Selectivity Control by Chemical Modification of the Recognition Sites in Two-Point Binding Molecularly Imprinted Polymer

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ABSTRACT: We demonstrated the possibility of modifying the selectivity of a two-point binding imprinted polymer by chemical modification of the binding sites inside the cavities. We used a thermally reversible bond for the preparation of the monomer−template complex, which allowed us to remove the template easily by means of a simple thermal reaction and to simultaneously introduce various functional groups into the cavity. A phenylmaleimide having an azidocarbonyl group was reacted with diethylstilbestrol (DES, template) to yield a monomer, where the template was linked to two polymerizable maleimido groups via a thermally reversible urethane bond. The polymerization of the monomer was carried out in the presence of ethylene glycol dimethacrylate (EGDMA) by the initiation with 2,2-azobis(isobutyronitrile) (AIBN) at 54 °C in DMF. The polymers were refluxed in 1,4-dioxane in the presence of a nucleophile such as water, methanol, or aniline. In this extraction step, the template molecules were removed from the polymer matrix, and simultaneously the isocyanato groups, which were generated by the thermal cleavage of the urethane bond, were converted to amino, urethane, or urea groups through their reaction with water, methanol, or aniline, respectively. The specific recognition ability of the imprinted polymers for the template and its structural analogues was dependent on the space between the two binding points as well as on the nature of the functional group. This method is especially propitious for developing artificial receptors for molecules lacking strongly interactive groups.

Introduction

Molecular imprinting constitutes a valuable method of preparing polymeric materials with specific binding properties, which have potential uses in applications such as chemical sensors, microreactors mimicking enzymes, stationary phases for high-performance chromatography, catalysts, and membranes for separating toxic chemicals.[1−7] The most conspicuous merit of molecular imprinting is that structurally three-dimensional recognition sites can be introduced into a polymer matrix with ease and low cost when compared with the complicated process of biological system for antigen and antibody. In the molecular imprinting process, a target molecule (template) is first complexed with a functional monomer and then frozen into a matrix by polymerization. The formation of a stable complex before polymerization is crucial for the imprinted polymer to have high rebinding ability. So most templates reported so far have functional groups that react or interact strongly with a monomer.

In this work, we demonstrated the possibility of modifying the selectivity of an imprinted polymer by chemical modification of the binding sites inside the cavities. We used a thermally reversible urethane bond for the preparation of the monomer−template complex, which allowed us to remove the template easily by means of a simple thermal reaction and to simultaneously introduce various functional groups into the cavity. This method is especially propitious for developing artificial receptors for molecules lacking strongly interactive groups. Only a few examples have been reported of imprinted polymers recognizing molecules other than the template.[8] Whitcombe and co-workers prepared an imprinted polymer showing high selectivity for a molecule that could not normally form a stable complex with a monomer.[8a−c] They used a functionalized template which was structurally analogous to this molecule. The template was linked to a monomer through a sacrificial spacer. After polymerization, the template and the spacer were removed to create a recognition site for the poorly functionalized molecule. Recently, Zimmerman and co-workers reported on a monomolecularly imprinted dendrimer which showed better selectivity for its structural analogues than the porphyrin imprinted originally.[8d]

Several studies have shown that the major factors governing the recognition ability of an imprinted polymer are the shape of the cavities and the orientation of the functional groups situated inside them, with the latter considered to be predominant.[1a] For this reason, multipoint binding imprinted polymer shows superior performance to the one-point binding one. The arrangement of the functional groups inside the cavities is determined by the formation of a template−monomer complex and its subsequent polymerization. Since the functional groups generated by the removal of the template are directly attached to the polymer matrix, chemical modification under mild conditions does not change their orientation significantly, but it does have an effect on the amount of space available inside the cavities.

