patterns in class 1, but, for those in class 1, there is a substantial reduction in the number of errors. The network typically corrects most of the errors or rejects a pattern as nonrecognizable. A network of 72 systems would be difficult to implement experimentally, but simulations of a smaller network, which could be implemented experimentally (*22*), show that this smaller network also possesses pattern recognition abilities, albeit to a lesser extent than the larger networks.

The chemical network has many similarities and some differences with a neural network of the Little (*15*) or Hopfield (*16–18*) type: patterns are stored in both the chemical and the Hopfield network by a Hebbian rule, but the connection weights (k_{ij}) may have either sign in a Hopfield network; the chemical systems must be bistable, but the neurons in a Hopfield network are typically monostable; and in both, stored patterns are stable steady states and are recalled when the network is initialized in their basins of attraction. In an electrical realization of a neural network (*17*), the neurons are amplifiers, the connections are wires, the connection weights are resistors; their analogs in the chemical computer are the bistable reaction mechanisms, mass transfer, and the mass transfer rates. The chemical network shares many of the desirable features of neural network models: both are robust in the presence of noise, both retain some computational power when damaged, and, in both, the computational abilities are not strongly dependent on model parameters. Because the connection weights can be either positive or negative in the Hopfield network, as well as in our earlier chemical networks (*1–3*), they perform better than the network presented here. The chemical implementation of parallel computers given here and in the earlier papers (*1–3*) provides a chemical basis of neural networks.

There are many biological reaction mechanisms and biological systems with multiple stationary states; mass transfer among compartments in biological systems is ubiquitous. These are the necessary components of the pattern recognition device presented here, and the components are at least available in living systems. The predictions for a small chemical network are experimentally verifiable.

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12. The necessary constants, taken from (*11*), are $k_A = 0.21\ M^{-1}\ s^{-1}$ and $k_B = 21000\ M^{-2}\ s^{-1}$. We chose $[I^-]_o = 8 \times 10^{-5}$ M, $[IO_3^-]_o = 0.001$ M, and $k = 0.00718\ s^{-1}$. Under these conditions, $[I^-] = 0.00013$ M and 0.00058 M in the two possible stable steady states and 0.000357 M in the unstable steady state for each isolated system whose kinetics is described by Eq. 2. The multiplier λ is 2.3×10^{-5} for Fig. 1 and 1.6×10^{-5} for Fig. 2.
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20. Stored patterns are generated randomly, and one of them is designated *p'*. A certain number of randomly chosen pixels are reserved (a system that is in the high $[I^-]$ state is set to the low $[I^-]$ state, or vice versa) in the pattern *p'* to generate the presented pattern.
21. The average time for the emergence of a stored pattern depends on the reaction kinetics, and the iodate–arsenous acid reaction is notoriously slow.
22. As an example of what is experimentally feasible, we cite the work of J.-P. Laplante and T. Erneux [*J. Phys. Chem.* **96**, 4931 (1992)] in which they performed experiments with a system of 16 coupled bistable systems (the chlorite-iodide reaction in a continuous-flow stirred tank reactor) with the possibility of 32 connections.

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Controlling Chemical Reactivity with Antibodies

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The remarkable specificity of an antibody molecule has been used to accomplish highly selective functional group transformations not attainable by current chemical methods. An antibody raised against an amine-oxide hapten catalyzes the reduction of a diketone to a hydroxyketone with greater than 75:1 regioselectivity for one of two nearly equivalent ketone moieties. The antibody-catalyzed reaction is highly stereoselective, affording the hydroxyketone in high enantiomeric excess. Similarly, the reduction of ketones containing branched and aryl substituents, including the highly symmetrical 1-nitrophenyl-3-phenyl-2-propanone, was enantioselective. The simple strategy presented herein may find general applicability to the regio- and stereoselective reduction of a broad range of compounds.

