strates, which indicates that the difference in activation enthalpies are near zero and that the KIEs are almost independent of temperature. Extrapolation of the data in Figure 1 to a zero reciprocal temperature leads to an isotope effect on the pre-exponential factor of the Arrhenius function, $A_H/A_D = 19.8 \pm 2.9$, which is markedly different from that expected for a classical transition-state barrier model ($A_H/A_D \approx 1$). These results are consistent with contribution of H-tunneling to the C9–H abstraction step.

Whether H-tunneling is a general event in desaturase reactions is now raised as an interesting question. In a recent review, Scrutton et al.[17] warned against the assumption that a measured KIE $< 7$ is indicative of classical hydrogen-atom transfer and emphasized that extensive tunneling could also occur in cases with low KIE values. Therefore, although KIEs in the range of 5.5 – 7.0 have been reported for other fatty acid desaturases thus far studied, it is possible that H-tunneling is a common mechanism of hydrogen-atom transfer in these oxidation reactions. Therefore, temperature dependence of the reaction rate and KIE should be determined in other desaturase enzymes to confirm that H-tunneling occurs. Further work along this line is in progress in our laboratory.

*Experimental Section*

The probes (9,9-$^2H_2$) tridecanoic acid (**2**) and (10,10-$^2H_2$) tridecanoic acid (**3**) were prepared following reported procedures.[5] The deuterium contents of the labeled substrates were determined by GC-MS analysis of their respective methyl esters and were found to be as follows: **2**: 89.0 % 2 D, 8.7 % 1 D, and 1.1 % 0 D; **3**: 88.2. % 2 D, 8.7 % 1 D, and 3.1 % 0 D.

Cryptoregiochemistry was determined following the previously reported procedure.[5]

In vitro temperature dependence of reaction rates and KIE values were determined using a gland culture procedure. These experiments were carried out using round bottom, 96-well plates. To each well, a 5 μL drop of incubation medium was added and the plates were placed in an incubator at the desired temperature (6, 15, 23 and 30 °C). The incubation medium consisted of Grace's saline (135 μL) and a 1:1 mixture of tridecanoic acid and **2** (10 mg mL$^{-1}$ each) in a dimethyl sulfoxide solution (15 μL). One-day-old virgin *S. littoralis* females, reared as reported elsewhere,[10] were briefly anesthetized on ice and the pheromone glands were excised, carefully cleaned, and immersed into a drop of the incubation medium at the given temperatures. Plates were sealed with adherent plastic covers and the incubation proceeded for 3 h. After this time, pheromone glands were collected and soaked in chloroform/methanol (2/1) at 25 °C for 1 h. The lipidic extracts thus obtained were base methanolyzed as described elsewhere[10] to obtain the fatty acid methyl esters. Methyl pentadecanoate was added as internal standard for quantification.

The extracts were analyzed by GC-MS at 70 eV on a Fisons gas chromatograph (8000 series) coupled to a Fisons MD-800 mass selective detector. The system was equipped with a nonpolar Hewlett Packard HP-1 capillary column (30 m × 0.20 mm i.d.) using the following temperature program: After an initial delay of 2 min at 120 °C, the temperature increased from 120 °C to 180 °C at 5 K min$^{-1}$ and then to 260 °C at 2 K min$^{-1}$. Analyses were carried out under selected ion monitoring (SIM) mode. Selected ions were 223, 224, 225, 226, 227, 228, 229, 230, and 231. The dwell was set at 0.02 and mass span at 0.5.

KIEs were calculated from the ratios of product formed from unlabeled substrate and that from the deuterated analogs, and were based on the abundance of the respective molecular ions of the various isotopomers of methyl (*Z*,*E*) tridecadienoate (**$d_0$** 224, **$d_1$** 225). Isotope effects were corrected for the exact proportion of unlabeled and labeled substrates administered, which was determined by GC-MS analysis of a $BF_3 \cdot MeOH$ derivatized sample of the applied mixture. Corrections were also made for incomplete deuterium incorporation in the substrates and for the natural abundance of carbon and oxygen isotopes in the ions monitored. These latter values were obtained from the GC-MS chromatograms of gland extracts incubated with the individual substrates.

Reaction rates ($k^*$) were defined as the relative amounts of nonlabeled or labeled diene formed with respect to the internal standard.

