ORGANIC LETTERS

2007
Vol. 9, No. 20
3865−3868

Synthesis and Photoresponsive Properties of a Molecularly Imprinted Polymer

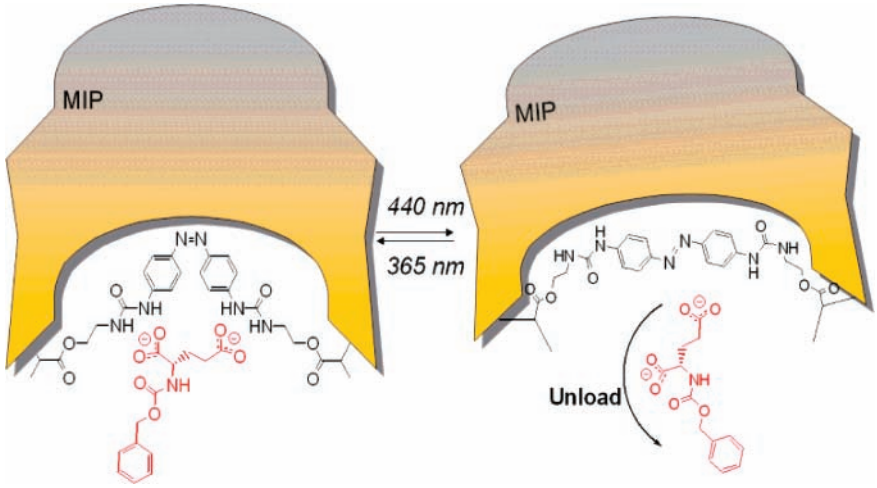
Christophe Gomy and Andreea R. Schmitzer*

Department of Chemistry, Université de Montréal, C.P. 6128 Succursale Centre-ville, Montréal, Québec, H3C 3J7 Canada

ar.schmitzer@umontreal.ca

Received June 6, 2007

ABSTRACT



A photoresponsive molecularly imprinted polymer was prepared from a di(ureidoethylenemethacrylate)azobenzene monomer, using a methotrexate analogue as template. Photoisomerization of the 3D crosslinked polymer matrix allowed switching the substrate affinity by altering the geometry and spatial arrangement of the receptor binding sites. As a result, controlled release and uptake of the template (or analogous ligands) were obtained.

Light offers many advantages as a means for manipulating systems of either microscopic or macroscopic size: photons can be applied with extreme spatial and temporal resolution using modern laser techniques. Photons are perfectly clean, leaving no remaining contamination and, in contrast to matter, the photons do not interact with each other (at moderate intensities) allowing for multiplexing. For these reasons molecular assemblies with optical triggers are ideal systems for studying molecular processes as well as for the construction of molecular machines. Azobenzene has been the most widely used optical trigger over the last decades for the design and synthesis of a large variety of photoresponsive systems.[1−8] Incorporation of azobenzene moieties into polymers creates materials with photocontrolled mechan-

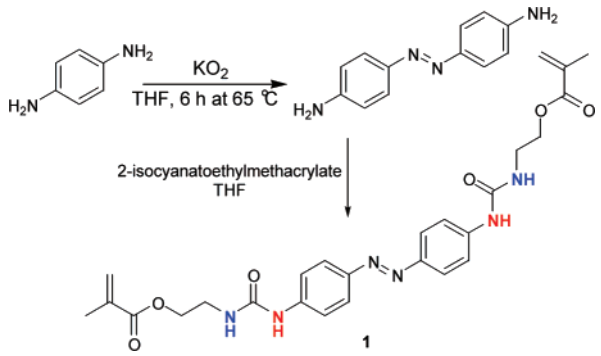
* To whom correspondence should be addressed. Phone: (+1) 514-343-6744. Fax: (+1) 514-343-7586.

10.1021/ol701618n CCC: $37.00
Published on Web 08/30/2007

(1) Willner, I.; Rubin, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 367−385.
(2) Willner, I. *Acc. Chem. Res.* **1997**, *30*, 347−356.
(3) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915−1925.
(4) Shibaev, V.; Bobrovsky, A.; Boiko, N. *Prog. Polym. Sci.* **2003**, 729−836.
(5) Natansohn, A.; Rochon, P. *Chem. Rev.* **2002**, *102*, 4139−4175.
(6) Pieroni, O.; Fissi, A.; Angelini, N.; Lenci, F. *Acc. Chem. Res.* **2001**, *34*, 9−17.
(7) Harada, A. *Acc. Chem. Res.* **2001**, *34*, 456−464.
(8) Rau, H. In *Studies in Organic Chemistry: Photochromism, Molecules and Systems*; Dürr, H. B.-L., H., Ed.; Elsevier: Amsterdam, 1990; Vol. 40, p 165−192.