It is known that the urethane bond formed between an isocyanate and a phenol is stable at room temperature, but the reversible cleavage occurs at elevated temperatures.[9−12] Diethylstilbestrol (DES) having two terminal phenol groups was chosen as a template. A monomer with an isocyanato group can be attached to DES on both sides by means of a thermally reversible

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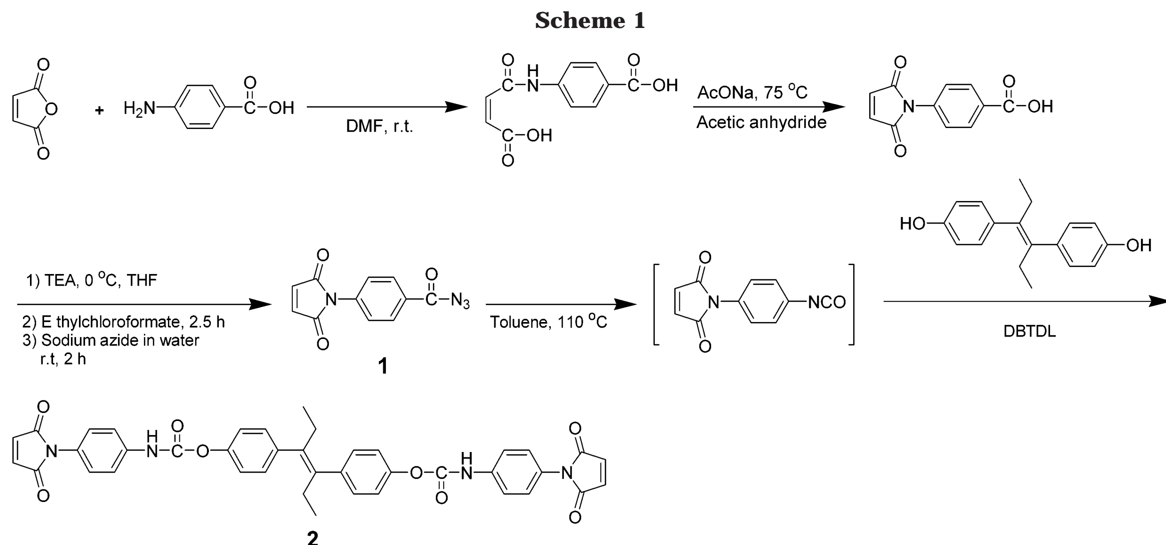
Scheme 1



urethane bond. The resulting monomer–template complex presents two point binding sites[13] into the polymer matrix after extraction of template molecules. Besides, the choice of DES as a template was motivated by environmental necessity of its detection. DES is a synthetic nonsteroidal estrogen, which is known for its ability to treat prostate cancer but which is suspected of being an endocrine disrupting chemical.[14]

Results and Discussion

Monomer Synthesis. Monomer **2** was prepared according to Scheme 1. Phenylmaleimide **1** having an azidocarbonyl group was converted to an isocyanate through Curtuis rearrangement by heating at 110 °C in toluene. Diethylstilbestrol was reacted with the isocyanate to yield monomer **2**, where the template was linked to two polymerizable groups via a thermally reversible urethane bond. The thermal cleavage of the urethane bond was investigated by ^{1}H NMR spectroscopy. Monomer **2** was dissolved in DMSO-d_6 containing moisture, and its ^{1}H NMR spectra were measured at various temperatures (Figure 1). The aromatic ring proton peaks appeared at 7.64, 7.31, and 7.29 ppm and the NH peak at 10.40 ppm at room temperature. The vinyl proton peak of a maleimido group showed up at 7.17 ppm. After increasing the sample temperature to 110 °C, the peak corresponding to the urethane group at 10.40 ppm completely disappeared, and the aromatic ring proton peaks corresponding to free DES appeared at 6.75 and 6.98 ppm. A peak corresponding to an amino group also showed up at 4.9 ppm, resulting from the reaction of an isocyanato group with the water present in DMSO-d_6 (Scheme 2).