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[1] J. Shanklin, E. B. Cahoon, *Annu. Rev. Plant Physiol. Mol. Biol.* **1998**, *49*, 611 – 641.
[2] P. H. Buist, D. M. Marecak, *J. Am. Chem. Soc.* **1992**, *114*, 5073 – 5080.
[3] P. H. Buist, B. Behrouzian, *J. Am. Chem. Soc.* **1996**, *118*, 6295 – 6296.
[4] P. H. Buist, B. Behrouzian, *J. Am. Chem. Soc.* **1998**, *120*, 871 – 876.
[5] A. Pinilla, F. Camps, G. Fabrias, *Biochemistry* **1999**, *38*, 15272 – 15277.
[6] T. Jonsson, M. H. Glickman, S. Sun, J. P. Klinman, *J. Am. Chem. Soc.* **1996**, *118*, 10319 – 10320.
[7] A. Kohen, J. P. Klinman, *Acc. Chem. Res.* **1998**, *31*, 397 – 404.
[8] J. Basran, M. J. Sutcliffe, N. S. Scrutton, *Biochemistry* **1999**, *38*, 3218 – 3222.
[9] A. Kohen, R. Cannio, S. Bartolucci, K. P. Klinman, *Nature* **1999**, *399*, 496 – 499.
[10] T. Martinez, G. Fabrias, F. Camps, *J. Biol. Chem.* **1990**, *265*, 1381 – 1387.
[11] A. E. Rettie, M. Boberg, A. W. Rettenmeier, T. A. Baillie, *J. Biol. Chem.* **1988**, *263*, 13733 – 13738.
[12] Results of the stereospecificity studies that show that the pro-(*R*) hydrogen atoms at both C9 and C10 are removed in the $\Delta^9$ desaturation of (*E*)-11-tetradecenoic acid, will be reported elsewhere.
[13] M. J. Nelson, S. P. Seitz, R. A. Cowling, *Biochemistry* **1990**, *29*, 6897 – 6903.
[14] M. H. Glickman, J. Wiseman, J. P. Klinman, *J. Am. Chem. Soc.* **1994**, *116*, 793 – 794.
[15] C. C. Hwang, C. B. Grissom, *J. Am. Chem. Soc.* **1994**, *116*, 795 – 796.
[16] J. C. Nesheim, J. D. Lipscomb, *Biochemistry* **1996**, *35*, 10240 – 10247.
[17] N. S. Scrutton, J. Basran, M. J. Sutcliffe, *Eur. J. Biochem.* **1999**, *264*, 666 – 671.

## Molecular Recognition with Introverted Functionality**

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Both chemical and physical forces play roles in molecular recognition. Functional group complementarity influences selectivity and affinity in chemical recognition, and these interactions can be arranged using synthetic receptors with open, cleftlike structures. For receptors that completely

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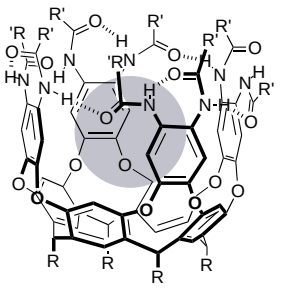


**1** • guest

Figure 1. Structure of the octaamide cavitand **1** with a generic guest species encapsulated, R = $C_{11}H_{23}$, R′ = $C_7H_{15}$.