ical[1−3] and optical[4] properties and has also found extensive applications in the search for innovative photoresponsive materials as well as for photomodulation of biological properties of peptides, proteins, and lipids.[2,5−7] This is because of the pronounced changes in geometry and polarity that result upon its light-induced cis → trans isomerization, leading to the high (photo)stability, the high isomerization, and quantum yields[8] as well as the high rate and full reversibility of the isomerization. This process is complete within 10 ps for the trans → cis and within 1 ps for the cis → trans.[9−13] At the photostationary states the trans and cis isomers are formed, depending on the irradiation wavelength. Conversely thermal cis→trans relaxation is a slow process, but leads to 100% trans isomer.[1] Less explored is the possibility of incorporating such stimuli-responsive receptors into rigid polymer matrices for controllable chemical and drug delivery, analyte separation, and extraction. Receptor sites that are capable of recognizing specific molecular species can be conveniently imprinted into rigid polymer matrices via a template-directed polymerization technique known as molecular imprinting.[14] With a suitable choice of functional monomers, molecularly imprinted polymers (MIPs) with a substrate affinity that can be switched by externally applied stimuli should be possible. While azobenzene has been frequently utilized as the backbone and in the side chains of polymers and hydrogels for the fabrication of photoresponsive materials,[15−18] its application in photoresponsive molecular recognition and host−guest binding in MIP materials has seldom been reported.[19−21] To our knowledge, all previously decribed MIPs contained photoresponsive azobenzene side chains. Incorporation of the azobenzene group in the MIP's backbone may lead to higher differences in host−guest recognition properties of the two isomers. In this work, new reported azobenzene-derivatived functionalized monomers, (Scheme 1) were used to fabricate a bulk MIP material containing bis(TBA)-*N*-*Z*-L-glutamate, a methotrexate analogue, as the molecular template. The

Scheme 1. Di(ureidoethylenemethacrylate)azobenzene Monomer Synthesis



thermodynamically less stable cis-form of the monomer was used for the MIP preparation. Photoisomerization of azobenzene located in the backbone allowed the obtained material to release the template most efficiently after irradiation at 440 nm and rebind the molecular template from solution after irradiation at 365 nm.

The design of the novel functional monomer **1** was based on the previously reported features that are relevant to the creation of selective MIPs for oxyanions.[22] First are the bisurea binding functionalities, which exhibit strong affinity for dicarboxylate moieties.[23] The second feature is the selection of polymerizable end groups. We chose methacrylate groups, commonly used in chemical cross-linking and known for providing materials with good thermal stability. The methacrylate group is placed two carbons away from the H-bond donating ureas to prevent destabilization of the template/monomer complex during the polymerization process. The monomer was synthesized from the *p*-phenylenediamine with KO_2 by heating under reflux for 6 h.[24] The resulting azobenzene-4,4′-diamine was treated in a one-pot reaction by *N*-hydro-*C*-alkylamino addition on 2-ethyliso-cyanatemethacrylate (Scheme 1). ^{1}H NMR titrations were performed with both cis and trans isomers of monomer **1**, using bis(TBA)-*N*-*Z*-L-glutamate as guest compound. The complexation-induced chemical shift ($\Delta(\delta)$) of the urea protons of both isomers of **1** was monitored. Addition of increasing amounts of bis(TBA)-*N*-*Z*-L-glutamate (0−10 equiv) to DMSO-d_6 solutions of **1** allowed extraction of monomer/guest interaction information for each isomer (Table 1). Titration data fit well to 1:1 binding isotherms and association constants (K_{ass}) were obtained by nonlinear least-squares fitting.[22] The competitive solvent DMSO-d_6 prevented self-association of monomer and/or guest to occur in the system. Although both inner and outer protons from *trans*-**1** are more acidic than those from *cis*-**1**, the cis monomer gives rise to higher $\Delta(\delta)$ than the trans isomer. This is compatible with the hypothesis that the cis isomer is topologically complementary to the oxyanion substrate. A