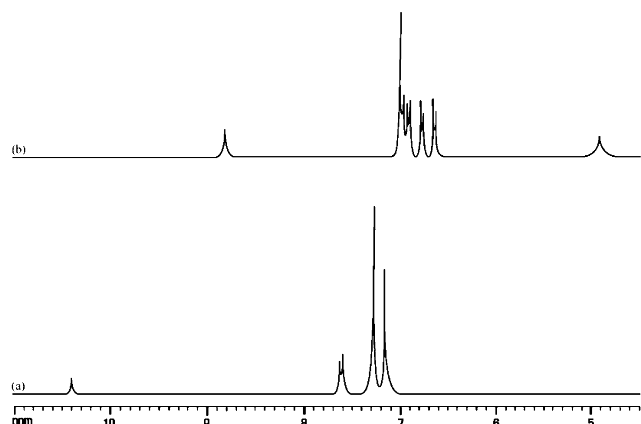
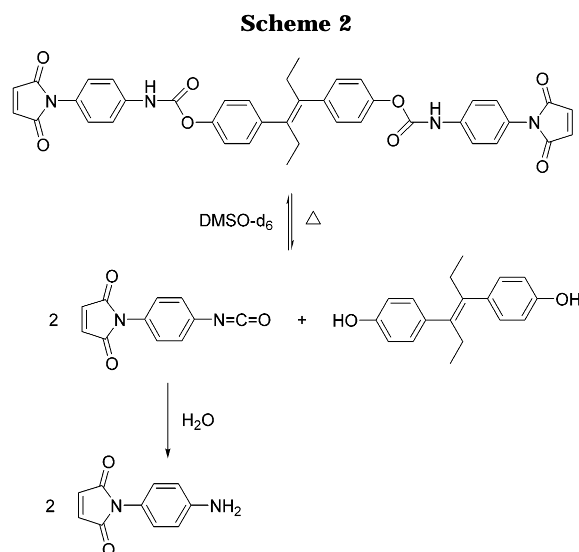


Figure 1. ^{1}H NMR spectra (DMSO-d_6 + moisture) of compound **2** obtained (a) at 25 °C and (b) at 110 °C.

