−NH), 1512 (dioxin skeleton); ^{1}H NMR (d_6-DMSO) δ (ppm) 5.12 (d, 2H, J = 11 Hz, *cis*-C*H*$_2$=CH), 5.69 (d, 2H, J = 18 Hz, *trans*-C*H*$_2$=CH), 6.65 (dd, 2H, $J(Z)$ = 11 Hz, $J(E)$ = 18 Hz, CH$_2$=C*H*), 7.18 (s, 2H, arom CH), 7.38, 7.43 (2×d, 2H, J = 9 Hz, arom CH), 7.82 (s, 2H, arom CH); ^{13}C NMR (d_6-DMSO) δ (ppm) 106.5, 109.0 (2×t), 112.1 (s), 116.4, (q), 118.1 (t), 124.9 (q), 126.7 (t), 131.2 (q), 136.2 (H$_2$C=*C*H), 139.0, 139.9, 140.7, (3×q) 152.3 (C=O). No molecular ion could be observed with EI- or FAB-MS; MALDI-ToF-MS showed a molecular ion at m/z = 572 (high-resolution mass measurement was not possible); EI-HRMS on the principal fragments: 1, calcd for $C_8H_9N^+$ 119.0735, found 119.0740; 2, calcd for $C_9H_9NO^+$ 145.0528, found 145.0517; 3, calcd for $C_{12}H_8N_2O_2Cl_2^+$ 281.9963, found 281.9965.

1,4-Bis(3/4-vinylbenzyloxy)benzene (3). Hydroquinone (10 g), 15 g of K_2CO_3, and 35 g of 3/4-vinylbenzyl chloride were heated to 70 °C in DMF and stirred for 1 h. The mixture was poured into water and extracted with diethyl ether. The organic extracts were washed with NaOH solution and water. After drying and evaporation the residue was recrystallized to yield **3** as a mixture of the three possible isomeric compounds (58%), mp 91−92 °C. IR (cm^{-1}) 3084 (=CH$_2$), 2907 (C−H), 2860 (OCH$_2$), 1509 (aryl), 1235 (C−O), 1022 (C−O); ^{1}H NMR ($CDCl_3$) δ (ppm) 4.99 (s, 4H, O−CH$_2$), 5.24, 5.26 (2×d, total integr. 2H, J = 11 Hz, *cis*-C*H*$_2$=CH), 5.75, 5.76 (2×d, total integr. 2H, J = 18 Hz, *trans*-C*H*$_2$=CH), 6.71, 6.72 (2×dd, total integr. 2H, $J(Z)$ = 11 Hz, $J(E)$ = 18 Hz, CH$_2$=C*H*), 6.90 (d, 4H, J = 3 Hz, arom CH), 7.4 (m, 8H, arom CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 70.4, 70.5 (2×O−CH$_2$), 114.0, 114.2 (2×H$_2$*C*=CH), 115.8 (*C*H=C−O), 136.4, 136.6 (2×H$_2$C=*C*H), 125.3, 125.7, 126.4, 126.9, 127.7, 128.7 (6×t), 136.8 137.2, 137.5, 137.8 (4×q), 153.1 (CH=*C*−O); EI-HRMS calcd for $C_{24}H_{22}O_3^+$ 342.1620, found 342.1620; isotope distribution calcd (found) 342 = 100 (100), 343 = 26.3 (27.3), 344 = 3.7 (4.0).

2-Methacryloyloxybenzoyl Chloride. Salicylic acid (25 g) was dissolved in 90 mL of pyridine, the solution was cooled in ice, and 33.5 g of methacrylic anhydride was added. The mixture was stirred overnight and then added to an excess of dilute HCl and crushed ice. The oily product was extracted with diethyl ether and the organic extracts were dried over $MgSO_4$ and evaporated. Pure 2-methacryloyloxybenzoic acid was obtained by repeated crystallization from hexane as colorless crystals, yield 42%; mp, IR, and ^{1}H NMR and in accordance with the literature.[27] The acid was placed in a 50 mL round-bottomed flask, fitted with a gas scrubber, and an excess of oxalyl chloride was added. The reaction was initiated by the addition of one drop of DMF. When gas production had ceased, the excess of oxalyl chloride was removed by applying a water-pump vacuum directly to the flask. The residue was dried under reduced pressure and the product was obtained by vacuum distillation as a yellowish oil (65%). IR (cm^{-1}) 1781 (Cl−C=O), 1743 (O−C=O), 1601, 1451 (2×aryl); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.07 (s, 3H, CH_3), 5.80 (s, 1H, *cis*-H$_3$C−C=C*H*$_2$), 6.37 (s, 1H, *trans*-H$_3$C−C=C*H*$_2$), 7.20 (dd, 1H, ^3J = 8 Hz, ^4J = 1 Hz, C-3), 7.39 (ddd, 1H, $^3J_{4,5}$ = 8 Hz, $^3J_{5,6}$ = 8 Hz, $^4J_{3,5}$ = 1 Hz, C-5), 7.67 (ddd, 1H, $^3J_{3,4}$ = 8 Hz, $^3J_{4,5}$ = 8 Hz, $^4J_{4,6}$ = 2 Hz, C-4), 8.21 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, C-6); ^{13}C NMR ($CDCl_3$) δ (ppm) 18.2 (p), 123.7(q, C-1), 124.2 (t, C-3), 126.3 (t, C-5), 128.2 (s), 134.1 (t, C-6), 135.0 (>*C*=CH$_2$), 135.9 (t, C-4), 150.4 (q, C-2), 164.3, 165.3 (2×C=O).