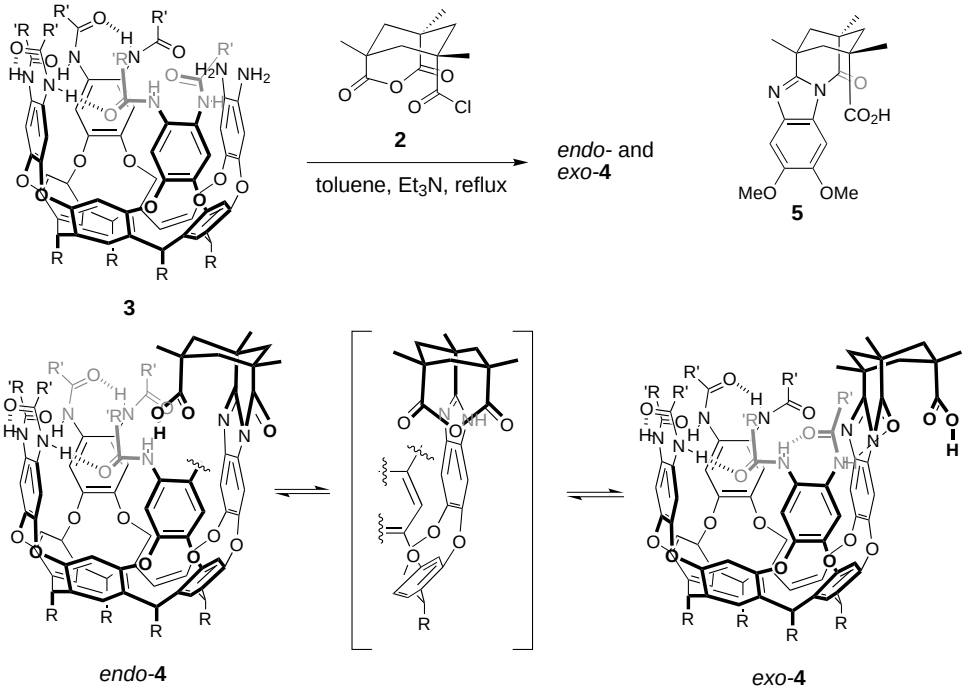
surround their targets, simple size and shape complementarity can distinguish one molecule from another. Herein, we present a synthetic receptor that surrounds a smaller target within physical barriers and offers a carboxyl function for contact with amines such as nicotine.

The cavitand **1** (Figure 1) has a vaselike shape and eight secondary amide functions on its upper rim. These groups provide a chain of intramolecular hydrogen bonds that resist changes in the conformation of the cavitand; the peripheral alkyl groups of the amide units enhance the solubility of the cavitand in organic media.[1] The interior surface of the cavitand is bleak and featureless. Most of the atoms that line the receptor are $sp^2$ hybridized with their p orbitals directed inward, as though the cavity was coated with a thin layer of negative charge. This offers weak C–H/π interactions and polarizability to size- and shape-complementary guests. Neutral molecules such as adamantane are recognized and bound reversibly as guests in the open-ended cavity host. The binding affinities are low ($\Delta G \sim 2$–$3\ kcal\,mol^{-1}$) but the energetic barriers to guest exchange are high ($\Delta G^{\neq} \sim 17\ kcal\,mol^{-1}$). The aromatic rings provide physical barriers to the entrance and exit of guests, and the seam of hydrogen bonds must be disrupted for guest exchange to occur.

We incorporated chemical recognition features by installing a carboxyl group on the rim of the vaselike cavitand. The condensation[2] of a Kemp's triacid[3] module **2** (Scheme 1) with the known diamine **3**[4] in boiling toluene gave the new receptor **4**. The benzimidazole nucleus formed raises the rim of the cavitand and maintains the space available in the cavity, and the carboxyl function may have an "inwardly" directed (introverted) OH bond as in *endo*-**4** or an outwardly directed one as in *exo*-**4**. Two geometrical isomers were obtained in roughly a 5:1 ratio from the condensation and both products displayed *m*/*z* signals in the mass spectrum that were consistent with the expected structures.[5] Given the affinity of *endo*-**4** for triethylamine (see below), it is likely that the presence of this base in the condensation reaction exerted a template effect and drove the presumed equilibrium (Scheme 1 bottom) toward the production of *endo*-**4**. The structures are inherently chiral, with the seam of head-to-tail secondary amides most likely to be that shown.[6]

The molecular recognition capabilities of the major product were revealed during its isolation, when signals of the adventitious guest, triethylamine, were identified in the NMR spectrum (Figure 2 a). Signals in the region upfield of 0 ppm are characteristic of bound species and reflect the anisotropy provided by the eight aromatic rings that line the interior of the gloomy capsule.[1] Exchange rates are slow on the NMR timescale (600 MHz, room temperature), and separate signals are seen for the free and bound guests. The binding is tight ($> 5 \times 10^4\ kcal\,mol^{-1}$) and beyond the limits for accurate determination by NMR titrations. The asymmetric environment of the cavity results in the splitting of the methylene signals of the triethylamine guest. In contrast, no upfield signals were observed in solutions of triethylamine with the minor product *exo*-**4**, the model compound **5**, or the parent structure **1**.

