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Toward an Artificial Aldolase

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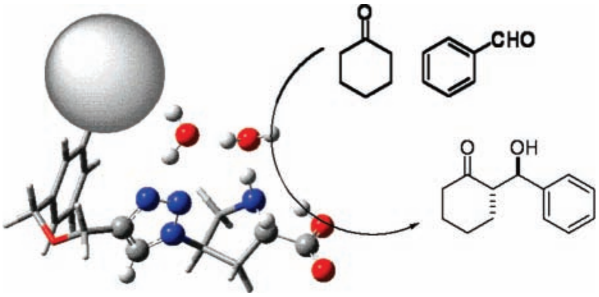
ABSTRACT



A new functional polymer where proline is bonded to polystyrene through a 1,2,3-triazole linker depicts characteristics targeted for an artificial aldolase. In spite of the hydrophobicity of the polymer backbone, the resin swells in water with building of an aqueous microenvironment. This property, arising from the formation of a hydrogen-bond network connecting the proline and 1,2,3-triazole fragments, is translated into a very high catalytic activity and enantioselectivity toward direct aldol reactions in water.

The aldol reaction, which is used by nature for the building of carbohydrate molecules through the use of aldolase enzymes,[1] is a most useful synthetic procedure for the construction of carbon−carbon bonds. The process can be catalyzed by simple amino acids and some of their derivatives which, in this context, have been considered as "micro-aldolases".[2,3] Macromolecular catalytic systems, able to act as artificial enzymes,[4] are of even greater interest since they can readily accommodate the structural information required for substrate recognition and can present suitably placed hydrophobic and hydrophilic regions, as true enzymes operating in aqueous, biological systems also do.[5]

Among macromolecular enzyme mimics, catalytic antibodies have been considered to date as the most advanced synthetic aldolases.[6] In addition to these, dendrimers[7] and polymers[8] have gained in recent times considerable ac-

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(5) Significant progress has been achieved in recent times on enzymelike molecules promoting enantioselective aldol reactions in the presence of water: (a) Mase, N.; Nakai, Y.; Ohara, N.; Yoda, H.; Takabe, K.; Tanaka, F.; Barbas, C. F., III. *J. Am. Chem. Soc.* **2006**, *128*, 734. (b) Hayashi, Y.; Sumiya, T.; Takahashi, J.; Gotoh, H.; Urushima, T.; Shoji, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 958. (c) Hayashi, Y.; Aratake, S.; Okano, T.; Takahashi, J.; Sumiya, T.; Shoji, M. *Angew. Chem., Int. Ed.* **2006**, *45*, 5527. (d) Guizzetti, S.; Benaglia, M.; Raimondi, L.; Celentano, G. *Org. Lett.* **2007**, *9*, 1247. Successful operation of these systems in purely aqueous media remains challenging: (e) Aratake, S.; Itoh, T.; Okano, T.; Usui, T.; Shoji, M.; Hayashi, Y. *Chem. Commun.* **2007**, 2524.

(6) (a) Wagner, J.; Lerner, R. A.; Barbas, C. F., III. *Science* **1995**, *270*, 1797. (b) Zhong, G.; Hoffmann, T.; Lerner, R. A.; Danishefsky, S.; Barbas, C. F., III. *J. Am. Chem. Soc.* **1997**, *119*, 8131. (c) Barbas, C. F., III; Heine, A.; Zhong, G.; Hoffmann, T.; Gramatikova, S.; Bjornestedt, R.; List, B.; Anderson, J.; Stura, E. A.; Wilson, I. A.; Lerner, R. A. *Science* **1997**, *278*, 2085.

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10.1021/ol702901z CCC: $40.75

ceptance as structural elements for the assembly of enzyme-like systems with catalytic properties, since they allow the multiple installment of catalytic sites and, to some extent, control of the hydrophilic/hydrophobic nature of the ensemble. However, performance of these catalytic systems in asymmetric aldol reactions has remained low.[9]

In an attempt to develop a general and practical solution to this problem, we have recently introduced resin **1a** (Figure 1), prepared through “click-chemistry”,[10] that efficiently catalyzes the asymmetric aldol reaction in water.[11] Noticeably, resin **1a** exhibited a much better catalytic performance than its monomeric counterpart **1b**, thus suggesting that the polystyrene backbone in **1a** can play a role similar to that of the large hydrophobic pocket found in type I aldolases.[5a,6c,12−14] Moreover, since **1a** was by far a superior catalyst for aldol reactions than similar hydroxyprolines directly supported on polystyrene, like **1c**,[15] it was also suggested that the electron-rich 1,2,3-triazole fragment was involved in some manner in the catalytic event.

