by these NMR assays. HRMS calcd for $C_8H_{13}O_2$ ($M^+ - C_4H_9$) 141.0915, found 141.0888. The stereostructures **62** and **63** were confirmed by comparison of their GLC retention times with those of authentic diepoxides prepared from (*E,E*)- and (*E,Z*)-5,7-dodecadienes, respectively, with *m*-chloroperbenzoic acid.

Conversion of 25 to the Diepoxide 64 and the Furan 65 (Entry 20). GLC: **64**, t_r 15.29 min (1%); **65**, t_r 5.11 min (76%; 5% PEG-HT on Uniport HP, 3-mm-o.d. × 3 m, 1.0 kg/cm², 110 °C; pentadecane as internal standard). **64**: ^{1}H NMR ($CDCl_3$) δ 1.2–2.2 (br, 8, 4 CH_2), 2.59 (d, 2, J = 5.3 Hz, 2 CHO), 2.85 (dd, 2, J = 5.3 and 1.3 Hz, 2 CHO); HRMS calcd for $C_8H_{12}O_2$ (M^+) 140.0837, found 140.0820. The 2,3-threo structure of **64** was confirmed by ^{1}H NMR analysis in the presence of the chiral shift reagent. Thus the peak at δ 2.59 (d) was separated into two peaks at δ 3.12 (d) and 3.14 (d) in the presence of Eu(hfc)$_3$ (a chiral shift reagent; 7.1 mol %) in $CDCl_3$. A 7:93 mixture of **64** and 2,3-erythro diepoxide (stereoisomer of **64**) was prepared by the diepoxidation of 1,2-dimethylenecyclohexane with *m*-chloroperoxybenzoic acid in CH_2Cl_2. Each of these two stereoisomers was isolated by preparative GLC (5% PEG-HT on Uniport HP, 12-mm-o.d. × 3 m). The 2,3-erythro isomer: ^{1}H NMR ($CDCl_3$) δ 1.6–1.9 (br, 8, 4 CH_2), 2.57 (d, 2, J = 5.3 Hz, 2 CHO), 2.72 (d, 2, J = 5.3 Hz, 2 CHO). This stereoisomer did not indicate any separation of peaks in the presence of the same chiral shift reagent, reconfirming the 2,3-threo structure of **64**. **65**: ^{1}H NMR ($CDCl_3$) δ 1.6–1.8 (m, 4, 2 CH_2), 2.4–2.7 (br, 4, 2 CH_2), 7.1 (s, 2, aromatic); ^{13}C NMR ($CDCl_3$) δ 20.1, 23.5, 121.5, 137.3.[75]

A Catalytic Decomposition of 2,3-Didehydro 1,4-Epiperoxides with $FeCl_2(PPh_3)_2$. The standard procedure for the reaction is illustrated by the conversion of 3,6-diphenyl-3,6-epiperoxycyclohexene (**18**) to the *syn*-diepoxide **50** (entry 8 in Table I).

To a solution of **18** (20.7 mg, 0.078 mmol) in CH_2Cl_2 (2 mL) $FeCl_2(PPh_3)_2$ (2.7 mg, 0.004 mmol) was added at −78 °C while being stirred under argon. After being stirred at this temperature for 9 h, the mixture was directly subjected to column chromatography on silica gel (2 g) using a 10:1 mixture of hexane and ether as eluant to give **50** (19.6 mg, 95%): TLC R_f 0.57 (1:1 ether/hexane); ^{1}H NMR ($CDCl_3$) δ 2.0–2.7 (m, 4, 2 CH_2), 3.32 (s, 2, 2 CHO), 7.2–7.7 (m, 10, Ar); ^{13}C NMR ($CDCl_3$) δ 26.3, 57.2, 57.9, 125.1, 127.7, 128.5, 140.5; MS, m/z 264 (M^+). Anal. Calcd for $C_{18}H_{16}O_2$: C, 81.79; H, 6.10. Found: C, 81.79; H, 6.14.

Table I lists experimental details for other cases (see entries 5, 7, 14, and 21). Unless otherwise stated, the reaction was conducted by a similar procedure.

Conversion of 16 to the Diepoxide 45 (Entry 5). LC: SiO_2 (5 g), ether/hexane (1:4 to 2:1, gradient) as eluant.

Conversion of Ascaridole (17) to the Diepoxide 46 (Entry 7). ^{1}H NMR analysis was conducted using methyl laurate as an internal standard.

Conversion of 26 to the Furan 66 (Entry 21). LC: SiO_2 (5 g), hexane/ether (5:1) as the eluant. **66**: ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 3, CH_3), 1.30 (s, 3, CH_3), 1.6–1.9 (m, 2, CH_2), 2.4-2.9 (m, 3, CHO and CH_2), 6.30 (br s, 1, Ar), 7.2–7.4 (m, 2, Ar).[4f]

Decomposition of 21 with $Co^{II}TPP$ (Entry 15). GLC was conducted using methyl laurate as the internal standard under such conditions as described in entry 11 in Table I.

Decomposition of 21 with $OsCl_2(PPh_3)_3$ (Entry 16). The reaction was conducted in a sealed glass tube under argon. GLC was conducted using methyl laurate as the internal standard under such conditions as described in entry 11 in Table I.

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of the diepoxides **62** and **63** (4 pages). Ordering information is given on any current masthead page.

(75) Okazaki, R.; Negishi, Y.; Inamoto, N. *J. Org. Chem.* **1984**, *49*, 3819.