The interplay of mechanical and chemical recognition in *endo*-**4** was revealed through its binding behavior with amine guests (Figure 2). Primary amines such as isobutylamine (b) and 1-aminoadamantane (c), and tertiary amines such as triethylamine (a), (*S*)-(−)-nicotine (d), and 3-picoline (e) form stoichiometric complexes. In contrast, cavitand **1** *does not* bind triethylamine, isobutylamine, nicotine, picoline, or even the corresponding acetate salts under these conditions. Only 1-aminoadamantane is bound by both *endo*-**4** and **1**, but even in this case there are differences. The guest is bound with its amine function directed at the floor of the cavity in **1**[7] but




Scheme 1. Synthesis of the cavitands *endo*-**4** and *exo*-**4** from the diamine **3** and the Kemp's triacid module **2**. The model compound **5** was prepared similarly. The introverted carboxyl function is shown in bold. R = $C_{11}H_{23}$, R′ = $C_7H_{15}$.

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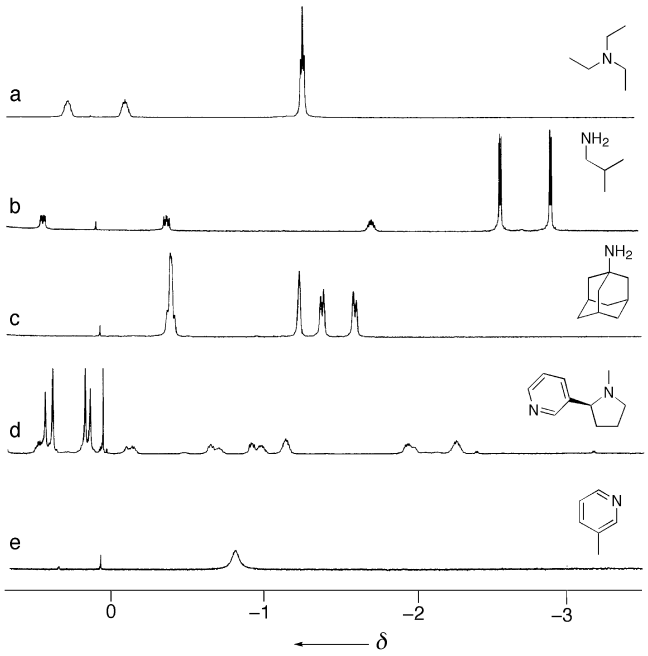


Figure 2. Signals of encapsulated guests from the upfield region of the $^1$H NMR spectra (600 MHz, $CDCl_3$, 295 K) of the receptor *endo*-**4**. The guest species are a) triethylamine, b) isobutylamine, c) 1-aminoadamantane, d) (*S*)-(−)-nicotine, and e) 3-picoline.

with the opposite orientation, namely with the amino group in contact with the acid, in *endo*-**4**. Adamantane itself is not bound by *endo*-**4** but is bound by **1**. Accordingly, the chemical recognition of *endo*-**4** is involved with amine guests. The high affinity is likely to involve the formation of a contact ion pair between the introverted carboxyl group and the guest amine.[8] The binding of 1-aminoadamantane represents the size limit for guests of *endo*-**4**. The slightly larger 1-adamantanylmethylamine was only weakly bound by *endo*-**4** and gave rise to very broad signals in the $^1$H NMR spectrum. The Kemp's triacid group partially obstructs the opening and provides size limitations in recognition: the guest is mechanically bound in all three dimensions within the host.

Diastereoselective binding in receptor *endo*-**4** was probed with a chiral guest. When an excess of (*R*)-(−)-1-cyclohexylethylamine was added to (racemic) *endo*-**4**, two equal-intensity sets of host signals were observed in the NMR spectrum; these signals correspond to the two diastereomeric complexes formed (Figure 3a). When a *racemic* mixture of 1-cyclohexylethylamine was added to *endo*-**4**, the same two sets of host signals were observed, but in a 3:1 ratio (Figure 3b). The binding diastereoselectivity for this guest is approximately 50% *de* at room temperature. In the case of (*S*)-(−)-nicotine, the pyrrolidine nitrogen atom is an additional stereogenic center and it is this atom that is in contact with the acid. A ROESY NMR experiment established that the pyrrolidine ring is bound deep in the cavity of *endo*-**4** and gave an exchange rate constant of $k \approx 0.5\ s^{-1}$. The presence of a number of isomers led to unresolved signals and precluded the determination of diastereoselectivity for this case.