The development of catalytic methods for the regio- and stereoselective synthesis of optically pure compounds has become an important focus in recent years (*1*). A number of chemical reagents have been designed for demanding asymmetric transformations, including titanium (IV) tartrate complexes for chiral epoxidations (*2*), rhodium and ruthenium catalysts for enantioselective hydrogenations (*3, 4*), osmium complexes for asymmetric dihydroxylations (*5*), and chiral boranes for stereoselective ketone reductions (*6*). However, while existing asymmetric catalysts have demonstrated impressive enantioselectivities, the rational design of such catalysts is still in its infancy, and high stereoselection is usually contingent upon neighboring ligands or restricted sets of substituents (*7*). Moreover, the ability to discriminate between chemically similar functional groups in the same molecule can often be achieved only by the application of extensive protecting group strategies, as in the synthesis of complex molecules such as the macrolide antibiotics, carbohydrates, and peptides (*8*).

The search for selective catalysts has also led to the increased use of enzymes in organic synthesis. Although high chemo-, regio-, and enantioselectivities have been achieved, enzymes often require expensive cofactors and are limited in number and selectivity (*9–11*). Given the extraordinary specificity of the immune system, we asked whether simple yet general strategies exist for generating antibodies that catalyze regio- and stereoselective functional group transformations. We chose the reduction of simple prochiral ketones as our initial target, specifically the regio- and stereoselective conversion of ε-diketone **5** to hydroxyketone **9** (Fig. 1). The similar chemical environments of the two carbonyl moieties

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in diketone **5** (distinguishable only by methoxy and nitro groups five and six atoms away) render this transformation extremely difficult to achieve by current chemical methods. Furthermore, in order to avoid the need for cofactor recycling, the inexpensive reductant sodium cyanoborohydride ($NaBH_3CN$) was chosen as the hydride donor for the antibody-catalyzed reaction.

Antibodies were raised against the racemic *N*-oxide hapten **1** (Fig. 1). Earlier studies demonstrated that antibodies elicited to an achiral, nitrobenzyl phosphonate hapten catalyzed the $NaBH_3CN$-dependent reduction of an activated α-keto amide (*12*). However, none of these phosphonate-specific antibodies appreciably catalyzed the reduction of the less activated ketone substrates **2** to **5**. We reasoned that antibodies specific for *N*-oxide **1** should not only stabilize the tetrahedral transition state resulting from nucleophilic attack on the carbonyl group but also direct regioselective addition of hydride to the nitrobenzyl-substituted carbonyl group of substrate **5**. Moreover, the chiral environment of an antibody combining site induced by one of the two enantiomers of hapten **1** should discriminate between the enantiotopic faces of a prochiral substrate, affording a highly stereoselective reduction. This simple hapten design would provide a versatile strategy for the reduction of a broad range of compounds because *N*-oxide haptens are readily synthesized by oxidations of the corresponding amines.

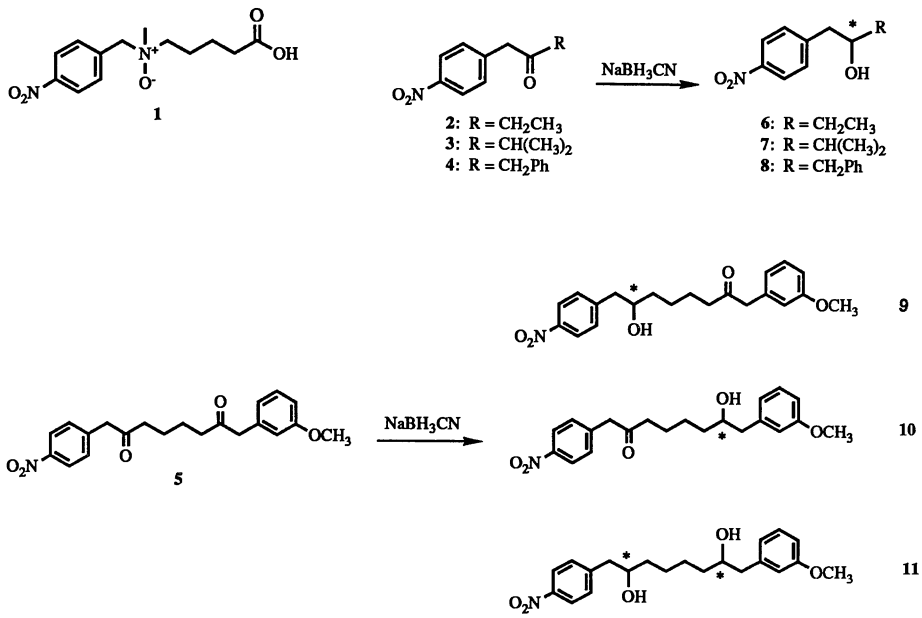

Fig. 1. Monoclonal antibodies raised against the keyhole limpet hemocyanin conjugate of hapten **1** catalyze the $NaBH_3CN$-dependent reduction of ketone substrates **2** to **4** and diketone **5**.

