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Imine exchange in *O*-aryl and *O*-alkyl oximes as a base reaction for aqueous 'dynamic' combinatorial libraries. A kinetic and thermodynamic study

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ABSTRACT: Kinetics and mechanisms of the imine exchange reactions of *O*-alkyl and *O*-aryl oximes with *O*-alkyl- and *O*-aryloxyamines, respectively, were studied by ^{1}H NMR spectroscopy in aqueous solutions. The reaction between benzaldehyde *O*-methyloxime and *O*-ethylhydroxylamine at 60 °C is first order in both oxime and the alkoxylamine (the second-order rate constant $k_2 = 0.86 \pm 0.08$ l mol^{-1} min^{-1} at pD 2.9), the reaction being subject to acidic catalysis. A similar imine transfer was studied in the reaction of 1,3-diaminooxypropane with bifunctional oximes. Testing of various additives as potential catalysts for the reaction revealed imidazole as a moderately effective catalyst. The exchange in *O*-aryl oximes was studied in the interaction between 3-pyridinealdehyde *O*-phenyloxime and *O*-(*p*-nitrophenyl)hydroxylamine. The reaction is first order in the oxime, but its rate is independent on the aryloxyamine concentration and pD. The proposed mechanism involves a rate-limiting hydration of the oxime molecule. Mechanisms of the exchange reactions are discussed in relation to their possible use to generate diverse pools of compounds for the recently proposed 'dynamic' combinatorial chemistry approach. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: imine exchange; oximes; aqueous 'dynamic' combinatorial libraries; kinetics; thermodynamics

INTRODUCTION

Reversible transformations attract only limited attention of organic chemists. Since the ultimate purpose of organic synthesis is to obtain the reaction product with the maximum yield, any process in which the product exists in a dynamic equilibrium with the starting material is usually avoided in synthetic routes. Such is also the case with the rapidly developing strategies of combinatorial synthesis, that in most cases are aimed at maximizing the yields of individual components in molecular libraries.[1]

Very recently, several groups have proposed a new approach to molecular diversity generation and screening that involves a reorganization of arrays (pools) of compounds, existing in a dynamic equilibrium, *via* their interactions with the target compound.[2–5] Such reorganization, that essentially represents a shift of the thermodynamic equilibrium, results in the formation of amplified amounts of those components that form the strongest complexes with the target.

The above approach appears to hold exceptional promise for combinatorial chemistry in that it allows one to combine technically simple methods of diversity generation with screening in one step and facilitate the identification of effective components due to their amplification in the 'dynamic' libraries. One serious limitation of the method is that the majority of the reversible transformations, such as peptide bond formation and cleavage,[2] photochemical isomerization,[3] Schiff base formation[4] and transesterification,[5] that have been used to interconvert library components, often interfere with side reactions with the solvent molecules. In particular, hydrolysis of the library components, *e.g.* esters, amides and imines, under the equilibration conditions may become a major problem in screening libraries of drug candidates in aqueous solutions. Therefore, this new method calls for a non-trivial quest for new or reinvented bond-making and-breaking reactions that could be performed with a substantial turnover and would not significantly intercede with the competing hydrolytic

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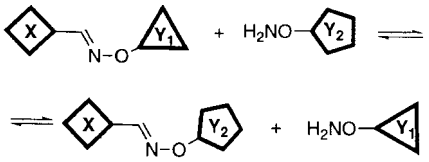
processes. In this paper, we introduce the imine exchange reaction between *O*-substituted hydroxylamines and their oximes as a transformation that may be used to generate and interconvert components of the 'dynamic' combinatorial pools with little or no hydrolytic decomposition in aqueous solutions.

The basic reaction that can be used for reversible exchange of building blocks Y in *O*-substituted oximes is depicted in Scheme 1. One can envision that by mixing various functionalities as the X and Y components, it may be possible to create diverse combinatorial pools of oximes. However, the stability and exchangeability of the pool components should be determined by the kinetics and thermodynamics of the imine exchange and competing hydrolysis of the oximes.



Scheme 1

Although mechanisms of carbon–nitrogen double bond *formation* in Schiff bases,[6] oximes,[7] semicarbazones,[8] etc. have been extensively studied in the past, only limited information is available on the imine *exchange* mechanisms.[9] In this paper, we describe the kinetics and discuss the mechanism and possible applications of the above reaction.

RESULTS

The exchange reaction was studied on compounds containing both alkyl and aryl residues as the Y units. Preliminary experiments showed that *O*-aryl oximes of aliphatic aldehydes possess relatively low stability in aqueous solutions, as followed from their incomplete formation from the aldehyde and amine components. For example, in the reaction of acetaldehyde with an excess of *O*-phenylhydroxylamine at pD 3, appreciable amounts of the original aldehyde and its water adduct were detected by NMR along with the resulting oxime. For this reason, further studies concentrated on the imine exchange in more stable O-alkyl and O-aryl oximes of aromatic aldehydes.