***N*-(2-(3,7,8-Trichlorodibenzodioxinyl))-2-methacryloyloxybenzamide (4).** 2-Amino-3,7,8-trichlorodibenzodioxin was obtained as a white solid by the reduction of the corresponding nitro compound (prepared by the published procedure,[22] see Scheme 2A) in a similar way as described above for 2,8-diamino-3,7-dichlorodibenzodioxin; GC-MS[22] m/z (M^+) 301. The crude amine (0.45 mmol) was dissolved in 7 mL

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of THF and 0.9 mmol of triethylamine. A solution of 0.45 mmol of 2-methacryloyloxybenzoyl chloride in 3 mL of THF/dichloromethane (6:4) was added dropwise. After stirring overnight under nitrogen, the mixture was filtered and the filtrate was evaporated. The evaporation residue was chromatographed on silica with diethyl ether/hexane (1:1). An intermediate fraction was collected, yielding 180 mg (82%) of an off-white solid, mp 177−179 °C. IR (cm^{-1}) 3394 (N−H), 3089 (=CH_2), 2963 (C−H), 1743 (O−C=O), 1681, 1529 (2×NH−C=O), 1493 (dioxin skeleton); ^{1}H NMR (d_8-THF) δ (ppm) 2.01 (s, 3H, CH_3), 5.79 (s, 1H, *cis*-$H_3C-C=CH_2$), 6.34 (s, 1H, *trans*-$H_3C-C=CH_2$), 6.82 (s, 1H, arom CH), 7.15, 7.17 (2×s, 1H, arom CH), 7.22 (dd, 1H, ^3J = 8 Hz, ^4J = 1 Hz, C-3), 7.36 (ddd, 1H, $^3J_{4,5}$ = 8 Hz, $^3J_{5,6}$ = 8 Hz, $^4J_{3,5}$ = 1 Hz, C-5), 7.54 (ddd, 1H, $^3J_{3,4}$ = 8 Hz, $^3J_{4,5}$ = 8 Hz, $^4J_{4,6}$ = 2 Hz, C-4), 7.83 (dd, 1H, ^3J = 8 Hz, ^4J = 2 Hz, C-6), 7.94 (s, 1H, arom CH); ^{13}C NMR (d_8-THF) δ (ppm) 18.5 (p), 112.3 (t, C-1′), 117.4 (t, C-4′), 118.6, 118.7 (2×t, C-5′, C-9′), 119.8 (q), 124.1 (t, C-3), 126.6 (t, C-5), 128.0 (s), 129.8 (q), 130.5 (t, C-6), 132.6 (q), 132.8 (t, C-4), 136.9 (>*C*=CH_2), 138.5, 140.7, 141.8, 141.9, 142.5, 142.8 (6×q), 149.8 (q, C-2), 164.4, 165.7 (2×C=O); EI−HRMS calcd for $C_{23}H_{14}NO_5Cl_3^+$ 488.9937, found 488.9930; isotope distribution calcd (found) 489 = 98.7 (100), 490 = 25.3 (27.7), 491 = 100 (99.6), 492 = 25.1 (26.0), 493 = 35.1 (34.9), 494 = 8.5 (8.5), 495 = 4.7 (4.5), 496 = 1.0 (1.0).

