Footprint Catalysis. X.[1—9] Surface Modification of Molecular Footprint Catalysts and Its Effects on Their Molecular Recognition and Catalysis

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Trimethylsilylation with hexamethyldisilazane was applied to a surface modification of silica(alumina) gel catalysts, on which surface the "molecular footprint" catalytic cavities had been marked by the authors' imprinting method. The modification provided for the more stable catalysts than the unmodified catalyst, retaining their molecular-recognition capabilities nearly intact.

We have succeeded in marking the "footprints" of molecules as a template on a surface of aluminium ion-doped silica gel. The footprints are cavities with complementary structures to the template molecules involving Lewis-acid sites within them. The Lewis-acid sites are an Al atom of aluminate incorporated by isomorphical substitution into the silicate matrix. The cavities possess catalytic abilities based on both their Lewis acidity and molecular-recognition capabilities. We have reported their unprecedented catalytic behaviors, termed "footprint catalysis," such as tailored substrate specificities and enzyme-like stereoselective catalyses.[1—11] The "molecular footprint" cavities should find further application besides catalyses, e.g., to affinity chromatography and chemical sensors. Practical use of the cavities might require more stable cavities. Since the cavities generate on a silica-gel surface, the stabilities of the cavities depend on those of the surface structures. A silica-gel surface is not sufficiently stable if surface silanol groups are present on the surface, that serve as clues regarding the degradation of the surface structures.

The present manuscript deals with a modification of the silica-gel surface loading the cavities. Trimethylsilylation with hexamethyldisilazane (HMDS) left the cavities intact and effectively blocked the surface silanol groups on the outside of the cavities. This modification provided more rigid, hydrophobic and stable silica-(alumina) gel catalysts without any significant changes in the molecular-recognition capabilities. The trimethylsilyl groups around the footprint cavities seemingly "deepened" the cavities, which caused an alteration in the catalytic properties through steric effects on the binding of substrate molecules.

Experimental

Materials. All of the chemicals were commercial products of guaranteed grade from Nacalai Tesque Co., Ltd., which were used without further purification, if noted otherwise specified.

Templates, Inhibitors, and Substrate. All of the compounds (**1**—**4**) were of the same preparation as those previously reported.[1,2,5]

N-Benzoylbenzenesulfonamide ($PhCONHSO_2Ph$),[1] **1**, Mp 148 °C (lit, 147 °C); (Found: C, 59.51; H, 4.26; N, 5.37%).

N-Benzoyl-4-(acetamido)benzenesulfonamide (*p*-(AcNH)-$C_6H_4SO_2NHBz$),[2] **2**, Mp 257 °C; IR (KBr) 3315 and 3274 (N–H), 1674 (C=O), 1538 (amide I), 1354 and 1165 cm^{-1} (S=O); ^1H NMR ($CDCl_3$) δ=7.22 (m, 9H, ArH), 1.47 (s, 3H, CH_3–); (Found: C, 56.47; H, 4.32; N, 9.28%).

4-(Acetamido)benzenesulfonamide,[2] **3**, Mp 219.5 °C (lit, 219 °C); (Found: C, 59.11; H, 8.00; N, 9.28%).

Benzoic anhydride,[1] **4**, was of reagent grade from Nacalai Tesque Co., Ltd., and was recrystallized from benzene–petr. benzine, Mp 42 °C.

Nucleophile. Potassium 2,4-dinitrophenolate, prepared and recrystallized as previously reported,[3] was dissolved in acetonitrile with 18-crown-6 to give a solution (5.0×10^{-3} mol dm^{-3}).

Silica gel: Merck Kieselgel 60, art. no. 7754, particle size 0.06—0.20 mm, mesh 70—230, was used.

Catalyst Preparation. The silica-gel catalysts loading molecular footprint cavities (footprint catalysts) were prepared according to the usual procedures, as previously reported.[3] They included successive surface activation, aluminium-ion doping, imprinting with templates, drying, and methanol extraction (Fig. 1). The details concerning the procedures were the same with those previously reported, if not specified.[3] The surface modification procedures were intruded between the drying and the methanol extraction, as follows (Scheme 1).

Kieselgel (100 g) was treated with conc hydrochloric acid (500 cm^3) under reflux for 5 h to generate free silanol groups on the surface.[12,13] The "surface-activated" gel was thoroughly washed with deionized water until the washings reached nearly neutral (pH 6—7). For aluminium-ion doping, the surface-activated gel was subjected to soaking in an aqueous aluminium chloride solution (0.2 mol dm^{-3}, 50 cm^3 to a gel portion equivalent to 10 g of dry weight) at pH 7—8 for 2 d. The "aluminium ion-doped" gel, isolated by decantation from precipitated aluminium hydroxide, was subsequently subjected to imprinting procedures. To the gel was added to an acetone solution of a template (2×10^{-3} mol template dissolved in minimum amount of acetone, to a gel portion equivalent to 10 g of dry weight). The mixture was adjusted to pH 4.0, and allowed to stand at room temperature for 1 week. The "template-treated" gel was collected by careful filtration so that the surface always remained wet, and was washed with very dil hydrochloric acid (pH 4.0). The wet gel was transferred into Petri dishes,