Catalysis by Cucurbituril. The Significance of Bound-Substrate Destabilization for Induced Triazole Formation[1]

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A mechanistic investigation is described for the cycloaddition induced between $RNH_2^+CH_2C{\equiv}CH$ and $RNH_2^+CH_2CH_2N_3$ (R = H, *t*-Bu) consequent to encapsulation by the polycyclic molecular receptor cucurbituril ($C_{36}H_{36}N_{24}O_{12}$). The reaction is shown to be substantially accelerated (ca. 10^5-fold), and the kinetic characteristics of catalytic saturation behavior, substrate inhibition, and slow product release are documented. For substrates with R = *t*-Bu a rotaxane product results, and inhibition kinetics with $NH_3^+CH_2CH_2C(CH_3)_3$ are also examined. A rate enhancement attributed to bound-substrate destabilization is detected. The significance of this effect and its connection with the phenomenon of nonproductive binding in catalytic systems are discussed.

Understanding enzymic catalysis represents perhaps the most severe challenge of mechanistic organic chemistry. Because of the complexity of proteins, analogues which mimic aspects of biochemical catalysis are desirable. Excellent progress has been made in devising synthetic molecular receptors, and much is being learned about *specificity* in molecular recognition from imaginatively engineered models of this nature.[2] Even more arduous is

(1) Taken in part from the Ph.D. Thesis of M. Adhya, University of Illinois, Chicago, 1986; *Diss. Abstr. Int. B* **1986**, *47*, 1056.

(2) Recent reviews: Breslow, R. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1986**, *58*, 1. Franke, J.; Vogtle, T. *Top. Curr. Chem.* **1986**, *132*, 135. Sutherland, I. O. *Chem. Soc. Rev.* **1986**, *15*, 63. Cram, D. J. *Angew, Chem., Int. Ed. Engl.* **1988**, *27*, 1009. Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 362. Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 90. Rebek, J. *Top. Curr. Chem.* **1988**, *149*, 189. Stoddart, F. *Chem. Brit.* **1988**, *24*, 1203.

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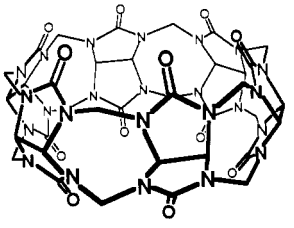
the securing of *kinetic acceleration* of reactions in such systems.[2,3] Certainly a major component of enzyme efficiency is the negation of entropic constraints through proper alignment of reacting substrate functionalities. However, additional catalytic benefit is in principle available to ligand–receptor systems which have potentially an *excess* of binding energy. We allude to the Pauling principle of catalysis, which states that complementarity between an enzyme and the *transition state* for its conducted reaction ought to be greater than that between enzyme and the reactants.[4] In that circumstance an additional promotion of the chemical transformation is attained, resulting in rates beyond that attributable to orientation considerations alone. The latter concept has assumed the status of dogma in theoretical enzymology, yet experimental verification has lagged. Consequently, acquisition of evidence in support of this principle is a critical objective.

Cucurbituril (1) is a novel nonadecacyclic cage compound with an exceptional capacity to encapsulate ammonium ions within its hollow core.[5] This synthetic receptor is readily assembled from the constituents glyoxal, urea, and formaldehyde: $CHOCHO + 2H_2NCONH_2 \rightarrow C_4H_6N_4O_2$ (glycoluril); $6C_4H_6N_4O_2 + 12CH_2O \rightarrow$ **1**, $C_{36}H_{36}N_{24}O_{12}$ (cucurbituril). Extensive molecular recognition studies have been carried out using **1**, with the following conclusions. Generally, the set of six *carbonyl dipoles* surrounding each occulus of **1** constitutes a *cation* binding site (i.e., for H_3N^+R). The collection of partially anionic oxygen atoms provides a fixed locus of attraction for positively charged ligands, particularly when they are H-bond donors. In the resulting complex, a properly sized substituent (R) on the bound cation may concurrently enter the interior of **1** with release of solvent molecules, thereby providing a favorable hydrophobic contribution to complexation. Because of the structural rigidity of **1**, exceptional selectivity is noted in the stoichiometric binding of ligands in the mode indicated.[6]



1

This article is concerned with *catalysis* by **1**. We find that simultaneous binding of a pair of appropriate substrates within **1** greatly facilitates a cycloaddition reaction. The kinetic characteristics of the reaction indicate operation of the Pauling principle of catalysis.[7]

Results

Alkynes undergo 1,3-dipolar cycloaddition with alkyl azides yielding substituted triazoles (**2** + **3** → **4** + **5**). In the case shown, the reaction proceeds slowly in aqueous formic acid at 40 °C, $k_0 = 1.16 \times 10^{-6}$ M^{-1} s^{-1}. The cycloadditions of azides have received intense mechanistic scrutiny previously; the process appears to be a typical concerted pericyclic reaction. No evidence has accrued for occurrence of any intermediate in such transformations.[8]

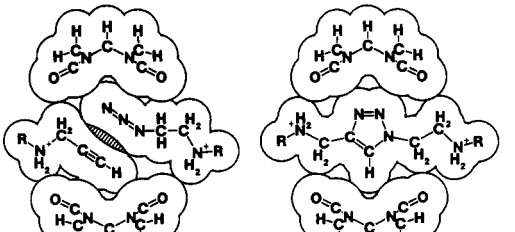
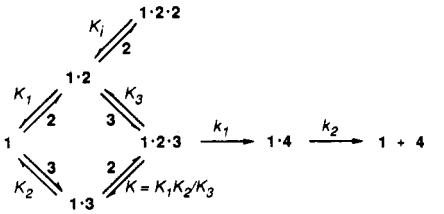
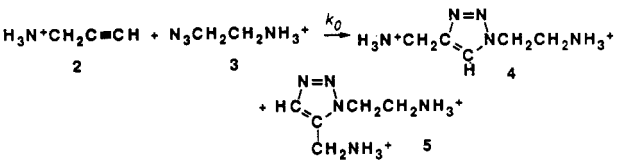


Figure 1. Conjectured cross-sectional representation of intermediate cycloaddition complexes: 1·substrates and 1·product (R = H or *t*-Bu). Outlines drawn to van der Waals radii (maximum projection for all atoms upon axial rotation of **1**, crystallographically determined interatomic distances for **1**). Shaded region corresponds to strain-induced compression of substrates, promoting reaction (see Discussion).