Reactions of *O*-alkyl oximes with *O*-alkyloxylamines

The mechanism of the exchange in oximes containing aliphatic substituents on the oxygen was first studied in the model reaction between benzaldehyde *O*-methyloxime (**1**) and *O*-ethylhydroxylamine in D_2O–methanol-d_4 (3:1, v/v) (**Scheme** 2). The reaction was monitored by ^1H NMR, and the reagent concentrations at any given time point were determined from integrals of the methyl group singlets in **1** (3.73 ppm) and methylhydroxylamine (3.66 ppm at pD 2.9) referenced to the internal standard.

Scheme 2

The reaction rate was found to be low at ambient temperature, even in acidic media where the exchange proceeded readily upon heating (see below). The second-order rate constant, estimated from the initial rate, was found to be 3×10^{-2} l mol^{-1} min^{-1} at 22 °C and pD 2.9, which corresponds to >8 h half-reaction time with 0.05 M ethylhydroxylamine (10-fold excess over the oxime and the highest concentration used in the kinetic series).

Further kinetic studies were performed at 60 °C. In excess of *O*-ethylhydroxylamine, the reaction followed a first-order kinetics with respect to **1**. Three runs performed at varying concentrations of the ethylhydroxylamine showed the exchange reaction to be also first order in the amine (see inset in Fig. 1), the second-order rate constant being 0.86 ± 0.08 l mol^{-1} min^{-1}. The level-off concentrations of the starting compounds and the products corresponding to the equilibrium (after more than five half-life times) were determined by NMR for various initial concentrations of the amine. The equili-

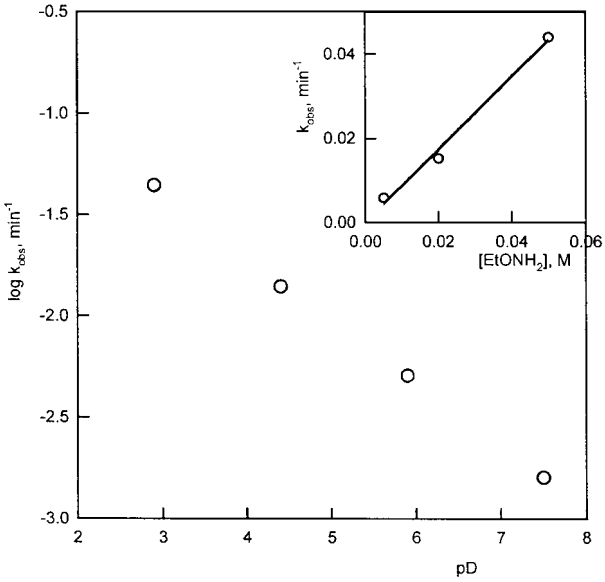


Figure 1. pD dependence of the observed pseudo-first-order rate constants for the reaction of **1** (5 mM) with *O*-ethylhydroxylamine (50 mM) at 60 °C in 25% CD_3OD in D_2O. Inset: plot of the pseudo-first-order rate constants for the above reaction at various concentrations of ethylhydroxylamine at pD 2.9

Table 1. Pseudo-first-order rate constants for the imine exchange reactions between 3C and 4 in aqueous solutions[a]

Additive	Temperature (°C)	pD	[**3**] (mM)	[**4**] (mM)	k_{obs} (min^{-1})
None	37	7.9	5.0	5.0	[b]
—	50	—	—	—	$4 \times 10-5$
Imidazole (0.5 equiv.)	10	—	3.9	4.7	[b]
—	50	—	—	—	2.3×10^{-4}
2-Aminoimidazole (0.5 equiv.)	10	—	4.27	4.7	[b]
—	50	—	—	—	1.4×10^{-4}
Amidase[c]	37	—	5	5	[b]
Carbonic anhydrase (0.5 mg)[d]	37	—	—	—	[b]