(9) Lednev, I. K.; Ye, T.-Q.; Hester, R. E.; Moore, J. N. *J. Phys. Chem.* **1996**, *100*, 13338−13341.
(10) Lednev, I. K.; Ye, T.-Q.; Matousek, P.; Towrie, M.; Foggi, P.; Neuwahl, F. V. R.; Umapathy, S.; Hester, R. E.; Moore, J. N. *Chem. Phys. Lett.* **1998**, *290*, 68−74. Natansohn, A.; Rochon, P. *Chem. Rev.* **2002**, *102*, 4139−4175.
(11) Nägele, R.; Hoche, R.; Zinth, W.; Wachtveitl, J. *Chem. Phys. Lett.* **1997**, *272*, 489−495.
(12) Fujino, T.; Arzhantsev, Y. S.; Tahara, T. *J. Phys. Chem. A* **2001**, *105*, 8123−8129.
(13) Satzger, H.; Spörlein, S.; Root, C.; Wachtveitl, J.; Zinth, W.; Gilch, P. *Chem. Phys. Lett.* **2003**, *372*, 216−223.
(14) Komiyama, M.; Takeuchi, T.; Mukawa, T.; Asanuma H. *Molecular Imprinting: From Fundamentals to Applications*; Wiley-VCH: Weinheim, Germany, 2003.
(15) Haitjema, H. J.; Buruma, R.; Alberda van Ekenstein, G. O. R.; Tan, Y. Y.; Challa, G. *Eur. Polym. J.* **1996**, *32*, 1437−1448.
(16) Howe, L. A.; Jaycox, G. D. *J. Polym. Sci., Part A: Polym. Chem.* **1998**, *36*, 2827−2833.
(17) Izumi, A.; Nomura, R.; Masda, T. *Macromolecules* **2001**, *34*, 4342−4348.
(18) Jaycox, G. D. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42*, 566−573.
(19) Gong, C. B.; Hon-Wah Lam, M.; Yu, H. *Adv. Funct. Mat.* **2006**, *16*, 1759−1767.
(20) Minoura, N.; Idei, K.; Rachkov, A.; Uzawa, H.; Matsuda, K. *Chem. Mater.* **2003**, *15*, 4703−4704.
(21) Minoura, N.; Idei, K.; Rachkov, A.; Choi, Y.-W.; Ogiso, M.; Matsuda, K. *Macromolecules* **2004**, *37*, 9571−9576.
(22) Gomy, C.; Schmitzer, A. R. *J. Org. Chem.* **2006**, *71*, 3121−3125.
(23) Hall, A. J.; Manesiotis, P.; Emgenbroich, M.; Quaglia, M.; De Lorenzi, E.; Sellergren, B. *J. Org. Chem.* **2005**, *70*, 1732−1736.
(24) Crank, G.; Makin, M. I. H. *Aust. J. Chem.* **1984**, *37*, 845−855.

Table 1. Extrapolated ^{1}H NMR Chemical Induced Shifts ($\Delta(\delta)$)[a] and Association Constant (K_{ass})[b]

	$\Delta(\delta)$ (ppm)		
	inner ^{1}H	outer ^{1}H	K_{ass} (M^{-1})
cis-**1**	3.09	3.35	2800
trans-**1**	2.83	3.23	2200

[a] The monomer concentration was 1 mM and the solvent was DMSO-d_6. [b] The association constants were determined from the experimental urea proton chemical shifts by the nonlinear refinement method[25].

600 M^{-1} increase in binding constant is observed for *cis*-**1**, relative to *trans*-**1** (Table 1). Though the difference is not overhelmingly impressive, it has an important meaning considering the nature of the measurement which has been done in solution. It has been reported previously that similar shorter receptors could form 2:1 assemblies, explaining the possible association for *trans*-**1**/guest in solution. Nevertheless, once contained in a preorganized cis conformer polymeric network, this effect should be attenuated, leaving place to a higher difference of affinity of the substrate for the cis relative to the trans isomer.