Scheme I





2 **3** **4** **5**

We find that a catalytic amount of **1** markedly accelerates the reaction depicted and renders it regiospecific, yielding only **4** as a product. This result is explained by formation of a transient ternary complex between **1**, **2**, and **3**. Simultaneous binding of both **2** and **3** (with one NH_3^+ coordinated to each set of carbonyls and with the substituents extending into the interior of **1**) aligns the reactive groups within the core of **1** so as to facilitate production of **4**. The proposed mechanism may be visualized with the aid of Figure 1.

This reaction has a number of convenient features which render it suitable for mechanistic investigations. Kinetics may be easily followed by disappearance of azide UV absorbance. Most importantly, saturation behavior is noted; with sufficient amounts of **2** and **3** the cycloaddition becomes independent of substrate concentration. As is familiar from enzyme catalysis, this indicates complete occupancy of all available **1** by the reacting species. We shall develop evidence showing that Scheme I properly describes the kinetic behavior for the catalyzed reaction.

The proposed sequence implies a random incorporation of either of the reactants into **1**, with subsequent formation of a common ternary complex (**1·2·3**), which undergoes conversion to product. Equilibrium binding of the reactants singly to **1** may be examined by NMR spectroscopy. The individual (binary) dissociation constants have been determined by previously described[6] competitive com-

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(4) Pauling, L. *Am. Sci.* **1948**, *36*, 51. For a discussion, see: Jencks, W. P. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 219.

(5) Freeman, W. A.; Mock, W. L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1981**, *103*, 7367.

(6) Mock, W. L.; Shih, N.-Y. *J. Org. Chem.* **1986**, *51*, 4440. Mock, W. L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1988**, *110*, 4706.

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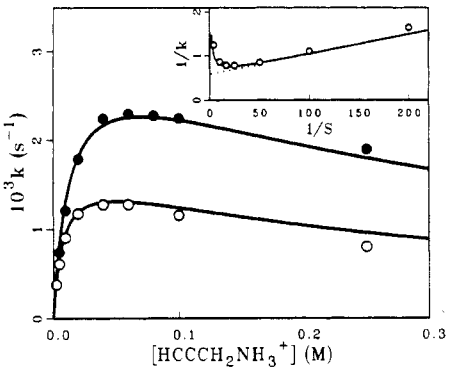


Figure 2. Dependence of apparent rate of cycloaddition within 1 upon substrate concentration for **2**. Open circles, azide (**3**) concentration 0.03 M; filled circles, 0.06 M. Inset: same data in double reciprocal (Lineweaver–Burke) format, emphasizing substrate inhibition (upward curvature in plot).

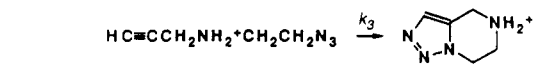
plexation experiments involving a reference standard; K_1 = 0.000 65 M for **2**, K_2 = 0.0025 M for **3**. In principle a value for K_3 (the dissociation constant for ternary adduct formation) and a rate constant for conversion to products should be accessible from kinetic measurements. It may easily be shown that *product release* from **1** (k_2 step in Scheme I) is rate-limiting for the overall catalytic reaction sequence. As may be seen from Figure 1, the openings into the interior or **1** represent a constriction through which branched-chain and cyclic guests have difficulty in traversing, a phenomenon which has previously been studied.[9] Rates of decomplexation are measured by a displacement technique, in which the velocity of substitution of one guest contained within **1** by an excess of another is measured by NMR or UV spectroscopy. It has invariably been found that the rate of exchange is independent of the nature or the concentration of the displacing agent, implying a dissociative mechanism. In the present instance the derived rate constant for release of **4** from a stoichiometric complex with **1** was found to be identical with that of the catalytic reaction under conditions of saturation of **1** with **2** and **3**, $k_2 = 1.7 \times 10^{-4}$ s^{-1}.

Since product dissociation is the slow step, no information about the actual cycloaddition stage is accessible from steady-state kinetics.[10] However, by using a relatively large amount of **1**, a "burst phase" at the initiation of reaction may be detected spectroscopically. This must correspond to "loading-up" of **1** with product **4**, and consequently the rate of the induced cycloaddition may also be examined by careful data collection at early stages of reaction. We have measured presteady-state kinetics for the cycloaddition reaction as a function of the concentrations of **2** and **3**, and representative *initial rate* data is given in Figure 2.

A complicating feature is immediately apparent. As expected, an increase in the concentration of **2** leads to a levelling-off in reaction velocity, corresponding to saturation of **1** by this substrate. However, at yet higher concentration of **2** the reaction velocity actually *decreases*. This is attributed to *substrate inhibition*; i.e., a complex containing a pair of molecules of **2** is produced, the second of which excludes **3** and thereby prevents reaction.[11] This is not unreasonable, since the two substrates are of similar dimensions and have nearly the same affinity for **1**. The phenomenon is incorporated into Scheme I with an inhibition constant K_i, corresponding to formation of **1·2·2**. However, this behavior introduces a major ambiguity into construing the kinetic data. Upon statistical analysis, the value for k_1 (the maximum rate for cycloaddition, corresponding to complete preliminary conversion of **1** into **1·2·3**) depends critically upon the relative magnitudes of K_i and K_3 (Scheme I, K_3 pertaining to dissociation of **1·2·3**). Mutually independent values for the latter two parameters may not be adequately secured by the usual nonlinear least-squares curve-fitting procedure. Under these circumstances the best solution is to assume that $K_i = K_3$; i.e., that both **2** and **3** bind equally well to a preexisting complex of **1·2**, as is chemically plausible. The curves drawn in Figure 2 are a best fit to the appropriate kinetic expression,[12] and the resulting parameters are as follows: k_1 = 0.019 (±0.002) s^{-1} and $K_3 = K_i$ = 0.30 (±0.04) M.