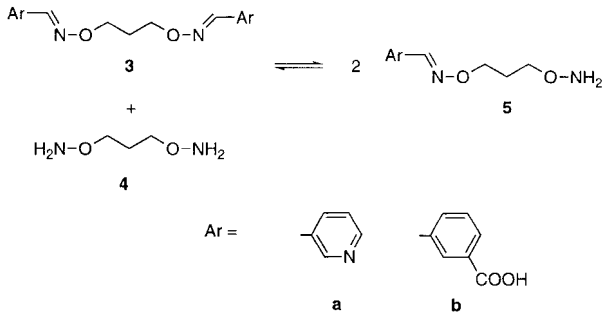
[a] Determined by ^{1}H NMR from the initial rates of accumulation of product **5**.
[b] The rate constant was below detection limit of 4×10^{-5} min^{-1}.
[c] *E. coli* (Sigma), 50 units.
[d] From bovine erythrocytes (Sigma), 4500 w-a units $mg.^{-1}$

brium constant K was estimated to be 0.72 ± 0.02. The plot of $[\mathbf{1}]_{eq}/[MeONH_2]_{eq}$ *vs* $([EtONH_2]_{total}/[\mathbf{2}]_{eq} - 1)$ at three $EtONH_2$ concentrations (5, 20 and 50 mM) yielded K as the slope. The exchange with the alkoxylamine was strongly pD dependent (Fig. 1).

Notably, the NMR spectra of the reaction mixtures recorded over *ca* 10 half-life periods of the imine exchange at varying pD and temperatures showed no signals other than those from the compounds shown in Scheme 2. Hence no competing hydrolysis or even partial addition of water to **1** or **2** occurred under those conditions.

In view of potential application of the imine exchange reactions in combinatorial chemistry, it seemed reasonable to study similar transformations in the compounds containing more than one oxime group. 1,3-Diaminoxypropane (**4**) was synthesized and its exchange with its oximes was studied. In order to eliminate possible *intra*molecular imine transfer reactions, we used the symmetrical *bis*oximes **3a** and **b** (Scheme 3). The product of this reaction, the monooxime **5**, could be formed only *via* the *inter*molecular imine exchange, similar to that shown in Scheme 2. The choice of the aldehydes used for oximation by **4** was dictated by the solubility of the resulting oximes in acidic (**3a**) and neutral (**3b**) media.

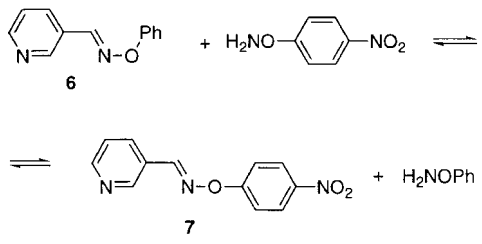
The effect of pD on the imine exchange between **3** and **4** was similar to that in the previous reaction. Thus, the pseudo-first-order rate constant for the reaction between **3a** and **4** (1.9×10^{-3} min^{-1}) was close to that expected for the exchange in **1** under similar conditions (the rate constant for the reaction shown in Scheme 2 is 1.8×10^{-3} min^{-1} at pD 4.1 and 60 °C, as estimated by the interpolation of the data in Fig. 1). The rate of reaction between **3b** and **4** in neutral media at 50 °C fell below the detection limit. A number of additives were tested as potential catalysts for the imine exchange in the hope of achieving acceleration through general base, nucleophilic or enzymatic catalysis. The results shown in Table 1 demonstrate that none of these additives was particularly effective, except for imidazole, which showed modest acceleration of the exchange in **3b** at pD 7.9.



Scheme 3

Reactions of *O*-aryl oximes with *O*-aryloxyamines

The exchange between *O*-aryloxyamines and corresponding oximes was studied in the reaction shown in Scheme 4. The reaction was monitored by ^{1}H NMR by the disappearance of the signals of oxime **6**. Formation of oxime **7** was clearly visible by the appearance of a new singlet from the CH=N proton (8.89 ppm), a new doublet of doublets from the nitrophenyl unit of **7** (8.37 and 6.95 ppm, $J = 9.2$ Hz), and other signals in the aromatic region.