1-Methoxy-3,5-bis(4-vinylbenzyloxy)benzene (5). A solution of 4.8 mmol of 3,5-dihydroxyanisole in 9.6 mL of 1 N methanolic KOH was evaporated under a nitrogen atmosphere. The residue was dissolved in 10 mL of DMF, the solution was stirred and heated to 60 °C, and 11.6 mmol of 4-vinylbenzyl chloride was added. After 30 min, the mixture was poured into water and extracted with diethyl ether. The organic extract was washed with 5 N KOH, then with water, and evaporated. The residue was chromatographed on silica with diethyl ether/hexane (1:9). Collection of an intermediate fraction yielded 180 mg of a colorless oil (10%). IR (cm^{-1}) 3087 (=CH_2), 2954 (C−H), 2870 (OCH_2), 2845 (OCH_3), 1602 (C=C), 1197 (C−O); ^{1}H NMR ($CDCl_3$) δ (ppm) 3.75 (s, 3H, $O-CH_3$), 4.99 (s, 4H, $O-CH_2$), 5.26 (d, 2H, J = 11 Hz, *cis*-CH_2=CH), 5.76 (d, 2H, J = 18 Hz, *trans*-CH_2=CH), 6.17 (d, 2H, J = 2 Hz, arom CH), 6.23 (d, 1H, J = 2 Hz, arom CH), 6.72 (dd, 2H, $J(Z)$ = 11 Hz, $J(E)$ = 18 Hz, CH_2=C*H*), 7.36 (d, 4H, J = 8 Hz, arom CH), 7.42 (d, 4H, J = 8 Hz, arom CH); ^{13}C NMR ($CDCl_3$) δ (ppm) 55.3 ($O-CH_3$), 69.8 ($O-CH_2$), 94.0, 94.5 (2×*C*H=C−O), 114.1 (H_2C=CH), 126.4, 127.7 (2×t), 136.3 (q), 136.4 (H_2C=*C*H), 137.3 (q), 160.6, 161.4 (2×CH=*C*−O); EI-HRMS calcd for $C_{25}H_{24}O_3^+$ 372.1725, found 372.1729; isotope distribution calcd (found) 372 = 100 (100), 373 = 27.5 (28.2), 374 = 4.2 (4.7).

Polymer Synthesis. All polymers in this study were prepared according to the following protocol: technical grade divinylbenzene was washed free of inhibitor with KOH solution, washed with water, and dried. A 0.5 g sample of a mixture of the appropriate monomers, 0.5 mL of porogen, and 1 mol % (relative to double bonds) of the radical initiator AIBN were mixed in a test tube and the mixture was degassed by repeated freezing, evacuating, and thawing. The tube was sealed under vacuum and heated in a water bath at 65 °C for 24 h. The block of polymer was removed from the tube, washed with methanol, dried, and ground to an average particle size of 10 μm.

Template Removal Procedures. (a) Reduction of P1−P6 with $LiAlH_4$. A 250 mg portion of ground polymer was suspended in 25 mL of THF and 125 mg of $LiAlH_4$ was added. The mixture was refluxed for 2 h. After cooling, destroying the excess $LiAlH_4$ with water, and acidifying with H_2SO_4, the mixture was filtered and the filter residue was washed with 2% H_2SO_4, water, methanol, diethyl ether, hexane, diethyl ether, and methanol. To deprotonate the cavity-borne amino groups, the polymers were further washed with 4% ammonia in water/methanol (1:1) and dried.

(b) Base Hydrolysis of P7−P15. A 250 mg portion of ground polymer was refluxed in 25 mL of 1 N methanolic KOH for 6 h. After cooling and acidifying with H_2SO_4, the mixture was filtered and the filter residue was washed successively with water, methanol, diethyl ether, hexane, diethyl ether, and methanol and dried.

Determination of the Degree of Template Removal. Samples of imprinted polymers before and after template removal were subjected to oxygen flask combustion and quantification of chloride by ion chromatography.

Determination of the Specific Surface Area. Polymer surface areas were determined from multipoint N_2 adsorption isotherms and calculated by using the BET equation. Polymers were degassed in vacuo overnight at room temperature before measurement.

Assessment of Binding of Polymers toward TCDD. Individual samples of polymer, 10 ± 0.1 mg, were weighed into screw cap vials, incubated for 24 h with 0.5 mL of the appropriate solvent (either nonane, acetonitrile, or methanol; all solvents were anhydrous) containing 1 pmol of [1,6-$^3H_2$]-TCDD, and filtered. The TCDD concentration in the filtrate was determined by scintillation counting and gave, by subtraction from the initial TCDD concentration, the amount of bound TCDD per unit weight of polymer. All experiments were done in 5 replicates.

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