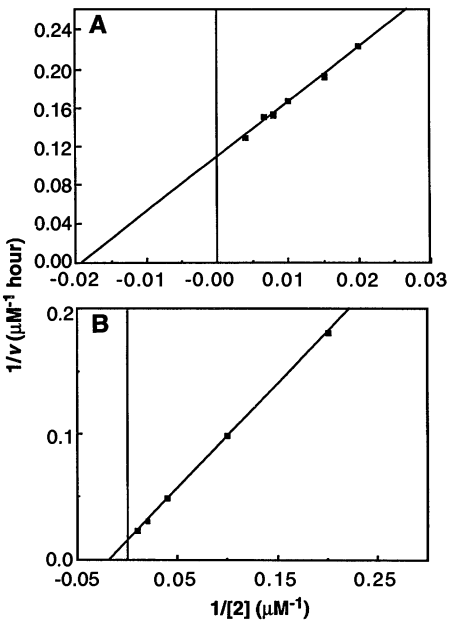

Fig. 2. Lineweaver-Burk plots for antibody-catalyzed ketone reduction. Reactions were performed in 45 mM NaCl, 50 mM MES buffer, pH 5.0, with (**A**) 1.6 or (**B**) 6.7 μM antibody and 5% (v/v) methanol. Velocities were determined at 22°C by monitoring the formation of alcohol with an HPLC assay (Rainin Dynamax Microsorb reversed-phase C_{18} column, 60% methanol in 0.1% aqueous trifluoroacetic acid). Product **5**, detected at 260 nm, was identified by coinjection with an authentic sample and quantified against an *o*-nitroanisole internal reference. Initial rates were determined by linear fitting of the product concentration at five time points. (A) The concentration of **2** was varied from 50 to 250 μM while [$NaBH_3CN$] remained constant at 50 mM. (B) The $NaBH_3CN$ concentration ranged from 5 to 100 mM while [**2**] was held at 150 μM.

Hapten **1** was conjugated to carrier proteins bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) through amide linkages (*13*). Twenty-five monoclonal antibodies specific for *N*-oxide **1** were generated by using standard protocols and purified by protein A affinity chromatography as described previously (*14*). Twelve of these antibodies accelerated the $NaBH_3CN$-dependent reduction of nitrobenzyl ketone **2**. Reduction was assayed at 22°C in 45 mM NaCl, 50 mM MES buffer, pH 5.0, by following the appearance of product with high-performance liquid chromatography (HPLC). The kinetic parameters for one antibody (37B.39.3) were characterized further.

Antibody 37B.39.3 displayed saturation kinetics with respect to both substrate **2** and $NaBH_3CN$ (Fig. 2). A Lineweaver-Burk analysis of the steady-state data at high $NaBH_3CN$ concentration (50 mM) afforded an apparent catalytic constant ($k_{cat,app}$) of 0.097 min^{-1}, a Michaelis constant ($K_{m,app}$) of 52 μM, and a second-order rate constant ($k_{cat,app}/K_{m,app}$) of 1.9×10^3 min^{-1} M^{-1} for substrate **2**. For comparison, the second-order rate constant for the uncatalyzed reaction (k_{uncat}) was 1.1×10^{-3} min^{-1} M^{-1}. Examination of the $NaBH_3CN$ dependence of the antibody-catalyzed reduction at fixed ketone concentration (150 μM) revealed rate constants $k_{cat,app}$ of 0.17 min^{-1} and $K_{m,app}$ of 57 mM (*15*). Greater than 25 turnovers per antibody molecule were measured with no observable change in activity, suggesting that $NaBH_3CN$ does not significantly inactivate the antibody. The specific activity of 37B.39.3 remained unchanged after further purification by anion (MONO-Q) exchange chromatography.