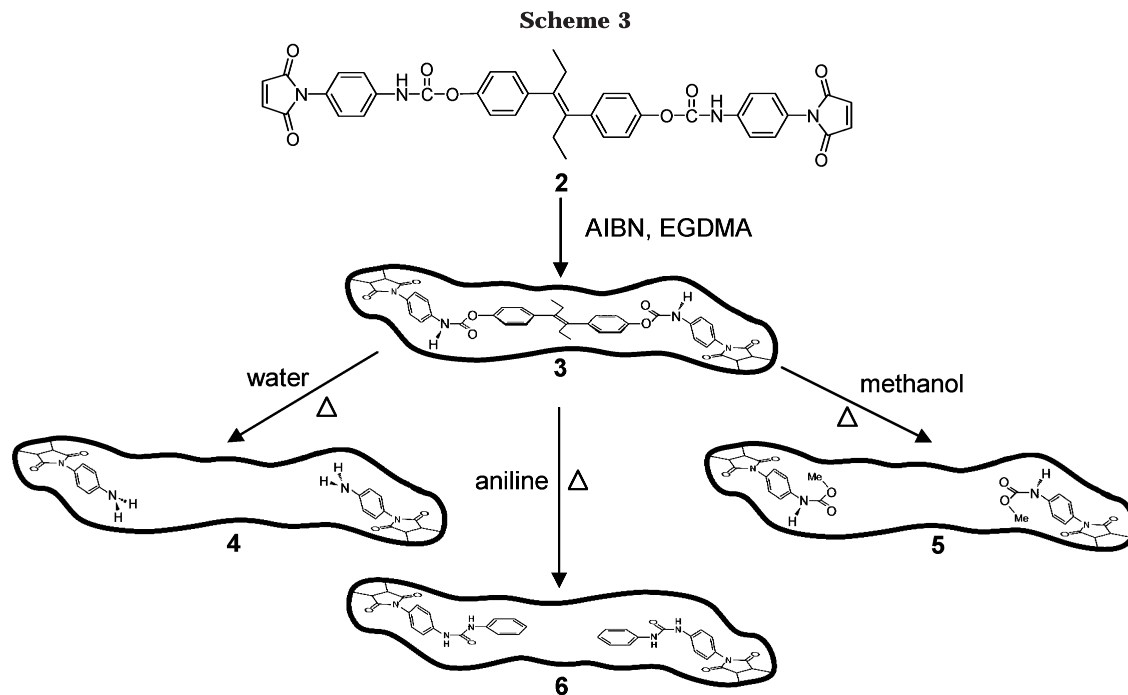
Scheme 2



Synthesis of Imprinted Polymer. The polymerization of monomer **2** was carried out in the presence of ethylene glycol dimethacrylate (EGDMA)[15] by the initiation with 2,2-azobis(isobutyronitrile) (AIBN) at 54 °C in DMF (Scheme 3).

To remove the template molecules from the polymer matrix, the polymer was refluxed in 1,4-dioxane in the presence of a nucleophile such as water, methanol, or aniline. In this extraction step, the template molecules were removed from the polymer matrix, and simultaneously the isocyanato groups, which were generated by the thermal cleavage of the urethane bond, were converted to amino, urethane, or urea groups through their reaction with water, methanol, or aniline, respectively. Extraction was monitored by UV–vis spectroscopy and continued until the absorption intensity for dissociated DES in the solvent reached the constant (Figure 2). The control polymer was also prepared following the same procedure as that used for the imprinted polymers, except that *N*-(4-acylazidophenyl)-maleimide (**1**) was used as a monomer in place of compound **2**.

Scheme 3



Solid-State ^{13}C CP-MAS NMR Analysis. The removal of the template and the resultant generation of the recognition site were investigated by solid-state ^{13}C NMR spectroscopy. In Figure 3, all of the strong peaks were assigned to the carbons of the cross-linker, by comparing them with the spectrum obtained from the polymerization of EGDMA only. After extraction in the presence of water, the peak at 153 ppm corresponding to the carbonyl carbon of the urethane group disappeared along with the peaks at 14 and 30 ppm for the CH_3 and CH_2 groups of the DES moieties, respectively, indicating that most of the template molecules were removed (Figure 3b). This spectrum coincided exactly with the spectrum of the control polymer. Figure 3c shows the spectrum of the imprinted polymer prepared by the extraction in the presence of methanol. The carbonyl group peak of the newly formed urethane bond and the methoxy carbon peak appeared at 154 and 52 ppm, respectively. In the spectrum of the imprinted polymer obtained by the reaction with aniline (Figure 3d), the peak for the urea carbonyl group showed up at 154 ppm, and the intensities of the phenyl carbon peaks increased.

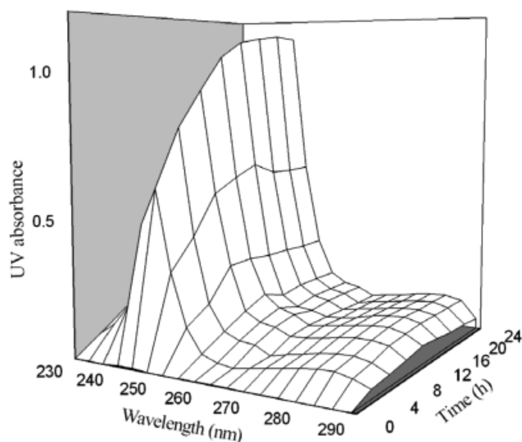


Figure 2. Extraction of template molecule from DES-bound polymer monitored by UV−vis spectroscopy ($\lambda_{max} = 243$ nm).