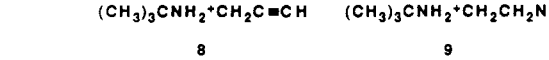
At this point the sometimes controversial matter of "catalytic acceleration" may be addressed. Because k_1 is a first-order rate constant (for spontaneous conversion of **1·2·3**), it is obviously inappropriate to compare its value with the previously provided rate constant (k_0) for the bimolecular reaction of **2** plus **3** in the absence of **1**. However, since K_3 is actually a Michaelis constant, the ratio k_1/K_3 constitutes a second-order rate constant for reaction between **3** and the saturated complex **1·2** (k_1/K_3 = 0.063 M^{-1} s^{-1}). Hence, the kinetic acceleration by comparison of *bimolecular* reactions is a factor of 5.5×10^4. This constitutes the proper index of effectiveness of 1.[13] However, it should be acknowledged that since $k_1 > k_2$, the true *catalytic* benefit (comparison of k_0 with k_2/K_3) is only 4.9×10^2 by this criterion.[13]

As an independent check, we have prepared model substance **6**, which undergoes uncatalyzed (intramolecular) conversion to **7** with a rate constant of $k_3 = 2.0 \times 10^{-5}$ s^{-1} under the standard conditions (HCO_2H–H_2O, 40 °C). Comparison of this number with k_1 indicates an acceleration of 9.4×10^2 for the previously described cycloaddition within **1**. The model **6** has free rotations about several bonds, which must be frozen in the transition state for its cycloaddition. Consequently, the relative unreactivity in formation of **7** may be explained.



6 **7**

However, there are two unsatisfactory aspects of the kinetic results obtained for the induced reaction between **2** and **3**. One has been previously alluded to; substrate inhibition by **2** allows only an extrapolated estimate for k_1, based upon an assumption about the relative magnitudes of K_3 and K_i. Secondly, there are practical limitations in the acquisition of kinetic data, stemming from the fact that the k_2 step is not much slower than that of k_1. At certain substrate concentrations the presteady-state initial rates are not well-enough delineated from the catalytic rate for meaningful separation of the burst phase. Consequently, we sought to modify the structure of our substrates so as to improve the quality of the data. The successful solution was N-*tert*-butylation of both substrates; i.e., partial repetition of the kinetic study with reactants **8** and **9**.



8 **9**

(9) Mock, W. L.; Shih, N.-Y. *J. Am. Chem. Soc.* **1989**, *111*, 2697.
(10) Product release as the *rate-limiting* step in enzymic reactions is a common phenomenon: Cleland, W. W. *Acc. Chem. Res.* **1975**, *8*, 145.
(11) Substrate inhibition is another frequently encountered characteristic of enzymic reactions.
(12) The fitted equation is $k_{app} = k_1[2][3]/\{[2][3] + K_3([2] + K_1(1 + [3]/K_2) + [2][2]/K_3)\}$, in which $K_i = K_3$.
(13) Since the uncatalyzed reaction rate (k_0) corresponds to formation of two regioisomers (in equal amount), the kinetic acceleration factor given could justifiably be doubled (statistical correction).

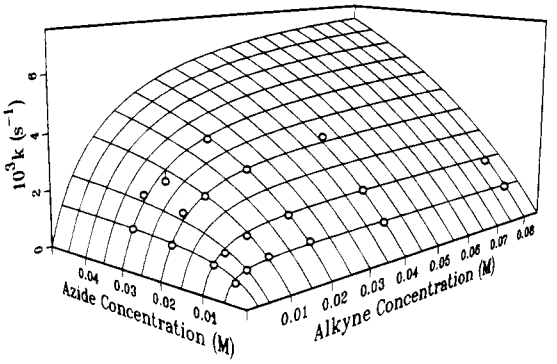
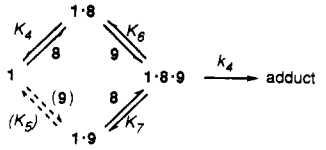


Figure 3. Three-dimensional presentation showing simultaneous dependence of apparent rate of cycloaddition within 1 upon substrate concentrations for **8** and **9**. Cross-sections on surface (grid lines) correspond to Michaelis curves as in Figure 2; circles are data points correlating with concentrations given by intersections in grid.

Scheme II



A *tert*-butyl group is too bulky to fit into or to pass through the interior of **1**.[6] Consequently, the reaction between **1**, **8**, and **9** yields a nondissociable complex, or *rotaxane*[14] (Figure 1, R = CMe_3). Because the cycloadduct may no longer escape at all from **1**, the reaction ceases to be catalytic in the strict sense. While this might seem to defeat the purpose of this investigation, it solves the second of the experimental limitations listed previously. With relatively high concentrations of **1**, valid initial rates may now be secured at all substrate concentrations, with confidence that only the cycloaddition step is being observed. Less obviously, the problem of substrate inhibition is also overcome. For reasons about which we shall subsequently speculate, in the induced reaction with **8** and **9** the characteristic fall-off in rate at high concentrations of alkyne seen in Figure 2 does not occur, and the kinetic analysis is correspondingly further simplified.

In this latter cycloaddition, the individual reactants were found to have similar affinities as the previous substrates for **1**. Independent determinations gave binary dissociation constants K_4 = 0.000 44 M for **8** and K_5 = 0.0035 M for **9**. The results from initial rate measurements for the cycloaddition in the presence of **1** are summarized in Figure 3. Saturation behavior with respect to **8** is cleanly observed at several different concentrations of **9**, with no evidence of substrate inhibition. It proved impracticable to saturate similarly **1** with **9**. The required acquisition of spectroscopic rate data involving a small change in a strongly absorbing species (**9**) would be inherently unreliable, so that only the concentration ranges depicted could be usefully examined. The surface drawn in Figure 3 corresponds to a simultaneous least-squares fit of the data to the appropriate equation for Scheme II, which is a simplified version of Scheme I.[15] In this instance the Michaelis constants K_6 and K_7 were independently inserted as adjustable parameters in the data processing, in order to provide an unbiased picture of the kinetic behavior of the system.