Scheme 4

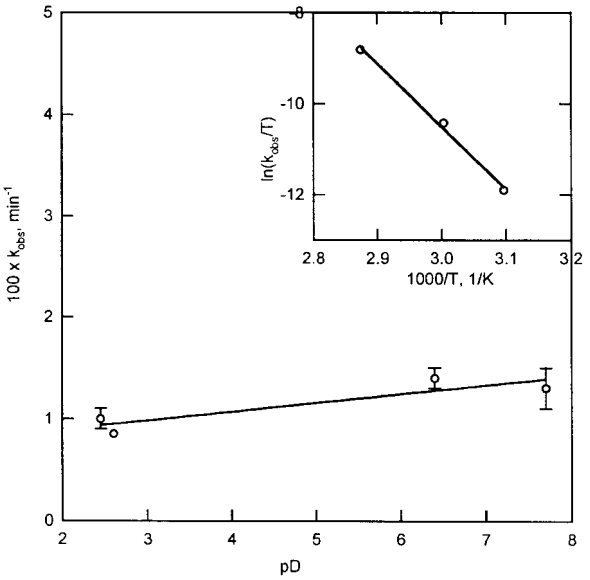


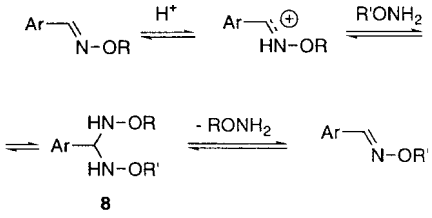
Figure 2. pD dependence of the observed pseudo-first-order rate constants for the reaction of **6** (3 mM) with *O*-(*p*-nitrophenyl)hydroxylamine (3 mM) at 60 °C in D_2O. Inset: linearized Eyring plot (see Experimental) for the above reaction at pD 3.0

The kinetics of the exchange in this system were found to be surprisingly different from those observed with the aliphatic analogs. The rate of reaction between oxime **6** and *O*-(*p*-nitrophenyl)hydroxylamine was independent of the aryloxylamine concentration in the range 2.5–9 mM, thereby indicating zero order in this reagent. Furthermore, the pD dependence of the exchange rate was found to be nearly negligible (Fig. 2). As in the case of alkyloxyamines, the exchange rate was strongly temperature dependent, being nearly undetectable at ambient temperature. Activation parameters of the exchange reaction were determined by fitting the first-order rate constants, observed at three different temperatures in the interval 50–70 °C, to the Eyring equation (see inset in Fig. 2). The linearized plot yielded values of $\Delta H^{\neq} = 116 \pm 8$ kJ mol^{-1} and $\Delta S^{\neq} = 26 \pm 3$ J mol^{-1} K^{-1}. Determination of the equilibrium point in this reaction was not possible because of the partial precipitation of product **7** and the formation of some unidentified side-products (signals at 7.0–7.1 ppm) in the later stages of the reaction.

The possibility of the imine exchange between the oxime and an *aldehyde* was also explored. A 1:1 mixture of 5 mM **6** and 3-carboxybenzaldehyde was incubated at 60 °C in D_2O (pD 2.9). However, no new oxime could be detected by ^{1}H NMR after overnight heating.

DISCUSSION

The observed kinetics of the exchange between alkoxylamines and their oximes are consistent with the mechanism shown in Scheme 5, similar to that proposed previously for the aminolysis of imines.[9a] The first reaction order in both reagents and the presence of acidic catalysis indicate that the transition state of the rate-limiting reaction step includes a protonated form of the oxime–alkoxylamine adduct. Most likely, the fast reversible protonation of the oxime nitrogen is followed by the attack of the alkoxylamine leading to the formation of the tetrahedral adduct that can further eliminate either $RONH_2$ or $R'ONH_2$, the former pathway leading to the formation of the new oxime.



Scheme 5

Both second and third steps in Scheme 5 could be rate-limiting, the two possibilities being indistinguishable by the kinetics of the oxime consumption. The mechanism suggested for many reactions of imine formation from carbonyl compounds suggests fast formation of the tetrahedral intermediate and its slow transformation into the product.[6–8] However, the rate-limiting nucleophilic attack at the protonated oxime is more consistent with the earlier studies of the imine exchange mechanism.[9] In the low pD region, the rate of the tetrahedral intermediate formation is also decreased owing to protonation of the amine (pK_a of $MeONH_2 = 4.58$[10]). The latter fact is probably responsible for the lower than unity pD-dependence slope in Fig. 1.*

As expected from the similar nature of methyl- and ethylhydroxylamines, the equilibrium constant found for the reaction shown in Scheme 2 is close to unity. This fact may have implications for the use of the reaction to generate dynamic combinatorial libraries in which most components would be present in comparable fractions under the equilibrium conditions.

The subtle effect of the additives tested as potential catalysts for the exchange (Table 1) reflects the common trend of general acid and base catalysis to be less

*Consideration of the oxime (Ox) and alkoxylamine (Am) protonation in Scheme 5 yields the following equation for the observed pseudo-first-order rate constant (k_{obs}) in the excess of Am:

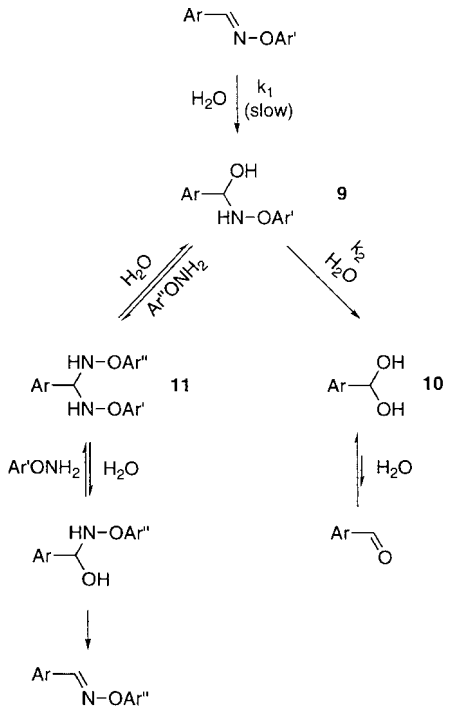
$$\log(k_{obs}) = \log(kK_{Ox}[Am]) - (pH + \log(K_{Ox}[H^+] + \log(K_{Am}[H^+] + 1)),$$

where k is the second-order rate constant for the reaction between OxH+ and Am, K_{Ox} and K_{Am} are the reactant's K_a values. As follows from this equation, a curve fitting of the data in the figure 1 with a straight line would not be justified. The observed data, within experimental error, reflect the part of the pH dependence with a changing fractional slope close to the amine pK_a value.

effective in the imine formation and exchange reactions than the acidic catalysis.[11]

The exchange of aryloxyamine with aryl oxime displays kinetics distinctly different from the exchange in alkyloxyamine systems. Since the rate of the reaction shown in Scheme 4 is independent of pD and of the concentration of the aryloxyamine, it is reasonable to assume that the rate-limiting step includes some transformation involving only the oxime substrate and the solvent. Such a transformation, however, would also occur in the absence of the aryloxyamine. To explore this possibility, we incubated oxime **6** alone under the conditions used for one of the exchange runs (D_2O, pD 8.1, 60 °C). Indeed, the ^1H NMR spectrum shows formation of several new sets of signals under these conditions. One new form of the pyridine moiety, showing a new H-5 (*meta*-proton) peak at 8.15 ppm, can be detected at the initial stage of the reaction. Later, formation of the H-5 signal from yet another form is observed at 7.67 ppm. These two new signals have been assigned to the products of stepwise hydration of the oxime, **9** and **10** (Scheme 6), respectively. Another proof for the formation of the tetrahedral intermediate comes from the appearance of a singlet at 6.38 ppm that corresponds to the proton at the tetrahedral carbon in **10** and was also detected in the aqueous solution of 3-pyridinealdehyde.

Integration of the H-5 signals for forms **9** and **10** over the reaction course and fitting the data to the equation for consecutive reactions allowed us to determine separately the rate constants k_1 and k_2 as $(1.5 \pm 0.1) \times 10^{-2}$ min^{-1} and $(2.2 \pm 0.4) \times 10^{-2}$ min^{-1}, respectively. Further transformation of form **10** into the aldehyde (Scheme 6) was also observed through the appearance of a minor singlet from the aldehyde proton at 10.18 ppm. The latter step, however, appears to be slow, and the molar amount of aldehyde present after 90 min of the reaction did not exceed 7% of the starting material.



Scheme 6

The above results, combined with the kinetics of the imine exchange, support the proposal that the formation of the hydrated oxime **9** is rate limiting in both imine exchange with aryloxymine and the hydrolysis reactions. This mechanism is also supported by the fact that the $k1$ value for the hydrolysis equals, within experimental error, the pseudo-first-order rate constants of the exchange given in Fig. 2. Likewise, the positive value of the activation entropy for the exchange indicates that the rate-limiting step is unlikely to include association of the oxime with the imine.

Further pathway of the imine exchange probably involves attack of the hydrated form **9** by the aryloxyamine (Scheme 6) followed by conversion of adduct **11** to the new oxime (*cf.* Scheme 5). On the basis of the kinetic data we cannot rule out the alternative pathway that includes the reaction of aryloxyamine with **10**, preceded by the dissociation of the original amine.

While the mechanism outlined in Scheme 6 reflects some degree of competing hydrolysis involved in the aryloxyimine exchange, the hydrolytic pathway can be almost completely redirected to the new oxime formation by the excess of the aryloxyamine. Thus, at the end of the exchange reaction 3-pyridinealdehyde was found to be present only in trace amounts in the reaction mixture. This observation is consistent with high thermodynamic stability of *O*-aryloximes in aqueous solutions (as compared with, *e.g.*, corresponding Schiff bases), which is also reflected in spontaneous formation of the oximes in water. For example, incubation of a 1:1 mixture of 5 mM 3- pyridinealdehyde and phenylhydroxylamine in D_2O at pD 2.5 led to the formation of the oxime and the hydrated aldehyde form in the ratio *ca* 2:1, only trace amounts of the starting compounds being present in the mixture.

