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Molecularly Imprinted Nanoreactors for Regioselective Huisgen 1,3-Dipolar Cycloaddition Reaction

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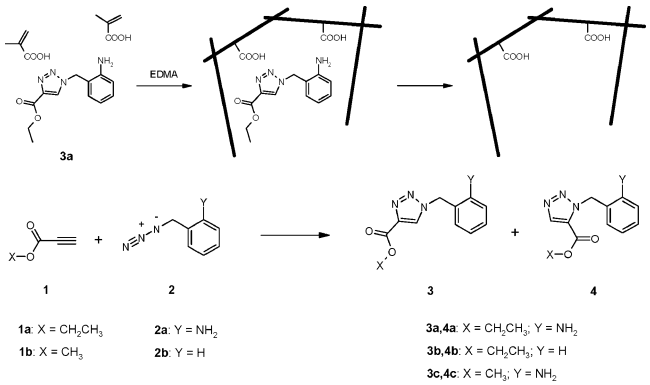
In nature, chemical transformations usually take place with exceptionally high efficiency and stereo-, regio-, and chemoselectivity inside well-defined nanosized environments, such as the catalytic sites of enzymes. A challenge for contemporary chemists is the development of nonenzymatic nanoreactors with selectivity and efficiency approaching that achieved in nature. Toward this goal, many synthetic low molecular weight organic receptors capable of encapsulating reagents have been designed.[1] However, the construction of such nanoreactors usually implies complicated multi-step synthesis, which severely limits their large-scale application. Therefore, other synthetically more accessible nanoreactors are highly desirable. As a new class of artificial receptors, molecularly imprinted polymers (MIPs) have shown great potential in this area due to their highly selective recognition capability, favorable mechanical, thermal, and chemical stability, and ease of preparation.[2] In general, molecular imprinting can be defined as the formation of specific nanosized cavities by means of template-directed synthesis. Until now, MIP materials have been utilized as nanoreactors in stereo- and regioselective syntheses,[3a−f] enzyme-like catalysis,[3g,h] and the anti-idiotypic imprinting of bioactive molecules.[4] In this paper, we describe extension of both the anti-idiotypic imprinting and in-cavity synthesis concepts into the modern drug discovery area by further exploring the feasibility of combinatorial drug or lead candidate "cloning"—a process that exploits properties (shape, size, and electronic features) embedded in the historical drugs or advanced drug candidates to identify novel biologically active entities as starting points in a variety of drug discovery programs.

Huisgen 1,3-dipolar cycloaddition of azides and alkynes has drawn great attention because of its efficiency and versatility to provide fast access to an enormous variety of medicinally interesting triazoles.[5] The reaction usually leads to a mixture of 1,4- and 1,5-regioisomers (e.g., the anti- (**3**) and syn-isomers (**4**) in Scheme 1).[5a] Recently, nanoreactors based on an organic capsule[6] and enzymes[7] have been utilized to affect different 1,3-dipolar cycloaddition reactions, resulting in high product regioselectivity due to the well-defined geometry of the organic capsule and the active center of the enzymes. Herein we demonstrate, for the first time, that a MIP can also act as highly regioselective nanoreactors for 1,3-dipolar cycloaddition of azides and alkynes. We should mention that, in the previous MIP-assisted regioselective reactions, the imprinted sites were utilized either as a supported reagent[3c,f] or to provide temporary functional group protection.[3e] In the latter case, the imprinted sites contained a reactive moiety that could be temporarily linked to certain steroid compounds, leaving the unprotected functional groups to be chemically modified. The objective of this work is to obtain noncovalently imprinted nanoreactors for regioselective bimolecular reactions using a model Huisgen 1,3-dipolar cycloaddition reaction. When the two reactants are encapsulated in the same site, they are correctly oriented to afford regioselective bimolecular reaction. Central to our approach is the use of only noncovalent interactions to encapsulate two reactants into the imprinted nanoreactor, the same being exploited in the enzyme systems.[7] Obviously, this method is generally applicable and easy to use.

Scheme 1. Preparation of **3a**-Imprinted Polymer (top) and the Huisgen 1,3-Dipolar Cycloaddition Reactions Investigated (bottom)



The imprinted polymer (MIP(3a)) was synthesized using **3a** as template in anhydrous toluene. Methacrylic acid (MAA) and ethylene glycol dimethacrylate (EDMA) were used as functional monomer and cross-linker, respectively (Scheme 1). ^{1}H NMR titration experiment suggested possible two-point interactions between MAA and **3a**: one between the carboxyl group of MAA and the amino group of **3a**, the other between MAA and the triazole ring of **3a** (see Supporting Information). Equilibrium binding experiment showed that the imprinted polymer (MIP(3a)) bound 2-fold more **3a** than the control polymer (CP(3a)). More importantly, binding of the minor syn-isomer (**4a**) on MIP(3a) was marginal, almost identical to that on CP(3a) (see Supporting Information). The superior binding of **3a** over **4a** makes MIP(3a) ideal nanoreactors for the regioselective 1,3-dipolar cycloaddition reaction.