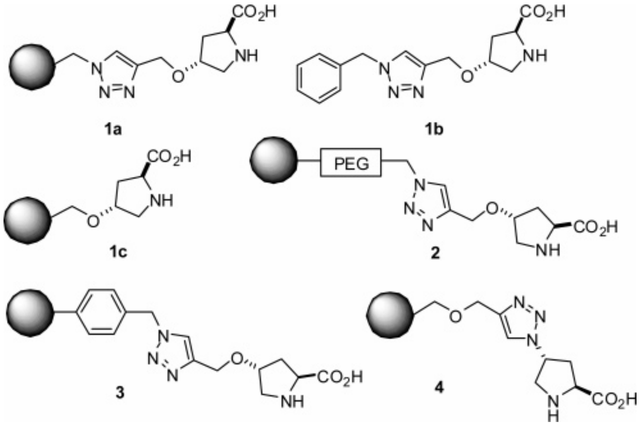
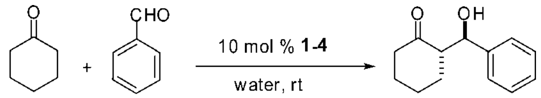


Figure 1. Polymer-supported prolines tested in water. On Merrifield resins (**1a**, **1c**, **4**); on PS-PEG NovaBioSyn resin (**2**); and on Argopore resin (**3**). Reference monomer (**1b**).

To test these two hypotheses, natural 4-hydroxyproline was grafted onto several resins using the same click-chemistry strategy as for **1a** (resins **2** and **3**), or following a complementary approach (resin **4**). Resins **1a**−**4** were next evaluated in the reaction of cyclohexanone with benzaldehyde in water, at room temperature (Table 1). Neither the introduction of a hydrophilic PEG region in **2**, nor the use of the macroporous resin **3** led to any improvement over **1a**. Resin **1c**, lacking the triazole moiety, led to similar yields but much poorer ee’s. Gratifyingly, resin **4** (see Supporting Information (SI) for experimental details) exhibited an optimal catalytic performance, with a notable rate acceleration and improved stereoselectivity over those of the other studied resins. The problem of low reaction rate, one of the most important limitations of organocatalytic aldol reactions in water,[5,11−13] finds a quite satisfactory solution with resin **4**. Thus, running the reaction at 40 °C, a high acceleration was observed (70% isolated yield in only 12 h) without deterioration in stereocontrol.

Table 1. Benchmark Aldol Reaction in Water Catalyzed by Resins **1a**−**4**



resin	t [h]	yield [%][a]	anti:syn[b]	ee [%][c]
1a (1% DVB)	84	67	95:5	95
1a (2% DVB)	60	58	94:6	92
1c (1% DVB)	60	70	93:7	40
2	60	10	n.d.	91
3	84	traces	n.d.	70
4	24	74	96:4	98
4[d]	12	70	93:7	97

[a] Isolated yield. [b] Determined by ^{1}H NMR. [c] Determined by HPLC. [d] Reaction at 40 °C.

Noticeably, and in sharp contrast with **1a**−**3** and with other polystyrene resins (**1c**), **4** perfectly swells in water. Indeed, whereas the reaction mixtures with **1**−**3** were multiphase systems, with resin **4** a gel-like single phase was formed (see Figure 2). TGA analysis of the swollen resin showed a water content up to 24% in weight. According to the resin functionalization, this corresponds to ca. 36 water molecules per proline unit.

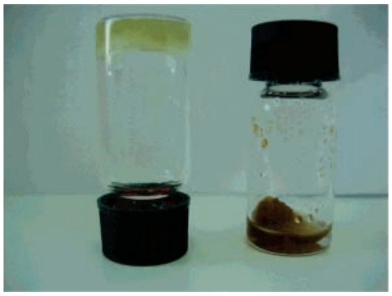


Figure 2. Phase behavior of resins **4** (left, gel) and **1a** (right, multiphase) under the benchmark reaction conditions in water.