The absence of acidic catalysis in the aryloxyimine exchange, that could have accelerated, *e.g.*, the formation of **9**, is surprising. The most reasonable explanation may come from the different pK_a values of *O*-aryl and *O*-alkyl oximes. While these values are too low to be evaluated experimentally, one can assume that the basicity of aromatic oximes is lower than that of aliphatic oximes.

CONCLUSION

We have shown that the imine exchange reactions of *O*-alkyl- and *O*-arylhydroxylamines with corresponding oximes of aromatic aldehydes can be used to maintain dynamic equilibrium of the formation and cleavage of

bonds between various structural units. Two features of such equilibrium seem to be of particular importance for the formation of 'dynamic' combinatorial pools. First, the equilibration rates in the studied reactions are low and negligible under physiological conditions, *i.e.* at ambient temperature in neutral aqueous media. As a result, the components of the pools based on *O*-substituted oximes could be isolated as stable compounds and used for biological testing (*O*-alkyl oximes and *O*-alkyloxyamines have been shown previously to possess biological activity and low toxicity, [12]and may therefore constitute viable scaffolds for libraries of drug candidates). The evolutionary selection–equilibration method reported by us previously[3] is particularly promising for the use with these pools since it involves separation of the binding and 'scrambling' sites. Thus, the exchange can be turned 'off' for selection on fragile biological ligands under physiological conditions and then back 'on,' by increasing the temperature and/or acidic catalysis, to bring the system to equilibrium.

The second important attribute of the imine exchange in the studied system is that the competing hydrolysis of the oximes is minimal or absent under equilibrating conditions. In that sense, the studied reactions represent a unique balance between stability and exchangeability and fit the requirements formulated in the Introduction for dynamic combinatorial pools. The different properties of the aliphatic and aromatic aminoxy compounds can be used for choosing the proper equilibrium reaction depending upon the selection conditions.

EXPERIMENTAL

General. All reagents were purchased from Aldrich and Fluka (*O*-phenylhydroxylamine) and used without further purification. NMR spectra were recorded on Varian Gemini 300 MHz and Unity 500 MHz spectrometers. pD values presented throughout the paper were obtained by adding the increment of 0.4 to the reading of a glass combination electrode in the D_2O solutions. Kinetic data were processed on a Macintosh computer with the aid of SigmaPlot 4.0 software.

Syntheses. 1,3-Diaminoxypropane (**4**) was synthesized by a modification of the previously described procedure.[13] A solution of *N*-hydroxy-5-norbornene-2,3-dicarboximide (9.94 g, 55 mmol), TEA (20 ml, 143 mmol) and dichloropropane (2.6 g, 23 mmol) in 50 ml of DMSO was refluxed for 15 h. After cooling, the reaction mixture was diluted with ethyl acetate and washed with water and brine. After drying over Na_2SO_4, the organic extract was concentrated and recrystallized from EtOH to yield 7.03 g (77%) of 1,3-diaminoxypropanebis(5-norbornene-2,3-dicarboxy)imide. ^{1}H NMR (300 MHz, $CDCl_3$), 6.19 (s, 4H), 4.17 (t, J = 6.1 Hz, 4H), 3.43 (brs, 4H), 3.19 (m, 4H), 1.98 (p, J = 6.1 Hz, 2H), 1.77 (d, J = 9 Hz, 2H), 1.51 (d, J = 9 Hz, 2H). The product of the previous reaction (6 g, 15 mmol) was dispersed in a solution of hydrazine hydrate (1.75 ml, 36 mmol) in 150 ml of ethanol and refluxed for 15 h. The reaction mixture was then acidified with 4 ml of 3 M HCl, refluxed for another 15 min and concentrated *in vacuo*. The crude product was washed with boiling EtOAc, filtered and crystallized from EtOH as the dihydrochloride. Yield, 1.35 g (50%). 1H NMR (500 MHz, DMSO-d_6), 10.98 (s, 6H), 4.06 (t, J = 6.2 Hz, 4H), 1.93 (p, J = 6.2, 2H); ^{13}C NMR (126 MHz, DMSO-d_6); 71.74, 27.08.