The effect of imprinted nanoreactors was demonstrated on 1,3-dipolar cycloaddition of ethyl propiolate **1a** and 2-aminobenzyl azide **2a**. The total amount of product (including both **3a** and **4a**) obtained with MIP(3a) was 6.7 times that of with CP(3a) and 1.6 times that of in the polymer-free system (Table 1, entries 1−3).[8] Most importantly, the product obtained with MIP(3a) contained 94% of the anti-isomer,[9] whereas the product obtained with CP(3a) and from the solution reaction contained only 75 and 69% of **3a**, respectively, suggesting that the **3a**-imprinted nanocavities acted as effective nanoreactors with pronounced effect on regioselectivity and kinetics of the studied reaction. It is somehow surprising that the reaction performed in the free solution provided more product than in the presence of CP(3a), although with a lower anti/syn ratio.

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Table 1. Effect of the Polymers on the Huisgen 1,3-Dipolar Cycloaddition Reactions

	Reaction condition			Product (nmol)						
entry	acetylene	azide	polymer	**3a**	**3b**	**3c**	**4a**	**4b**	**4c**	anti/syn
1	**1a**	**2a**	MIP(3a)	4.29			0.27			15.9
2	**1a**	**2a**	CP(3a)	0.51			0.17			3.0
3	**1a**	**2a**		1.98			0.88			2.3
4	**1a**	**2b**	MIP(3a)		1.37			0.30		4.6
5	**1a**	**2b**	CP(3a)		1.26			0.26		4.8
6	**1a**	**2b**			2.30			1.25		1.8
7	**1a**	**2a**, **2b**	MIP(3a)	4.81	2.29		0.42	0.93		11.5 (**3a/4a**), 2.5 (**3b/4b**)
8	**1a**	**2a**, **2b**	CP(3a)	0.96	2.27		0.35	0.87		2.7 (**3a/4a**), 2.6 (**3b/4b**)
9	**1a**	**2a**, **2b**		2.34	2.73		1.07	1.31		2.2 (**3a/4a**), 2.1 (**3b/4b**)
10	**1b**	**2a**	MIP(3a)			0.52			0.16	3.3
11	**1b**	**2a**	CP(3a)			0.45			0.20	2.3
12	**1b**	**2a**				1.04			0.79	1.3

The same polymer effect was observed in other experiments (Table 1, entries 5, 6, 8, 9, 11, and 12). The reason is not fully understood, but may be due to the nonspecific adsorption of azides onto the polymer surface; the decreased azide concentration in the solution phase would result in a slower conversion. Furthermore, protonation of 2-aminobenzyl azide by the acidic CP(3a) might cause a perturbation of the electronic environment on the 1,3-dipole, resulting in the change of regioselectivity.

To further verify that the imprinted cavities were responsible for the product regioselectivity and the accelerated reaction rate, the 1,3-dipolar cycloaddition of benzyl azide **2b** and ethyl propiolate **1a** was carried out under the same conditions. The interaction between MIP(3a) and **2b** should be rather weak due to the lack of the amino group in **2b**. Therefore, it is expected that MIP(3a) would only have little effect on this reaction system, and the reactions with MIP(3a) and CP(3a) should provide more or less the same anti/syn ratio with similar reaction speed. This hypothesis was indeed confirmed by the experimental results (Table 1, entries 4 and 5).

By keeping the amino group in azide **2a** intact and instead making an incremental change in the acetylene structure (**1b**), we tested if the **3a**-imprinted cavities would assist the regioselective formation of other triazoles that are more similar to **3a**. As it turned out, addition of MIP(3a) in the reaction between **2a** and **1b** indeed resulted in higher anti/syn product ratio than the corresponding reaction in solution or in the presence of CP(3a) (Table 1, entries 10−12).

Finally, a multicomponent reaction was carried out by heating a mixture of azides (**2a** and **2b**), acetylene **1a**, and MIP(3a) in anhydrous toluene. Within the **3a**-imprinted cavities, we expected that the reaction between **2a** and **1a** should not be affected by the competing benzyl azide **2b**. As seen in Table 1, the anti/syn product ratio from the reaction between **2a** and **1a** was only slightly decreased due to the competing reaction from **2b** (Table 1, entries 1 and 7). These results further demonstrated that the imprinted cavities had indeed functioned as effective nanoreactors for the 1,3-dipolar cycloaddition of **2a** and **1a**. The imprinted cavities were responsible for the highly regioselective formation of product with increased rates. The improvement of regioselectivity was also substrate dependent; the maximum cavity effect was obtained for the reaction leading to the "best fit" product.

In summary, we have demonstrated that a MIP can function as effective nanoreactors for Huisgen 1,3-dipolar cycloaddition of azides and alkynes, providing high product regioselectivity and faster reaction kinetics. Furthermore, the MIP nanoreactors showed remarkable selectivity toward the reactant structures. Unlike the previously reported regioselective MIP nanoreactors, the present imprinted cavities bind reactants by means of only noncovalent molecular interactions. Considering the simple MIP preparation and their high stability, we believe that the new imprinted nanoreactors have potential to parallel their biological counterparts in studies of site-directed chemical reactions. In addition, presented data support the notion of using such systems in early stages of drug discovery to "clone" known biologically active compounds of interest and identify structurally novel chemotypes amenable to further optimization by medicinal chemists.

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Supporting Information Available: ^{1}H NMR titration, polymer preparation, and equilibrium binding results, MIP-assisted 1,3-dipolar cycloaddition reactions and kinetic studies (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The yield of **3a** from the reaction involving MIP(3a) has been corrected by subtracting the template leakage (Supporting Information).

(9) An even higher anti-isomer ratio (97%) was observed in the MIP(3a)-bound product. The polymer-bound product was recovered by methanol: acetic acid wash.

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