Recognition Test for DES. The ability of polymer **4** to recognize the template was investigated. In the rebinding test, polymer particles were added to the solution of the template in 10% (v/v) tetrahydrofuran−chloroform at various concentrations. Imprinted polymer **4** had two binding points in a cavity. The amount of an analyte in a sample solution was decided on the basis of the capacity of imprinted polymers, calculated theoretically.[16] After incubating for 24 h at room temperature, the polymer particles were isolated by filtration. The amount of template absorbed by the polymer was determined by measuring the residual analyte in the filtrate by reverse phase HPLC. Figure 4 shows the amount of template (DES) bound to polymer **4** and to the control polymer according to the sample concentration. The imprinted polymer exhibited a much higher recognition ability than the control polymer. The superior rebinding ability of the imprinted polymer, compared to the control polymer, proved that the target molecules were not simply adsorbed at the polymer surface but were trapped in the cavities through hydrogen bonding.

Selectivity Test. We also investigated the specific recognition ability of the imprinted polymers for the template and its structural analogues (Figure 5). Molecular modeling revealed that the space between the binding points in the cavity decreased in the order of an amino, a methylurethane, and a phenylurea group, while the relative direction of the two functional groups in the cavity was essentially unchanged. The rebinding test was carried out in the same manner as described above. The polymers were incubated into a solution (1 mM) of an analyte in 10% (v/v) tetrahydrofuran−chloroform. Except for **A6** and **A7** in Figure 5, all of the analogues possessed functional groups capable of hydrogen bonding. As expected, the imprinted polymer (**4**), containing amino groups inside the cavities, showed the highest recognition ability for the template (DES) and the lowest recognition ability for **A6** and **A7**. The lower response for **A1**−**A5** was attributed to their smaller sizes than that of the template. They would plausibly

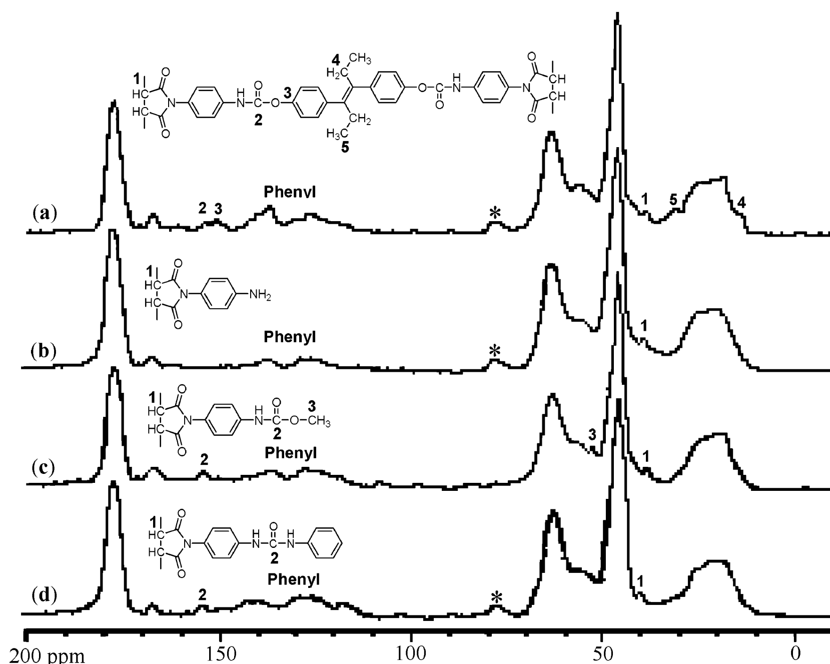


Figure 3. Solid-state ^{13}C NMR spectra of (a) polymer **3** before removal of the template molecules, (b) polymer **4**, (c) polymer **5**, and (d) polymer **6**. The asterisk (*) denotes a spinning sideband.

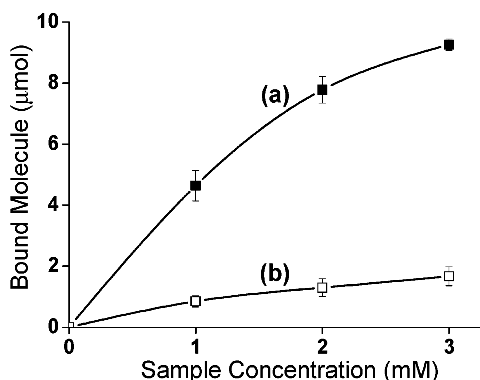


Figure 4. Amount of bound DES (a) by polymer **4** and (b) by the control polymer according to the sample concentration.

enter into the cavity with little steric hindrance but would not be able to form stable two-point binding.