A molar EDMA crosslinker /monomer/ bis(TBA)-*N*-*Z*-L-glutamate ratio of 8/4/1 was used for the 2,2′-azobis-(2,4-dimethylvaleronitrile) (ABDV) initiated crosslinking polymerization in DMSO as solvent. Higher and lower crosslinker/monomer ratios were tested, but the resulting polymeric materials displayed no photoisomerization properties. It is possible that at low crosslinker/monomer ratio, azobenzene monomers tend to aggregate, leading to the reduction of free volume necessary for the reorientation of the azobenzene chromophores during isomerization. The monomer is first irradiated at 365 nm and the polymerization mixture is maintained under UV irradiation to maintain the thermodynamically less stable cis configuration throughout the polymerization process. For the MIP preparation, the monomer and the bis(TBA)-*N*-*Z*-L-glutamate solution were kept at 40 °C for 30 min, to allow the monomer to bind the bis(TBA)-*N*-*Z*-L-glutamate prior to addition of the crosslinker and the polymerization initiator. The resultant bulk polymers were ground using a combination of coffee grinder and ball mill, then dry-sieved using a sieve shaker. Template removal was achieved by Soxhlet extraction and repeated methanol washing. The control polymer was prepared and treated in exactly the same way as the MIP, except that no template was used in the polymerization procedure.

Figure 1 shows the spectroscopic behavior of the resultant MIP, imprinted with bis(TBA)-*N*-*Z*-L-glutamate, toward irradiation at 365 and then 440 nm. Responses of the MIP material to photoexcitation are similar to that of the monomer, indicating that the azobenzene chromophore of the monomer still possesses its photoswitching properties after incorporation into a 3D crosslinked polymer network. Photoisomerization was also observed in the control polymer material (non-imprinted). Compared to the control material, the relatively faster cis → trans isomerization rate of the MIP material suggests that the imprinted receptor sites in the bulk

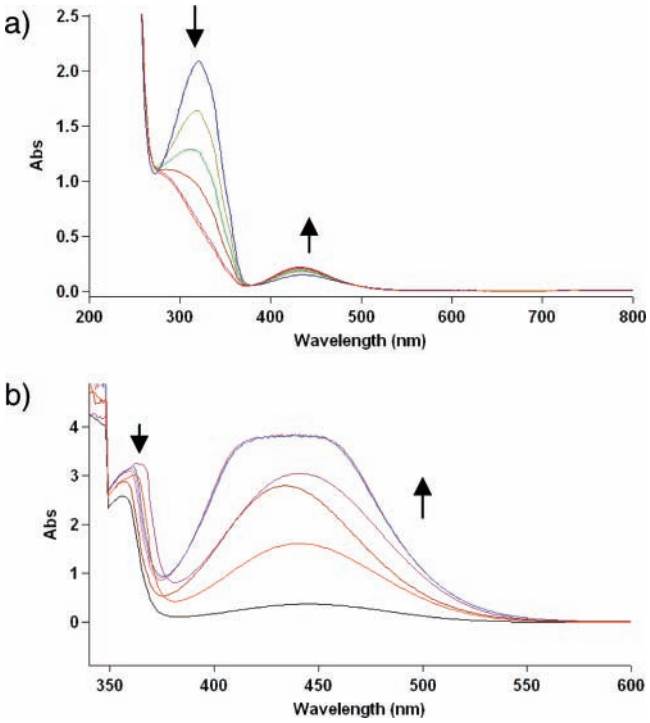


Figure 1. UV−vis spectra of the isomerization of monomer **1** and MIP: (a) trans → cis isomerization of monomer **1** after 20 min irradiation at 365 nm (50 μM in DMSO); (b) MIP toward irradiation at 365 nm (1 mg in 2 mL DMSO, 20 min of irradiation).

polymer may be able to provide more free volume for the photoswitching of azobenzene chromophores (data not shown).

The ability of the empty MIP material to rebind bis(TBA)-*N*-*Z*-L-glutamate was studied using batch-type rebinding assays in DMSO and is shown in Figure 2. The density of the imprinted receptor sites in the MIP was found to be 501 μmol/g *cis*-MIP and 270 μmol/g *trans*-MIP.

Figure 2 shows the change in free bis(TBA)-*N*-*Z*-L-glutamate in DMSO in the presence of bulk MIP and control material under alternating irradiation at 365 and 440 nm. The addition of the empty MIP (10 mg) allowed binding of 3.5 μmoles of bis(TBA)-*N*-*Z*-L-glutamate from solution into the MIP material. Taking into account the 0.5 μmoles of bis(TBA)-*N*-*Z*-L-glutamate that was bound by nonspecific sites in the polymer revealed from the control material and from the amount that remain bound to MIP following release at 440 nm a total of 3 μmoles of bis(TBA)-*N*-*Z*-L-glutamate was bound by specific sites in 10 mg MIP, which is equivalent to the uptake of 300 μmol/g *cis*-MIP. This also implies that 60% of the receptor sites in the *cis*-MIP material are occupied by the imprint molecules. Irradiation at 440 nm caused a steady increase in the amount of bis(TBA)-*N*-*Z*-L-glutamate in the DMSO solution. A total of 2.8 μmoles of bis(TBA)-*N*-*Z*-L-glutamate was released from the MIP material sites. This photoregulated release from the MIP material by 440 nm irradiation can be attributed to the photoinduced cis trans isomerization of the azobenzene