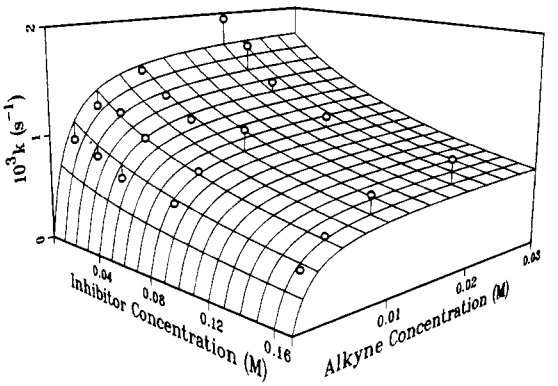


Figure 4. Three-dimensional presentation showing dependence of apparent rate of cycloaddition within **1** upon concentration of inhibitor **10**, with various concentrations of **8**, and with **9** at concentration 0.01 M. Grid surface as for Figure 3.

The least-squares rendering of kinetic data yields the following parameters (for Scheme II): k_4 = 0.023 (±0.008) s^{-1}, K_6 = 0.095 (±0.040) M, and K_7 = 0.033 (±0.014) M. The value for the rate constant k_4 is, within experimental error, the same as previously observed for conversion of **1·2·3** (i.e., k_1). However, a problem arises when our data is checked for internal consistency. The equilibrium constants in Scheme II must as a matter of thermodynamic necessity conform to the relationship $K_4 \cdot K_6/K_7 = K_5$. When the derived values for these constants from data processing are inserted into that equation, the "calculated" value for K_5 (namely, 0.0013 M) is at variance with the independently measured value of 0.0035 M, as obtained by direct equilibrium competition studies. The discrepancy factor of 2.7 is unexplained; it may reflect experimental error (particularly in kinetic measurements involving elevated concentrations of **9**) or it may indicate that Scheme II is an oversimplification. For example, the order of association of substrates with **1** may not be completely random, or perhaps an additional rate constant is needed, or some substrate inhibition (by **9**) may also be intruding in this case, etc. Regardless, from Figure 3 the value of K_7 would seem to be well-determined, so that we may calculate a second-order rate constant defined as k_4/K_7 (0.7 M^{-1} s^{-1}). Once again a substantial kinetic acceleration is indicated, in this case a factor of 6×10^5 when compared to the reaction of **2** and **3** in the absence of **1**.

Because of the previously mentioned incongruency in substrate binding affinities (K_5) and because of some other interpretational ambiguities for the induced cycloaddition mechanism, we undertook an *inhibition* study for the reaction between **1**, **8**, and **9**. By deliberate incorporation of a third, nonreactive alkylammonium ion at suitable concentrations in the reaction mixture, it should be possible to retard the cycloaddition by competitive exclusion of one or the other of the reactants from the binding loci of **1**. Such a study is desirable for elucidation of the phenomenon of *nonproductive binding* of substrates, which is a major concern subsequently to be discussed.

The inhibitor chosen for detailed kinetic examination is neohexylamine (**10**, $Me_3CCH_2CH_2NH_2$). In acid solution it provides an ammonium ion for coordination to the cation binding site of the receptor (dissociation constant with **1**, K_8 = 0.057 M, measured by competition experiments[6]), while at the same time the alkyl moiety is *excluded* from the interior of **1** by the bulk of the *tert*-butyl residue (as is evidenced by NMR spectroscopy of the complex with

(14) Schill, G. *Catenanes, Rotaxanes and Knots*; Academic: New York, 1971.

(15) The fitted equation is $k_{app} = k_4[8][9]/\{[8][9] + K_6([8] + K_4) + K_7[9]\}$. Because K_4, K_5, K_6, and K_7 are interdependent constants, the known value for K_5 was excluded in the data processing and was instead subsequently used for verification of the results.

1). Consequently, **10** may serve as a nonfunctional surrogate for the similarly dimensioned substrates **8** or **9**.[16]

The effect of several different concentrations of **10** on the induced cycloaddition between **8** and **9** are displayed graphically in Figure 4 for a fixed concentration of **9** and various concentrations of **8**. Quite evidently the reaction suffers a systematic retardation in the presence of **10**. By appropriate manipulation, it is possible to extract an inhibition constant (K_i' value) from this data, which provides a measure of the affinity of **10** for the partially occupied complexes of **1·8** or **1·9**. This has been done statistically by an expansion of Scheme I. Additional equilibria are incorporated; namely, allowance is made not only for formation of the directly observed complex **1·10**, but also for ternary complexes **1·8·10** and **1·9·10**, all of which diminish the observed rate of cycloaddition.[17] Fitting of an amalgamation of the data shown in Figures 3 and 4 to the appropriately elaborated expression[18] then gives K_i' = [**1·8**][**10**]/[**1·8·10**] = [**1·9**][**10**]/[**1·9·10**] = 0.114 (±0.030) M; i.e., a value for the dissociation constant of externally ligated **10** in **1·8·10** or **1·9·10**. The numerical magnitude of this parameter is only twice that of the (binary) equilibrium dissociation constant for **1·10**, showing that occupancy of **1** by a solitary substrate molecule only marginally perturbs *external* ligation to the unoccupied occulus of **1** by a second ammonium ion, although such binding does effectively prevent the cycloaddition reaction.[19]

Discussion

The premier observation from this study is the substantial "kinetic acceleration" of ca. 10^5 seen in the reaction between an appropriate pair of cycloaddition participants bound within **1**.[20] Although the net catalytic benefit in the formation of **4** is diminished by virtue of product release from **1** being rate-limiting, the actual chemical conversion step is nonetheless markedly enhanced, even in comparison with the unimolecular model reaction **6** → **7**. For the most part this may be attributed to an overcoming of *entropic* constraints. As Figure 1 depicts, simultaneous binding of both substrates within **1** would appear to orient them optimally, leaving little freedom for any motion other than that leading to fusion of the reactants.