The antibody-catalyzed reaction was completely inhibited by the addition of 50 μM hapten. Fluorescence quenching experiments performed in the reaction buffer at 22°C afforded dissociation constants (K_d) of 33 nM for racemic *N*-oxide **1** and 150 nM for substrate **2**, indicating that the antibody binds the transition state analog more tightly than the substrate (*16*). Similar experiments in which racemic product **6** was used gave a K_d of 200 nM, close to that of the sp^2-hybridized substrate.

Further analysis revealed that the reduction of substrate **2** was highly stereospecific: secondary alcohol **6**-(*S*) was obtained in enantiomeric excess (ee) of

96.0% (*17*) (Table 1). Interestingly, while antibody 37B.39.3 demonstrated a strong ability to discriminate between the enantiotopic faces of nitrobenzyl ketone **2**, it tolerated a broad range of side chains. The reduction of substrates **3** and **4**, which contain branched and aryl side chains, resulted in 95.5 and 86.7% ee, respectively. These results are consistent with earlier studies which suggest that antibody binding specificity is relatively insensitive to elements of the hapten and substrate close to the conjugation site (*12, 18*). At the same time, the high stereoselectivity obtained for the reduction of substrate **4** (in which the carbonyl substituents are distinguishable only by the nitro group) reflects the extraordinary specificity attainable with the antibody molecule. Screening of additional antibodies should provide a catalyst that produces alcohols of absolute (*R*)-configuration because there is no apparent stereochemical bias in the immune response.

In order to investigate the regioselectivity of antibody 37B.39.3, the reduction of diketone **5** was assayed in 45 mM NaCl, 50 mM MES buffer, pH 5.0 (*19*). The antibody-catalyzed reduction of diketone **5** was highly regioselective, affording alcohol **9** in 95.5% yield (2.5 hours, 57% conversion). Only minor amounts of products **10** and **11** were observed (0.6 and 3.9%, respectively). The ratio of the reduction rates (V_{rel}) of the nitrobenzyl carbonyl moiety relative to the methoxybenzyl carbonyl moiety was ~75. In contrast, the nitrobenzyl carbonyl group was reduced more slowly than the methoxybenzyl carbonyl group in the uncatalyzed reaction (V_{rel} = 0.74), consistent with an acid-catalyzed mechanism. In addition to exhibiting high regioselectivity, the antibody-catalyzed reduction was stereoselective, affording the (*S*)-enantiomer of product **9** in 96.3% ee.

This model study suggests the potential for exploiting the specificity of antibody molecules to perform highly selective functional group transformations, regardless of chemical environment and substrate complexity. The generation of catalytic antibodies with predetermined selectivities is more easily achieved than the design of small molecule catalysts. Although the smaller size of the latter may provide broader substrate specificities, the simple strategy presented herein may find general applicability to the regio- and stereoselective reduction of a wide range of complex compounds.

Table 1. Enantioselectivity of the antibody-catalyzed reduction of nitrobenzyl ketones **2** to **5**. The reported values represent the average of two experiments.

Alcohol product	ee (%)*
6 R = CH_2CH_3	96.0
7 R = $CH(CH_3)_2$	95.5
8 R = CH_2Ph	86.7
9	96.3

*The enantiomeric excess for the antibody-catalyzed reaction was determined by extracting the products into 2-butanone and analyzing the composition by HPLC (Chiralpak AD column, Daicel Chemical Industries, 95:5 hexanes/2-propanol, 1.0 ml/min). Products were identified by coinjection with authentic samples and compared against the corresponding uncatalyzed reaction by using an *o*-nitroanisole internal standard. Products **6, 7**, and **8** were obtained after 33, 13, and 37% conversion, respectively. Reaction mixtures contained 250 μM substrate, 50 mM $NaBH_3CN$, and 4.4 μM antibody. Product **9** was analyzed after >99% conversion by using 50 μM substrate, 7 mM $NaBH_3CN$, and 27 μM antibody.