Our major concern in this test was to determine whether the selectivity of the imprinted polymers could be controlled by tailoring the nature of the functional groups as well as by adjusting the space between them in the cavity. In this regard, Figure 6 clearly shows that the approach we were taking was on target. When methylurethane groups were introduced into the cavity, the imprinted polymer (**5**) showed the highest affinity for 4,4′-biphenol (**A2**), which has a smaller size than the template. Since **A2** had two hydroxyl groups as the template, the selectivity change was likely due to the space contraction between the two binding points. More striking results came from polymer **6**, wherein phenylurea groups were introduced into the cavity. This polymer showed the highest recognition ability for *trans*-stilbene (**A7**), which contains two phenyl groups. The $\pi-\pi$ interaction between the phenyl urea group and *trans*-stilbene seemed to play a key role, leading to a two-point binding.[17] Molecular modeling showed that in the cavity of the polymer the urea group was screened by the bulky phenyl group, and accordingly, its ability of hydrogen bonding with an analyte would be obstructed.

Conclusions

We demonstrated how to control the selectivity of two-point binding imprinted polymers. In our approach, after molding the overall shape of the imprint cavity by means of the template molecule, the affinity of the cavity was tailored by adjusting the space between the two binding points as well as by changing the nature of the functional group. We utilized a thermally reversible urethane bond in the template–monomer complexation, which allowed us to readily introduce various functional groups into the cavity, during the process of template removal after polymerization. The rebinding test results were very encouraging for the development of an

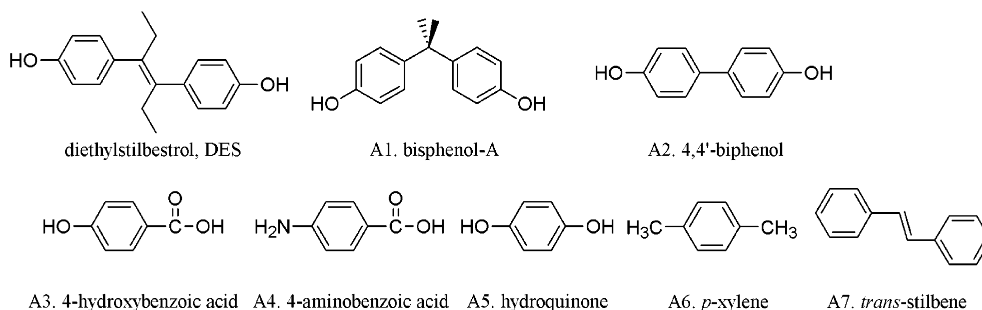


Figure 5. Template molecule (DES) and its analogues.

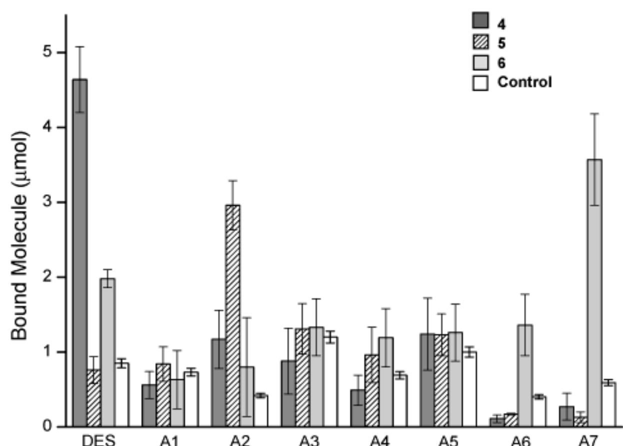


Figure 6. Biding selectivity of polymers **4**−**6** and the control polymer. In all experiments, 0.1 g of the polymers was added to 7.4 mL of the sample solutions (1 mM) in chloroform/THF (9/1 v/v).

artificial receptor for an unfunctionalized molecule. Further studies are in progress along these lines.