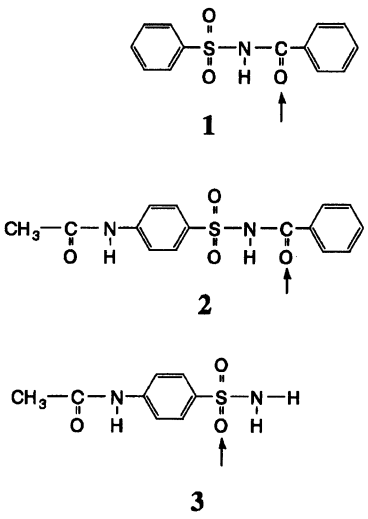


Fig. 1. Template molecules. The arrows indicate the location of a Lewis-base site in the template molecules.

and then air-dried at room temperature until it reached a constant weight. The "template-treated and air-dried" gel was subsequently submitted to methanol extraction using a Soxhlet extractor. Extraction was continued until the release of template molecules into the extracts was no longer observed by optical density (O.D.) monitoring at 220 nm. The "methanol-extracted" gel was transferred into a desiccator and dried under gradually reduced pressure (atmospheric to 3 mmHg) at room temperature for 1 d (1 mmHg=133.322 Pa). Finally, it was exhaustively dried under 3 mmHg at 140 °C for 1 h before use. The imprinted catalytic cavities with the template **1**, **2**, and **3** will be referred to as {**1**}, {**2**}, and {**3**}, respectively, in the text.

Surface Modification. The "template-treated and air-dried" gel sample mentioned above served as a sample for the present surface modification. Trimethylsilylation with hexamethyldisilazane (HMDS) was carried out according to the procedures reported by Bohemen, et al.[14]

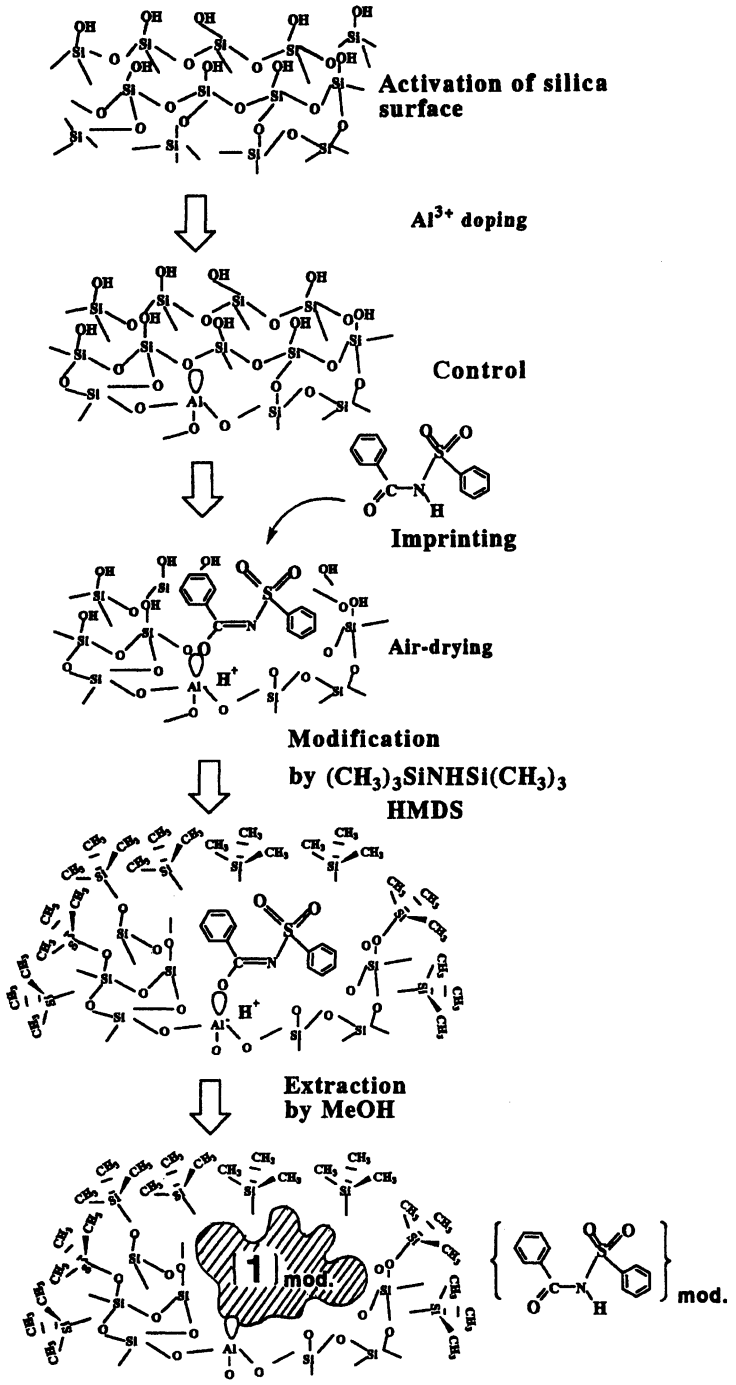
A portion of the gel sample (5.0 g) was fully dried under reduced pressure (1 mmHg) at 130—150 °C for 2 h. The still hot gel sample, kept from moisture, was immediately added into a solution of HMDS (3 cm^3) in petr. benzine (20 cm^3). The mixture was refluxed for 1 h. To the mixture was added 1-propanol (2.0 cm^3) and allowed to stand for 2 d to decompose excess HMDS. To complete the decomposition, the mixture was refluxed for 4—5 h. The "surface-modified" gel was collected and washed twice with petr. benzine (30 cm^3), twice with 1-propanol (30 cm^3), and finally twice with petr. benzine (30 cm^3) by decantation. The gel was then collected by filtration, and dried in a desiccator for 2 d under reduced pressure. Following methanol extraction and drying, the procedures were the same as those described above. The modified catalytic cavities are designated as $\{\mathbf{1}\}_{mod.}$ in the text (Scheme 1).