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(9) (a) Reymond, J. L.; Chen, Y. *J. Org. Chem.* **1995**, *60*, 6970. (b) Kofoed, J.; Darbre, T.; Reymond, J.-L. *Org. Biomol. Chem.* **2006**, *4*, 3268.

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(12) For recent alternative approaches to supported proline for aldol reactions in the presence of water, see: (a) Giacalone, F.; Gruttadauria, M.; Marculescu, A. M.; Noto, R. *Tetrahedron Lett.* **2007**, *48*, 255. (b) Gruttadauria, M.; Giacalone, F.; Marculescu, A. M.; Lo Meo, P.; Riela, S.; Noto, R. *Eur. J. Org. Chem.* **2007**, 4688.

(13) For a dendrimeric aldol catalyst operational in water, see: Wu, Y.; Zhang, Y.; Yu, M.; Gang Zhao, G.; Wang, S. *Org. Lett.* **2006**, *8*, 4417.

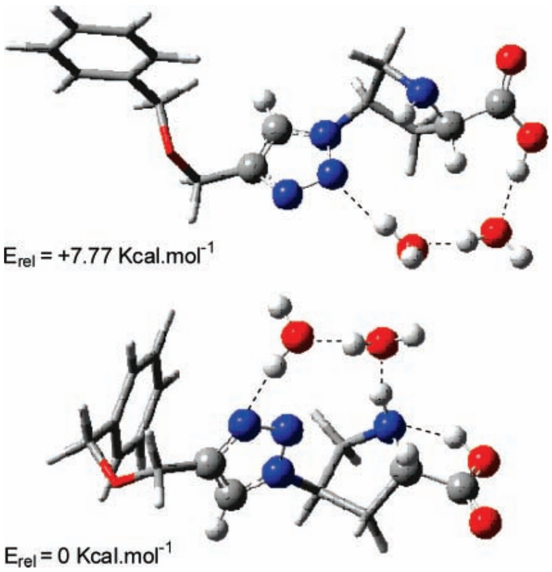
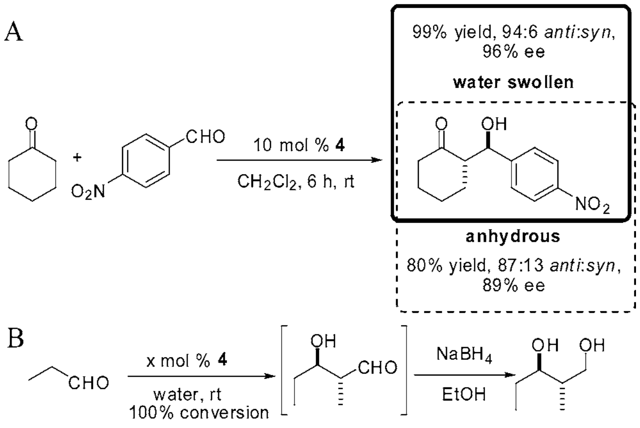


Figure 3. ONIOM (MP2-FC/6-31G(d,p):PM3/ZDO) calculated conformers of the hydrogen-bonded diaquo-triazolylproline model system. High-layer atoms (MP2) are in ball-and-stick form. Low-layer atoms (PM3) are in tube form. O, red; N, blue; C, gray; H, white.

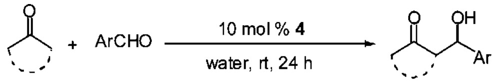
Scheme 1. Effect of Water in the Aldol Reaction Catalyzed by **4**: (A) Activity of Water-Swollen vs Anhydrous Resin in Organic Solvent; (B) Reaction in Water Using Water-Soluble Reactants

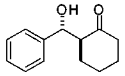
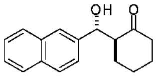
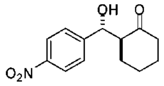
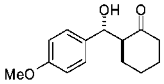
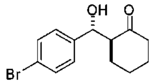
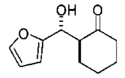
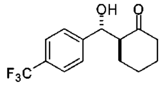
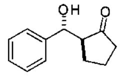


4 [mol %]	time [h]	*anti*:*syn*[a]	ee *anti* [%][b]
1	24	5:1	97
10	7	4:1	96

[a] Determined by ^{1}H NMR on a crude sample of the 1,3-diol. [b] Determined by HPLC on a chiral stationary phase after conversion to the monobenzoyl ester.