However, close scrutiny of the kinetic parameters for the induced reaction suggests that another factor may be of demonstrable significance in this cycloaddition. In both of the cases here examined the values of the ternary dissociation constants, K_3 = [**1·2**][**3**]/[**1·2·3**] or K_7 = [**1·9**]-[**8**]/[**1·8·9**] as obtained from saturation kinetics, are numerically larger than the corresponding binary dissociation constants, K_2 = [**1**][**3**]/[**1·3**] and K_4 = [**1**][**8**]/[**1·8**] as measured independently by direct observation. Such negative cooperativity means that the presence of one reactant within the interior of **1** *diminishes* the thermodynamic affinity of the second reactant for **1**. This in turn implies that the cavity within **1** is slightly undersized for optimal binding. Therefore, the substrates experience a *strain*[21] when they are simultaneously incorporated, which tends to *compress* them together. The shading in Figure 1 is intended to represent this. Previous examination of macroscopic pressure effects in 1,3-dipolar cycloadditions[22] as well as common sense indicate that the strain energy would tend to promote reaction. This should have additional kinetic benefits beyond that attributable to orientational effects alone, provided only that the *transition state* for the cycloaddition were a better fit to the cavity within **1**, as can readily be imagined.[8,23]

What we have suggested is an example of the Pauling principle of catalysis, which holds a prominent place in current theories of enzyme efficacy.[4] In the present instance it may be quantified as the ratio of the ternary to the binary dissociation constants, a factor of 120 in the case of **2** reacting with **3**, although only a factor of 75 in the case of **8** plus **9**. (The latter substrates presumably provide more reliable kinetic data, for reasons previously described.) While these numbers may seem modest, the existence of the effect appears secure. If the entirety of this strain were relieved in the transition state, a 2–3 kcal/mol reduction in the activation energy would ensue. Furthermore, what is shown may actually represent a *lower limit*; i.e., the true magnitude of the phenomenon should not be less and might in fact be considerably larger in this instance. We should like to briefly elucidate why this is so, for it provides some practical insight into the problems of designing catalytic systems which take advantage of the Pauling principle.

The uncertainty in establishing an actual magnitude for strain activation of bound substrates stems from the possibility of *nonproductive binding*. In addition to the *productive* mode depicted in Figure 1, one can readily imagine an alternative *strain-free* configuration in which *one* of the substrates is bound similarly (with reactive moiety internal to **1**), but in which the *second* substrate has only its ammonium portion coordinated to an occulus of **1**, with the reactive group (and *tert*-butyl, if present) remaining in the exterior region (i.e., a nonproductive complex). If the strain energy associated with the productive configuration were severe enough, the kinetically detected species under conditions of saturation of **1** with substrates could actually have the hypothesized nonproductive geometry. The chemical mechanism for product formation would be unchanged, except that the reactive species would be a *minor* participant in a prefatory equilibrium involving productive and nonproductive complexes. However, in this situation the kinetically derived ternary dissociation constants (K_3 and K_6 or K_7 in Schemes

(16) *tert*-Butylamine could not be employed as inhibitor because of the insolubility of its salts in the reaction medium.

(17) A possibility of substrate inhibition by **9** was also allowed, since it cannot be excluded by the data.

(18) The fitted equation pertinent to a modified Scheme I/II is $k_{app} = k_4[8][9]/\{[8][9] + [9]K_6(1 + ([9][10]/K_i') + (K_5/[9])(1 + [10]/K_8) + ([8]/K_4)(1 + [10]/K_i')\}$, into which have been introduced additional terms corresponding to inhibitor complexes **1·10**, K_8 = 0.057 M; **1·8·10**, K_i' = adjustable parameter; **1·9·10**, same K_i'; and **1·9·9**, substrate inhibition by **9**, also same K_i'; with the identities in K_i' stipulated in order to limit the number of adjustable parameters. Substrate inhibition by **8** is not incorporated, since the data appears to exclude it.

(19) The difference is partly statistical; **1·8** has only a single unoccupied occulus, whereas empty **1** has two.

(20) This quantity is best regarded as an approximation. For the pair of cases examined its value differs by an order of magnitude (see also ref 13). Furthermore, since $K_6 \neq K_7$ the calculated acceleration factor depends upon which substrate is used to saturate **1** in the kinetic analysis. This latter ambiguity is unavoidable.

(21) Strictly speaking, the distortion of **1** induces a *stress* in **2·3**; the reflexive term strain is used by chemical convention; Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman: New York, 1985; p 342. Although 5-membered rings are bound optimally within **1**, a *benzene* ring (which may approximate the combined volume of substrates poised for cycloaddition) experiences compression when ensconced within **1** (ref 6).

(22) For a number of 1,3-dipolar cycloaddition reactions a large *negative* volume of activation ($\Delta V^{\ddagger}$ ca. −20 cm^3 M^{-1}) has been observed: Yoshimura, Y.; Osugi, J.; Nakahara, M. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 680. Yoshimura, Y.; Osugi, J.; Nakahara, M. *J. Am. Chem. Soc.* **1983**, *105*, 5414. Swieton, G.; Jouanne, J. v.; Kelm, H.; Huisgen, R. *J. Org. Chem.* **1983**, *48*, 1035. This is further discussed in ref 8.

(23) The apparent excellent fit of the product is actually nonbeneficial, for it contributes to the slowness of product release, which is in fact rate-limiting. Actually, C–C and C–N bonds exocyclic to the triazole ring must experience some bending in the product complex, in order that the 1,3-substituent CH_2 units may be directed to the portals in **1**. Consequently **1·4** is also not without some unique strain, which may be absent in the transition state for cycloaddition. This may facilitate product release.