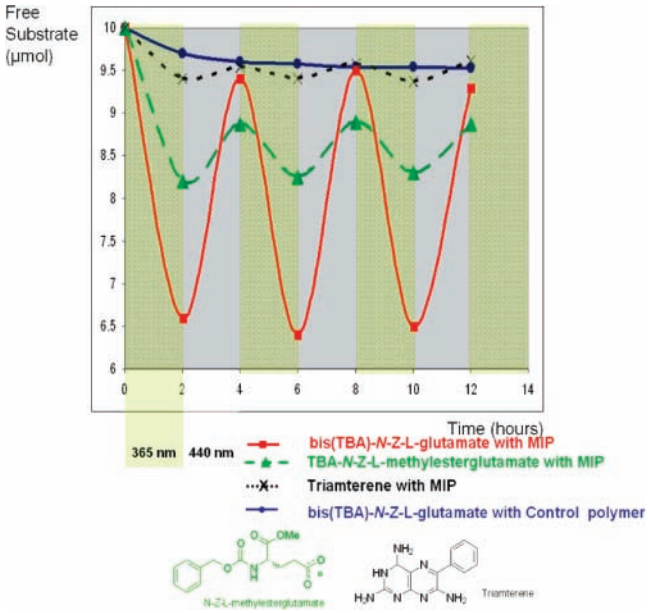


Figure 2. Photoregulated release and uptake of bis(TBA)-*N*-*Z*-L-glutamate by the MIP and the control polymer. The initial amount of ligands was 10 μmol for 10 mg MIP in 2 mL of DMSO.

chromophores in the MIP receptor sites, resulting in changes in the receptor geometry. The host−guest interaction is therefore weakened and the imprint is released into the solution, by the switch of substrate affinity of the MIP receptor sites. The near-quantitative uptake of the imprint molecule after the trans → cis isomerization is an evidence of the reversibility of the receptor-site configuration and imprint affinity, without loss of specificity after three irradiation cycles.

To examine the substrate specificity of the MIP material, we studied the photoregulated release and uptake of TBA-*N*-*Z*-L-methylesterglutamate and triamterene, two substructural models of the methotrexate drug. At the same substrate concentration, the MIP material bound only 1.8 μmoles of TBA-*N*-*Z*-L-methylesterglutamate and 0.4 μmoles of triamterene. Irradiation of the MIP material at 365 and 440 nm also allowed the partial release (40%) and uptake of TBA-*N*-*Z*-L-methylesterglutamate. In the case of triamterene (Figure 2), no significative release/uptake was observed. These results indicate that the receptor sites in the MIP material possess a specific affinity for bis(TBA)-*N*-*Z*-L-glutamate, and TBA-*N*-*Z*-L-methylesterglutamate is less sensitive than its analogue to the change in receptor geometry.

We have demonstrated the utility of a new di(ureidoethylememethacrylate)azobenzene monomer for the preparation of photoregulated MIP. The photoisomerization properties of the azobenzene chromophore are retained in the rigid polymer. Photoinduced cis → trans isomerization of the azobenzene-containing polymer backbone is able to regulate the receptor sites' geometry and can regulate the release and uptake of a substrate. An important factor to consider is that this new cross-linking monomer combines interactive monomer functionality with a cross-linking format. This new cross-linking agent is readily copolymerizable under mild conditions and has been used for noncovalent MIPs preparation with potential improved performance, that more functionality can be introduced without suffering performance losses due to reduced cross-linking. Development of stimuli-responsive MIP is advantageous for the improvement of the imprint molecule's release and may find applications in smart drug delivery systems.

Acknowledgment. We are grateful to NSERC, FQRNT, the Canadian Foundation for Innovation (CFI) and the Université de Montréal for financial support. We thank colleagues for careful reading and discussion of this manuscript.

Supporting Information Available: Experimental procedure for the synthesis of monomer, polymers, and photoregulated release and uptake of the substrates. This material is available free of charge via the Internet at http://pubs.acs.org.

OL701618N

(25) Connors, K. A. In *Binding Constants: The Measurement of Molecular Complex Stability*; John Wiley and Sons Ltd: New York, 1987.