Experimental Section

Materials and Methods. Maleic anhydride, *p*-aminobenzoic acid, sodium acetate, sodium azide, 2,2-azobis(isobutyronitrile) (AIBN), dibutyltin dilaurate (DBTDL), and ethylene glycol dimethacrylate (EGDMA) were obtained from Aldrich. AIBN was purified by recrystallization from methanol. EGDMA was dissolved in diethyl ether, washed with 1 M aqueous NaOH three times, and dried over anhydrous $MgSO_4$. After filtration, it was purified by distillation under reduced pressure. (*E*)-Diethylstilbestrol (DES) was purchased from Sigma. Bisphenol A, 4,4-biphenol, 4-hydroxybenzoic acid, 4-aminobenzoic acid, hydroquinone, *p*-xylene, and *trans*-stilbene were obtained from Aldrich. Acetic anhydride, toluene, and triethylamine were purchased from Junsei Chemical (Japan). Chloroform, methanol, water, and tetrahydrofuran for HPLC analysis were purchased from J.T. Baker.

The ^{1}H and ^{13}C NMR spectra were obtained on Bruker Avance DPX-300 (300 MHz) spectrometer and Bruker Avance DPX-500 (500 MHz). FT-IR spectra were recorded on a Perkin-Elmer Spectrum 2000 equipped with a temperature controller. The solid-state ^{13}C NMR spectra were obtained on Unity/Inova200 solid-state NMR 50 MHz spectrometer equipped with a CP-MAS probe (Varian). Samples were spun in air at approximately 5 kHz. Extraction of template molecules was monitored by a SCINCO S-3150 UV−vis spectrophotometer. Reverse phase HPLC analysis was carried out using a M930 solvent delivery system, a M720 UV−vis detector (YOUNG LIN Instrument Co., Ltd., Korea), a MetaSil 5u ODS column from Metachem (Torrance, Canada) with methanol for DES, bisphenol A, 4,4-biphenol, hydroquinone, *p*-xylene, and *trans*-stilbene, or water/methanol for 4-hydroxybenzoic acid and 4-aminobenzoic acid, as an eluent at a rate of 1.0 mL/min at room temperature. For each analysis 20 μL of sample was injected.

***N*-[4-(*N*-Diethylstilbestroxycarbonylamino)phenyl]-maleimide (2).** Toluene was dried by azeotropic distillation with a Dean−Stark trap for 24 h. *N*-(4-Azidocarbonylphenyl)-maleimide **(1)** (3.00 g, 12.39 mmol), prepared following the procedures in the literature,[18] was dissolved in toluene (70 mL). After refluxing for 3 h, diethylstilbestrol (DES 1.67 g, 6.22 mmol) and dibutyltin dilaurate (DBTDL, 1 mL) were added to the solution. The solution was refluxed for 5 h. The resulting precipitates were isolated by filtration and purified by column chromatography on silica gel (ethyl acetate:hexane = 5:4 v/v). Yield: 1.63 g (38%).