I and II) would pertain to the nonproductive binding mode, and the strain energy which provides kinetic acceleration to the productively bound species would be *negated* by an unfavorable equilibrium within the overall saturation complex. In an extreme circumstance, the mechanistic step of conversion of nonproductive to productive complex could actually become rate-limiting, rather than the cycloaddition chemistry itself. Indeed, it can be argued that catalytic efficiency theoretically would be maximized if these two steps were of equal velocity. The overall rate for sequential reactions is of the following form: $k_{net} = k_a k_b/(k_a + k_b)$. If factors tending to *increase* k_b (such as excessive strain) were accompanied by an unavoidable and compensatory *decrease* in preceding step k_a (formation of strained complex, as here stipulated), it may easily be shown that maximum throughput corresponds to the compromise situation where $k_a = k_b$.

Clearly, the major practical constraint in realizing substantial strain acceleration in a catalyzed reaction is the *minimization of nonproductive binding*. Not only must the reactants be compressed in a particular configuration, but there must be a careful avoidance of any alternative binding mode of comparable stability. With enzymes this problem of exclusion of nonproductive binding modes has been solved by natural selection. Much of the precise but cryptic disposition of apparently nonfunctional residues in active site regions may exist for this inconspicuous reason. Attempts at de novo catalyst design involving this principle of bound-substrate destabilization (including the immunological or "abzyme" approach), or even the use of mutagenic techniques to "improve" an existing enzyme, may unwittingly encounter this aspect of catalysis as a genuine manifestation of what has been known jocularly as Murphy's law.

Caveats aside, what can be said in this regard about the reactions induced by 1? Does the saturation behavior which we observe correspond to a nonproductive mode of binding, in which case the true strain activation could actually be much greater? The near identity of rate constants k_1 and k_4 does suggest otherwise. However, the question is not easily resolved because the ternary complex itself may not be examined. The inhibition study with neohexylamine (**10**) was undertaken as an indirect way of exploring this possibility. As previously indicated, due to its steric bulk **10** may only bind in external fashion to **1** (analogously to the nonproductive mode). However, as a primary amine **10** provides a *mono*substituted ammonium ion, which might be expected to participate with *threefold* H-bonding to an occulus of **1**. This ought to yield a stronger ion–dipole interaction at this center than would be the case for either **8** or **9**, which may only form two H-bonds. (This reasoning may be extended to provide a plausible explanation as to why **2** exhibits substrate inhibition, while **8** does not.[24]) Therefore, one may argue that the nonproductive binding mode ought to be weaker for **8** or **9** than for **10**. But if the pertinent data is examined, it will be noted that the value of K_i' for **10** (0.114 M) is threefold *larger* than the kinetically obtained ternary binding constant K_7 for **8** (0.033 M). Because this latter number corresponds to a tighter experimental binding affinity for a substrate than one might reasonably expect for nonproductive binding, we infer that our kinetic constants do indeed pertain largely to *productive complexation* (Figure 1). Although some compressional repulsion apparently exists in this ternary complex, its formation is nevertheless energetically *just as favored* as the putative nonproductive mode, probably because of a hydrophobic effect and/or favorable dispersion forces operating on the reactive substrate moieties.

In conclusion, the idea behind the Pauling principle of catalysis is that potential binding energy in *excess* of the minimum necessary to bring the substrates together may in favorable cases be channeled to facilitate their further conversion to products. As we have indicated, objective evidence for this phenomenon is that binding of the second substrate to the catalyst in the presence of the first should be less favorable than in its absence. This would seem to be a necessary condition for claiming a demonstration of the effect, but even then ambiguity remains for the general case. On one hand the magnitude of the phenomenon could easily be masked by nonproductive binding, and on the other hand such negative cooperativity in substrate binding need not automatically be relieved entirely in the transition state for reaction.

The proper criterion for measuring kinetic acceleration in catalyzed bimolecular reactions is a comparison of second-order rate constants, which entails knowledge of velocities for one substrate reacting with a saturated complex of the second substrate plus catalyst (in the present case an observed factor of ca. 10^5 for the induced reaction itself). In enzymology these parameters (e.g., k_1/K_3) are known as *specificity constants*. It is relevant to note that any catalytic benefit arising from *reactant destabilization* as previously evinced for the cycloaddition is *not reflected* in the specificity constant, due to a cancellation of the opposing consequences for substrate binding (K_3) and for the velocity of the ensuing reaction (k_1). In order to produce a Pauling principle effect in this kinetic parameter, there must be unique, positive (attractive) intermolecular forces between transition state and catalyst, which are specifically absent in the initial complex of reactants. There is no autonomous method of experimental detection of such forces, in a manner analogous to that shown here for bound-substrate destabilization. Although Pauling principle phenomena may be central to biocatalytic efficiency, unambiguous substantiation in a magnitude greater than here provided remains problematical.

Experimental Section

Materials. Cucurbituril (**1**) was prepared as a multihydrate by a modification of the procedure of Behrend.[25] Propargylamine (**2**) and neohexylamine (**10**) were obtained from a commercial source, and azidoethylamine (**3**) was prepared as previously described.[26] *N-tert*-Butylpropargylamine (**8**) was obtained by the reaction of excess *tert*-butylamine with propargyl bromide (CH_3OH, 50 °C, 5 h), bp 112–125 °C. It was converted to the hydrochloride, which was purified by recrystallization, mp 192–196 °C (from $C_6H_5CH_3$–CH_3OH). Anal. Calcd for $C_{17}H_{13}N$·HCl: C, 56.94; H, 9.56; N, 9.49. Found: C, 56.72; H, 9.59; N, 9.20. *N-tert*-Butylazidoethylamine (**9**) was obtained from *N-tert*-butylchloroethylamine hydrochloride[27] and sodium azide (H_2O, 75 °C, overnight), followed by alkaline steam distillation, vacuum distillation (45 °C, 3.5 mm), and conversion to the hydrochloride, mp 126–129 °C (from $C_6H_5CH_3$–CH_3CH_2OH). Anal. Calcd for $C_6H_{14}N_4$·HCl: C, 40.36; H, 8.46; N, 31.36. Found: C, 40.59; H, 8.70; N, 31.46. The bifunctional amine **6** was obtained similarly from azidoethylamine and propargyl bromide. While stable enough to be isolated, it spontaneously cyclized to **7**. The rate of the latter transformation was monitored spectroscopically on

(24) Inspection of Figure 1 shows that *productive* binding of **2** may only yield two H-bonds (see also ref 6). Hence, the nonproductive mode with **2** may compete better than in the case of **8**.