Table 2. Aldol Reactions in Water Mediated by Resin **4**



entry	product	yield [%][a]	*anti*:*syn*[b]	ee [%][c]
1		74	96:4	98
2		82	97:3	>99
3		80[d]	95:5	96
4		16	92:8	96
5		90	97:3	96
6		60	84:16	94
7		98	97:3	96
8		99	84:16	94

[a] Isolated yield. [b] Determined by ^{1}H NMR on a crude sample. [c] Determined by HPLC on a chiral stationary phase. [d] Isolated yield after 5 h.

To understand the interaction of water with **4**, a Monte-Carlo simulation (MMFF) was performed on a catalyst model in a cage containing 30 water molecules. Removing from the model water not involved in hydrogen bonding with the catalyst led ultimately to two distinct minima, both involving only two water molecules connecting the amino acid and the 1,2,3-triazole moieties. These minima were then re-optimized with an ONIOM approach (Figure 3).[16]

The structure involving a dual role (donor and acceptor) of the proline N−H moiety is predicted to be more stable by 7.77 kcal·mol^{-1}. In any case, the important point is that high-level theoretical calculations predict that a very small amount of essential water is sufficient to establish a hydrogen-bonding network between the triazole moiety and the amine and carboxylic acid functions in proline. Thus, the results of the theoretical calculations provide an explanation for the ability of **4** to swell in water.

To ascertain whether these water molecules can be mechanistically involved in the aldol reaction catalyzed by **4**, as occurs in type-I aldolases,[17] the following comparative experiment was performed: A sample of resin **4** was swollen in water, excess water was then removed, and the resin was transferred to dichloromethane, where the aldol reaction was studied. In a parallel experiment, the same reaction was performed with an anhydrous sample of **4**. Interestingly, the

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(14) For related backbone hydrophobic effects on asymmetric Michael additions, see: Alza, E.; Cambeiro, X. C.; Jimeno, C.; Pericàs, M. A. *Org. Lett.* **2007**, *9*, 3717.

(15) Kondo, K.; Yamano, T.; Takemoto, K. *Makromol. Chem.* **1985**, *186*, 1781.

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reaction performed with water-swollen **4** was faster, more diastereoselective, and more enantioselective than the corresponding one with anhydrous **4** (Scheme 1A). In sharp contrast with these results, the related resin **1a** behaves as a much less active catalyst in organic media (DMF) when dissolved amounts of water are present.[11] Although the elucidation of the exact role exerted by water in aldol reactions mediated by **4** would require further investigation, its unique behavior in this respect parallels the effect of *essential water* on some natural enzymes used in organic media.[18]

An additional experiment was performed to exclude the possibility of the reaction taking place in a segregated organic phase constituted by the reactants.[5e] The self-condensation of propanal, which is readily soluble in water, was catalyzed by resin **4** (Scheme 1B). As it can be seen, 1 mol % of **4** is enough to induce the complete conversion of propanal in 24 h. The corresponding aldol product was formed with high diastereoselectivity and enantiomeric purity.

The scope of applicability of resin **4** in the direct aldol reaction of a family of cyclic ketones in water was next tested with a variety of aldehydes (Table 2). Excellent yields, diastereoselectivities, and enantioselectivities were recorded in all the studied cases. As a matter of fact, the catalytic performance of **4** in the studied process equals or surpasses that of small molecule mimics previously used with the same purpose. Furthermore, resin **4** could be recycled and reused for at least five times without any apreciable loss in yield or in stereoselectivity (see SI).

In summary, resin **4** represents a substantial progress toward the conceptual development of an artificial aldolase. The particular functional arrangement in the monomer plus linker ensemble in the resin appears to facilitate the establishment of a hydrogen bond-based aqueous microphase around the hydrophobic resin that appears to play a fundamental role in its catalytic activity. Thus, **4** is able to induce fast and highly enantioselective aldol reactions in water or, whenever the aqueous microenvironment is provided, also in organic solvents.

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Supporting Information Available: Experimental procedures and characterization, and calculated atomic coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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