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13. Hapten **1** was synthesized by treating *p*-nitrobenzaldehyde and δ-aminovaleric acid with one equivalent (eq) of $NaBH_3CN$ in methanol at 25°C to afford *N*-(*p*-nitrobenzyl)-δ-aminovaleric acid. Following recrystallization from acetone/H_2O, this compound was treated with 40% aqueous formaldehyde and 1.6 eq $NaBH_3CN$ in H_2O at 25°C. The product, *N*-methyl-*N*-(*p*-nitrobenzyl)-δ-aminovaleric acid, was purified by silica-gel chromatography and treated with 1 eq of *m*-chloroperbenzoic acid in 0.1 M tris-HCl at pH 8.0 to give hapten **1**. Hapten **1** was purified by silica-gel chromatography and coupled to carrier proteins BSA and KLH by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. Epitope densities of five to ten haptens per carrier monomer were determined by ultraviolet absorbance (280 nm); protein concentrations were determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)]. Substrate **2** was synthesized as follows: carbonyl diimidazole–mediated condensation of propanoic acid and ethyl *p*-nitrophenylacetate produced the ester, which was isolated by silica-gel chromatography. Acid hydrolysis and decarboxylation afforded ketone **2** after purification by silica-gel chromatography. Substrates **3** and **4** were prepared from isobutyric acid or phenylacetic acid by using a similar strategy.
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16. Fluorescence quenching was measured in 45 mM NaCl, 50 mM MES buffer, pH 5.0, with 1.0 μM antibody. The sample was excited at 280 nm, and fluorescence was detected at 348 nm. Dissociation constants were determined by Scatchard analysis according to the method of K. Taira and S. J. Benkovic [*J. Med. Chem.* **31**, 129 (1988)].
17. Absolute configuration of the alcohol product was determined as follows: the antibody-catalyzed reaction was performed on a 10-mg scale in 45 mM NaCl, 50 mM MES buffer, pH 5.0, with 5.63 μM antibody, 5% (v/v) methanol, 2 mM substrate **2**, and 150 mM $NaBH_3CN$. Isolation of the product by preparative thin-layer chromatography, followed by ^1H nuclear magnetic resonance (400-MHz) analysis of the MTPA (Mosher) ester identified the major product as the (*S*)-enantiomer [J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.* **95**, 512 (1973)].
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19. Diketone **5** and products **9** to **11** were synthesized as follows: carbonyl diimidazole–mediated condensation of adipic acid, monomethyl ester, and ethyl *m*-methoxyphenylacetate afforded the diester. Purification by silica-gel chromatography followed by acid hydrolysis and decarboxylation gave the carboxylic acid, which was treated with diazomethane. The resulting methyl ester was purified by silica-gel chromatography and reduced with sodium borohydride ($NaBH_4$). Following purification, the alcohol was protected as the *tert*-butyldimethylsilyl ether. The product was isolated by silica-gel chromatography and hydrolyzed with sodium hydroxide to give the ε-hydroxy carboxylic acid with the protecting group intact. Carbonyl diimidazole–mediated condensation of this compound with ethyl *p*-nitrophenylacetate afforded the ester after silica-gel chromatography. Acid hydrolysis and decarboxylation yielded the product **10**, which was purified as above. Oxidation with pyridinium chlorochromate afforded substrate **5** after silica-gel chromatography. Reduction with 0.9 eq of $NaBH_4$ at 0°C afforded a mixture of products **9, 10**, and **11**. Products **9** and **10**, however, could be separated by preparative HPLC for subsequent analysis. Reduction was assayed as follows: reaction mixtures contained 45 mM NaCl, 50 mM MES, pH 5.0, with 5% (v/v) methanol, 50 μM substrate, and 15 mM $NaBH_3CN$. Velocities at 22°C were determined by using a Dynamax C_{18} column with a mobile phase of 60 to 80% methanol in 0.1% aqueous trifluoroacetic acid and a flow rate of 0.6 ml/min. Products were identified by coinjection with authentic samples. Average retention times of 20.7, 22.9, 21.9, and 24.9 min were obtained for compounds **5, 9, 10**, and **11**, respectively.

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