In summary, the receptor *endo*-**4** features both mechanical and chemical bonding. The concave surface provides a cavity

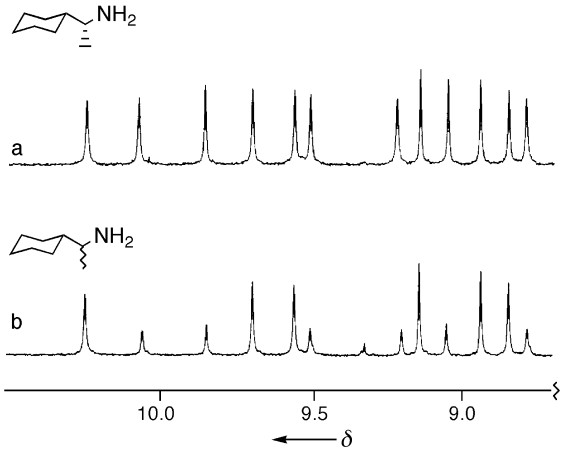


Figure 3. Downfield amide resonances for the receptor *endo*-**4** with either a) (*R*)-(−)-1-cyclohexylethylamine or b) racemic (±)-1-cyclohexylethylamine as the guest.

isolated from bulk solution and the introverted carboxyl group is directed towards the guest inside. The cavity is asymmetric and capable of diastereoselection in the binding of chiral substrates. Convergent functional groups,[9] concave reagents,[10, 11] molecular tweezers,[12] macrocyclic compounds,[13, 14] and endohedral functions[15] also feature reactive atoms in sterically constrained environments, but typically there is not enough room to both surround a guest completely and present useful functionality to it. Additionally, there is the often insurmountable problem of doing chemistry on concave molecular surfaces. The combination of the recognition attributes of **4**, many of which are shared by enzymes, augurs well for the development of synthetic molecules that approach the functional features of naturally occurring macromolecules.

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[1] D. M. Rudkevich, G. Hilmersson, J. Rebek, Jr., *J. Am. Chem. Soc.* **1998**, *120*, 12216–12225.
[2] J. Rebek, Jr., L. Marshall, J. McManis, R. Wolak, *J. Org. Chem.* **1986**, *51*, 1649–1653.
[3] D. S. Kemp, K. S. Petrakis, *J. Org. Chem.* **1981**, *46*, 5140–5143.
[4] A. R. Renslo, F. C. Tucci, D. M. Rudkevich, J. Rebek, Jr., *J. Am. Chem. Soc.* **2000**, *122*, 4573–4582.
[5] ESI-MS *m*/*z*: 2484 ([*M*H+$^{13}$C]$^+$ calcd for $C_{155}H_{224}N_8O_{17}{}^{13}C$: 2484).
[6] The arrangement shown allows for an additional hydrogen-bonding interaction between an amide N–H in the hydrogen-bonding seam and the carbonyl group of the acylbenzimidazole wall.
[7] The binding orientation is determined by comparison of the relative upfield shifts for different protons in the guest, see ref. [1].
[8] The model compound **5** is sparingly soluble in $CDCl_3$, but freely soluble upon the addition of triethylamine.
[9] J. Rebek, Jr., *J. Mol. Recognit.* **1988**, *1*, 1–8.
[10] U. Lüning, *J. Mater. Chem.* **1997**, *7*, 175–182.
[11] U. Lüning, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1996**, *24*, 43–60.
[12] S. C. Zimmerman, Z. Zeng, W. Wu, D. E. Reichert, *J. Am. Chem. Soc.* **1991**, *113*, 183–196.
[13] S. Goswami, D. Van Engen, A. D. Hamilton, *J. Am. Chem. Soc.* **1989**, *111*, 3425–3426.
[14] H.-D. Lutter, F. Diederich, *Angew. Chem.* **1986**, *98*, 1125–1127; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1125–1127.
[15] K. Goto, R. Okazaki, *Liebigs Ann. Chem.* **1997**, 2393–2407.

  1433-7851/00/3918-3283 $ 17.50+.50/0 