Benzaldehyde-*O*-methyloxime (**1**), Bis(aryloximes) **3**, and 3-pyridinealdehyde-*O*-phenyloxime (**6**) were synthesized in 75–85% yield by stirring the mixtures of the equivalent amounts of corresponding alkyl- or aryloxyamines and aldehydes in pyridine overnight, concentrating the solution *in vacuo* followed by crystallization of the products from EtOAc–EtOH. Analytical data. **1**: ^{1}H NMR (300 MHz, $CDCl_3$), 8.07 (s, 1H), 7.59 (m, 2H), 7.38 (m, 3H), 3.99 (s, 3H); ^{13}C (75 MHz, $CDCl_3$), 148.6, 132.2, 129.8, 128.7, 127.0. **3a**: ^{1}H NMR (300 MHz, DMSO-d_6), 8.91 (s, 2H), 8.75 (d, J = 5.0 Hz, 2H), 8.42 (s, 2H), 8.34 (d, J = 8.1 Hz, 2H), 7.73 (q, 2H), 4.30 (t, J = 6.5 Hz, 4H), 2.12 (p, J = 6.4 Hz, 2H); ^{13}C NMR (75 MHz, D_2O), 151.57, 150.88, 148.69, 146.71, 139.30, 134.72, 79.15, 35.37; Calculate for $C_{15}H_{16}N_4O_2^{\bullet}$ 2HCl: C, 50.43, H, 5.08, N, 15.68. Found: C, 50.44, H, 5.19, N, 15.27%. **3b**: ^{1}H NMR (500 MHz, DMSO-d_6), 13.19 (br s, 2H), 8.36 (s, 2H), 8.18 (s, 2H), 7.95 (d, J = 7.6 Hz, 2H), 7.84 (d, J = 7.6 Hz, 2H), 7.53 (t, J = 7.6 Hz, 2H), 4.25 (t, J = 6.4 Hz, 4H), 2.07 (p, J = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-d_6), 168.0, 149.4, 133.6, 132.6, 132.0, 131.6, 130.3, 128.7, 71.7, 40.2. **6**: ^{1}H NMR (300 MHz, CD_3OD), 8.78 (s, 1H), 8.55 (d, J = 4.8 Hz, 1H), 8.47 (s, 1H), 8.15 (dt, J = 1.8 Hz, 8.1 Hz, 1H), 7.45 (dd, J = 5.1 Hz, 7.8 Hz, 1H), 7.30 (t, J = 6.9 Hz, 2H), 7.20 (d, J = 7.8 Hz, 2H), 7.02 (t, J = 6.9 Hz, 1H); ^{13}C (75 MHz, CD_3OD), 160.6, 151.7, 150.1, 149.4, 136.1, 130.5, 129.7, 125.6, 123.8, 115.5.

For the synthesis of *O*-(*p*-nitrophenyl)hydroxylamine, a solution of *N*-hydroxy-5-norbornene-2,3-dicarboximide (3.07 g, 15 mmol) and anhydrous K_2CO_3 (2.61 g, 18 mmol) in anhydrous DMF (50 ml) was stirred at room temperature for 40 min, then 1-fluoro-4-nitrobenzene (1.6 ml, 15 mmol) was added, and the reaction mixture was stirred at 50 °C overnight. After removing the solvent *in vacuo*, the residue was diluted with 100 ml of saturated NaCl and extracted twice with $CHCl_3$ (350 ml). The organic layers were washed with brine, dried over Na_2SO_4 and, after evaporation of the solvent, yielded 4.16 g (92%) of the intermediate *N*-(4′-nitrophenyloxy)-5-norbornene-2,3-dicarboximide. ^{1}H NMR (300 MHz, $CDCl_3$), 8.23 (d, J = 7.5 Hz, 2H), 7.09 (d, J = 7.5 Hz, 2H), 6.32 (m, 2H), 3.54 (brs 2H), 3.41 (m, 2H), 1.86 (d, J = 9.0 Hz, 1H), 1.60 (d, J = 9.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$), 166.2, 157.5, 139.9, 130.9, 121.5, 109.8, 47.2, 40.5, 38.8. The solution of the intermediate

(300 mg, 1 mmol) in CH_2Cl_2 was mixed with hydrazine monohydrate (76.6 mg, 1.5 mmol) (suspension) and refluxed overnight. Evaporation of the solvent followed by column chromatography (silica, CH_2Cl_2) yielded 111 mg (72%) of *O*-(*p*-nitrophenyl)hydroxylamine. ^{1}H NMR (300 MHz, $CDCl_3$), 8.19 (d, J = 7.5 Hz, 2H), 7.25 (d, J = 7.5 Hz, 2H), 6.05 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$), 166.3, 141.8, 125.6, 113.3; MS (FAB); *m*/*z* 154.0 (M^+).

Kinetic studies. Kinetic runs were performed in 0.8–1 ml volumes in NMR tubes that were temperature controlled at the indicated temperatures and cooled to ambient temperature prior to taking the NMR spectra at every time point. Unless noted otherwise, the reactions were performed in deuterium oxide of 99.8% enrichment. The pD of the reagent stock solutions was adjusted by the D_2O solutions of TFA or HCl and/or NaOH before mixing. At least 16 acquisitions of the ^{1}H spectra were obtained for each time point. The spectra were processed with the aid of the authentic Varian software and the SwanMR software for Macintosh.[14] In most cases, the reaction course was monitored by integration of the signals of the starting oxime referenced to the signal of internal standard (1,4-dioxane) or the total integral of the aromatic signals for the starting compound and the products.