IR (KBr): 3350 (NH stretching), 2959−2870 (CH stretching in DES), 1743 (C=O in urethane bond), 1717 (C=O in maleimide), 1611 cm^{-1} (C=C in DES). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.40 (s, 2H, NH), 7.64, 7.31 (dd, 8H, $J \approx$ 9.0, 99 Hz, phenyl protons), 7.29 (s, 8H, phenyl protons), 7.17 (s, 4H, vinyl protons), 2.16 (q, 4H, CH_2), 0.78 (t, 6H, CH_3). ^{1}H NMR (300 MHz, THF-d_8) δ (ppm): 9.39 (s, 2H, NH), 7.61, 7.32 (dd, 8H, $J \approx$ 8.9, 87 Hz, phenyl protons), 7.23 (s, 8H, phenyl protons), 6.91 (s, 4H, vinyl protons), 2.20 (q, 4H, CH_2), 0.81 (t, 6H, CH_3). ^{13}C NMR (500 MHz, THF-d_8) δ (ppm): 169.74 (C=O of maleimide), 151.79 (C=O of urethane), 151.20, 139.56, 139.32, 138.63, 134.47, 129.71, 127.29, 126.83, 121.45, 118.57, 28.20 (CH_2 in DES), 12.18 (CH_3 in DES). Anal. Calcd for $C_{40}H_{32}N_4O_8$: C, 68.96; H, 4.63; N, 8.04. Found: C, 69.24; H, 4.85; N, 7.78.

Preparation of Imprinted Polymer 4. A mixture of monomer **2** (1.50 g, 2.15 mmol), EGDMA (13.60 g, 68.61 mmol), and DMF (20 mL) was charged to a polymerization tube (50 mL), and AIBN (0.17 g, 0.73 mol % with respect to polymerizable double bonds) was added. After three freeze−thaw cycles under N_2, the tube was sealed and placed in an oil bath at 54 °C for 24 h. The cross-linked polymer was washed with THF and dried in vacuo at room temperature for 48 h. The polymer (**3**) was ground with a mechanical mortar and pestle. The polymer particles were refluxed in 1,4-dioxane/water (7/1 v/v). The process of extraction was monitored by UV spectroscopy. The absorption intensity at 243 nm for the dissociated DES in the solution reached a constant after 24 h.[19] The polymer particles were isolated by filtration, washed with 1,4-dioxane, THF, methylene chloride, and acetone, and dried in vacuo at room temperature for a week.

Preparation of Control Polymer. The control polymer was synthesized in the same manner for the preparation of the imprinted polymer (**4**), except that *N*-(4-azidocarbonylphenyl)maleimide (**1**) was used instead of monomer **2**. Azido groups in the polymers were converted to amino groups when the polymers were refluxed in 1,4-dioxane/water (7/1 v/v).

Preparation of Imprinted Polymer 5. DES bound polymer **3** (3.00 g) was added to a solution of 1,4-dioxane (50 mL) and methanol (10 mL). The mixture was refluxed for 24 h. The resulting mixture was isolated by filtration, washed with 1,4-dioxane, THF, methylene chloride, and acetone, and dried in vacuo at room temperature for a week.

Preparation of Imprinted Polymer 6. DES bound polymer **3** (3.00 g) was added to a solution of 1,4-dioxane (50 mL) and aniline (10 mL). The mixture was refluxed for 24 h. The polymer was isolated by filtration, washed with DMSO, DMF, 1,4-dioxane, THF, methylene chloride, and acetone, and dried in vacuo at room temperature for a week.

Rebinding Test for DES. Imprinted polymer **4** (0.1 g) or the control polymer was added to a solution of DES in 10% (v/v) tetrahydrofuran−chloroform (7.4 mL) at various concentrations (1, 2, and 3 mM). After incubating for 24 h at 25 °C, the polymer particles were isolated by filtration and washed with chloroform and THF. The filtrate was concentrated to dryness by evaporation of the solvent before HPLC analysis.

Selectivity Test. An imprinted polymer or the control polymer (0.1 g) was added to a solution (1 mM) of an analyte listed in Figure 5 in 10% (v/v) tetrahydrofuran−chloroform (7.4 mL). After incubating for 24 h at 25 °C, the polymer particles were isolated by filtration and washed with chloroform and THF. The filtrate was concentrated to dryness by evaporation of the solvent before HPLC analysis.

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(16) The capacity of an imprinted polymer was calculated on the assumption that all monomer−template complexes produced the imprinted sites. For example, capacity of imprinted polymer **4** = [sample polymer (g)] ÷ (mol wt of 4-aminophenylmaleimide × 2 + mol wt of EGDMA × 32).

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