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freshly prepared material in acidic solution. The cyclization product 7 was isolated as the hydrochloride, mp 187–190 °C dec. Anal. Calcd for $C_5H_8N_4 \cdot HCl \cdot H_2O$: C, 33.62; H, 6.21; N, 31.37. Found: C, 34.09; H, 6.13; N, 31.64.

A preparative reaction yielding 4 was carried out by allowing a hydrochloric acid solution containing 2 and 3 plus a catalytic amount of 1 to stand for several days. Solvent evaporation and recrystallization of the residue gave 4 as the hydrochloride, mp 264–266 °C (from H_2O–CH_3CH_2OH). Anal. Calcd for $C_5H_{11}N_5 \cdot 2HCl$: C, 28.05; H, 6.72; N, 32.71. Found: C, 28.47; H, 6.12; N, 32.47. A stoichiometric adduct was preparatively obtained by allowing 1 to react with a slight excess of 8 and 9 in dilute hydrochloric acid for 14 days. Evaporation of solvent and recrystallization yielded the rotaxane as a hydrate, mp >300 °C (from H_2O–CH_3CH_2OH). Anal. Calcd for $C_{36}H_{36}N_{24}O_{12} \cdot C_{13}H_{17}N_5 \cdot 2HCl \cdot 12.5H_2O$: C, 38.01; H, 5.86; N, 26.24. Found: C, 37.84; H, 6.19; N, 26.57.

NMR spectra consistent with the structures of all substrates and products were obtained.[1]

Kinetics. The standard reaction medium employed throughout this work consists of a 1:1 (v/v) mixture of 88% formic acid plus water at 40.0 (±1.0) °C.[6] Rate measurements were obtained by adding 1.0 mL of a freshly prepared 40 °C stock solution of substrate azide (0.015–0.09 M) to 2.0 mL of a stock solution of 1 (0.003–0.0075 M) and substrate alkyne (0.00375–0.12 M) in a spectrophotometer cell at 40.0 °C. The initial decrease in azide absorbance (281 nm) was recorded, and the tangent to the absorbance vs time curve was determined by a computer-assisted, iterative least-squares line-fitting procedure. The total absorbance change after the reaction had run to completion was subsequently obtained, for securing rates in concentration units.[28] Duplicate kinetic runs concordant within 10% were routinely observed. The same techniques were employed for the inhibition study involving 10. Tolerances listed in this article are standard errors from least-squares analysis, for the most part.

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(28) This procedure was essential for the catalytic reaction of 2 plus 3 and was found to be quicker and more reproducible than a full exponential fit of the absorbance vs time plot for the induced reaction of 8 plus 9 (probably because of the long-term instability of 9). However, the latter procedure was employed for 6, for measuring product release from 1-4, and for the uncatalyzed control reaction of 2 plus 3 (second-order fit), with use of a quantitative NMR analysis.

Selective Reduction of Aryl Halides and α,β-Unsaturated Esters with Sodium Borohydride–Cuprous Chloride in Methanol and Its Application to Deuterium Labeling

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A reducing system, $NaBH_4$–Cu_2Cl_2/MeOH, was developed for dehalogenation of aryl halides, conjugate reduction of α,β-unsaturated esters, and deuterium labeling in a chemo- and regioselective manner. These reactions proceeded without reduction of isolated olefins. The Cu_2Cl_2 is assumed to function as the catalyst which generates a transient species of copper hydride as an active reducing agent, on contact with $NaBH_4$. Deuterium-labeling studies indicated that (i) the hydrogen which was transferred to the 4-position of methyl 4-iodobenzoate originates from MeOH and (ii) the hydrogens which were transferred to the α- and β-positions of the conjugated ester originate from MeOH and $NaBH_4$, respectively.

$NaBH_4$ is widely used as a common reagent for selective reduction of carbonyl compounds[1] and, in some cases, in combination with metal salts and various solvents provides a variety of reducing systems.[2] Expansion of the capability of these reducing systems would be useful, and it is also desirable to improve their chemo- and regioselectivity.

We needed a convenient method of preparing deuterated compound [4'-^{2}H]-1 for synthetic studies, and one attractive route was the selective replacement of the iodine atom of aryl iodide 2 with a deuterium atom. Only two methods for such a reaction have been reported.[3,4] The reduction of *o*-iodonitrobenzene to *o*-deuterionitrobenzene was reported to be readily achieved by using $NaBH_4$ in a 4:1 mixture of DMSO–D_2O,[3] but 2 did not undergo reduction under similar conditions. Bosin and co-workers[4] have successfully used $NaBH_4$ in MeOH in the presence of $PdCl_2$ for selective reduction of *p*-chlorobenzoic acid to the corresponding para-deuterated derivative, but application of this method to 2 generated 21% of the undesired overreduction product 3 besides 34% of the desired product [4'-^{2}H]-1.

Thus we decided to find a method useful for this selective transformation. One possibility for improving reduction selectivity is the use of a transition-metal salt as the catalyst for the $NaBH_4$ reduction. Although a variety of reductions using combinations of $NaBH_4$ with many other transition-metal salts such as $NiCl_2$,[5] $CoCl_2$,[6] $CuCl_2$,[6a,7] $CeCl_3$,[8] $RhCl_3$,[9] and $AlCl_3$[10] have been cited in the

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