The pseudo-first-order rate constants were determined by non-linear curve fitting to the first-order reaction equation or from the initial rates (in the cases of slow reactions and where the pseudo-first-order conditions were not held). Activation parameters were determined by fitting the data to the linearized form of the Eyring equation:

$$\ln(k_{obs}/T) = \ln(k/h) + \Delta S^{\neq}/R - \Delta H^{\neq}/(RT).$$

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REFERENCES

1. For recent reviews on combinatorial chemistry see: (a) L. A. Thompson and J. A. Ellman, *Chem. Rev.* **96**, 555–600 (1996) (b) X. Williard, I. Pop, L. Bourel, D. Horvath, R. Baudelle, P. Melnyk, B. Deprez and A. Tartar, *Eur. J. Med. Chem.* **31**, 87–98 (1996); (c) F. Balkenhohl, C. von dem Bussche-Hünnefeld, A. Lansky and C. Zechel, *Angew. Chem. Int. Ed. Engl.* **35**, 2288–2337 (1996).
2. P. G. Swann, R. A. Casanova, A. Desai, M. M. Frauenhoff, M. Urbancic, U. Slomczynska, A. J. Hopfinger, G. C. Le Breton and D. L. Venton, *Biopolymers* **40**, 617–625 (1996).
3. (a) A. V. Eliseev and M. I. Nelen, *J. Am. Chem. Soc.* **119**, 1147–1148 (1997); (b) A. V. Eliseev and M. I. Nelen, *Chem. Eur. J.* **4**, 825–834 (1998).
4. I. Huc and J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **94**, 2106–2110 (1997).
5. (a) S. J. Rowan and J. K. M. Sanders, *Chem. Commun.* 1407–1408 (1997); (b) P. A. Brady and J. K. M. Sanders, *J. Chem. Soc. Perkin Trans. 1* 3237–3253 (1997); (c) P. A. Brady and J. K. M. Sanders, *Chem. Soc. Rev.* **26**, 327–336 (1997).
6. E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.* **84**, 832–837 (1962).
7. (a) W. P. Jencks, *J. Am. Chem. Soc.* **81**, 475–481 (1959); (b) T. K. Korpela and M. J. Mäkelä, *Anal. Biochem.* **110**, 251–257 (1981).
8. (a) B. M. Anderson and W. P. Jencks, *J. Am. Chem. Soc.* **82**, 1773–1777 (1960); (b) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.* **84**, 826–831 (1962).
9. (a) K. Koehler, W. Sandstrom and E. H. Cordes, *J. Am. Chem. Soc.* **86**, 2413–2419 (1964); (b) L. do Amaral, W. A. Sandstrom and E. H. Cordes, *J. Am. Chem. Soc.* **88**, 2225–2233 (1966).
10. J. Mollin, F. Kaspárek and J. Lasovsky, *Chem. Zvesti* **29**, 39–43 (1975).
11. W. P. Jencks, Catalysis in Chemistry and Enzymology. McGraw-Hill, New York, (1969).
12. (a) J. van Dijk and J. M. A. Zwagemakers, *J. Med. Chem.* **20**, 1199–1206 (1977); (b) A. Niwa, H. Iwamura, Y. Nakagawa and T. Fujita, *J. Agric. Food Chem.* **38**, 514–520 (1990); (c) R. M. Khomutov, T. Hyvönen, E. Karvonen, L. Kauppinen, T. Paalenen, L. Paulin, T. Eloranta, R.-L. Pajula, L. C. Andersson and H. Pösö, *Biochem. Biophys. Res. Commun.* **130**, 596–602 (1985); (d) T. Hyvönen, L. Alakuijala, L. Andersson, A. R. Khomutov, R. M. Khomutov and T. O. Eloranta, *J. Biol. Chem.* **263**, 11138–11144 (1988) (e) L. Persson, A. R. Khomutov and R. M. Khomutov, *Biochem. J.* **257**, 929–931 (1989); (f) R. Poulin, J. A. Secrist III and A. E. Pegg, *Biochem. J.* **263**, 215–221 (1989); (g) T. O. Eloranta, A. R. Khomutov, R. M. Khomutov and T. Hyvönen, *J. Biochem.* **108**, 593–598 (1990).
13. A. Rougny and M. Daudon, *Bull. Soc. Chim. Fr.* **5–6**, 833–838 (1976).
14. G. Balacco, *J. Chem. Inf. Comput. Sci.* **34**, 1235–1241 (1994).