Kinetic Measurements. The reaction conditions and assay procedures were the same as those in previous studies.[3] An acetonitrile solution of a substrate (49 cm^3, 0.9—4.5×10^{-3} mol dm^{-3}) was equilibrated with a catalyst (50 mg) at 30 °C for 10—30 min. To the mixture was added a nucleophile solution (1 cm^3, 1.5×10^{-2} mol dm^{-3}) to initiate the reaction. The catalyzed 2,4-dinitrophenolyses were followed by triple-wavelength spectrophotometry. The change in the optical densities at 400, 430, and 500 nm due to free 2,4-dinitrophenolate were determined at proper intervals using a Shimadzu UV-160 spectrophotometer. In inhibition studies, catalysts were preincubated with an inhibitor in acetonitrile for 1 h at 30 °C. They were then equilibrated with substrate solutions for an additional 10 min. The subsequent procedures were the same as those described above. Pseudo-first-order rate constants, k_{obsd}s, were calculated from the linear plots of log O.D. vs. time. The thus-obtained k_{obsd}s obeyed Michaelis–Menten kinetics with respect to the substrate concentration. The kinetic parameters, K_ms and V_{max}s ($=k_{obsd,max}$s), were obtained graphically from double-reciprocal plots through a least-square method, and k_{cat}s were calculated from the V_{max} by dividing with catalytic site titers (see below). The catalytic sites molarities were determined by a kinetic titration method through irreversible poisoning of the catalytic sites with pyridine.[3] To a substrate solution containing a sample catalyst (50 mg) was added specified amounts of pyridine in acetonitrile (5—15 cm^3, 5.0×10^{-5} mol dm^{-3}). The volume of the mixtures were made up to 49 cm^3 with acetonitrile. The mixtures were incubated for pyridine poisoning at 30 °C for 1 h under gentle stirring. To them was then added a nucleophile solution in order to initiate the catalyzed reaction by surviving catalytic activities. The subsequent procedures were the same as those described above. An extrapolation of the plots for the obtained k_{obsd}s vs. pyridine amounts gave an intercept on the abscissa. The numerical value of the intercept directly showed the molarity of the catalytic sites per 1 gram of the catalyst, since pyridine in 50 cm^3 of the reaction mixtures poisoned a catalyst of 50 mg under these conditions.

Surface Analysis. The surfaces of the modified silica-(alumina) gels were analyzed by diffuse reflectance infrared Fourier-transform spectroscopy (DRIFTS) using a Perkin–Elmer 1740 FTIR spectrometer equipped with a SPECTRA-TECH collector. The observed spectra were transformed to Kubelka–Munk functions by the aid of Perkins' "KUBEL.OY" program.

Results and Discussion

DRIFTS-Spectra of Modified Surfaces. Several peaks were observed in the region between 4000—1500 cm^{-1} on the diffuse reflactance spectra of the footprint catalyst imprinted with the template **1**. A few peaks, however, significantly changed upon a modification with HMDS. The peaks at 3654 cm^{-1} and at 3452 cm^{-1} observed before the modification could be attributed to isolated pairs of adjacent SiOH groups (vicinal) making mutual hydrogen bonding,[15] and water molecules adsorbed on the isolated pairs of SiOH groups, respectively. The former peak and most of the latter peak disappeared upon the modification, and a new peak distinctly appeared at 2967 cm^{-1} that was attributed to C–H stretching of the methyl groups.[16] These findings supported the idea that most of the surface silanol groups were successfully trimethylsilylated by the action of HMDS under the present reaction con-



Scheme 1. Preparation of modified footprint catalyst by HMDS.

ditions.

Acidic Properties. The acid-strength determination of footprint catalysts was carried out according to the method of Benesi with Hammett indicators,[17] and Lewis-acid detection was performed with phenolphthalein.[18] All of the modified catalysts, including the modified control catalyst, were positive to the following Hammett indicators, i.e., Neutral Red (pK_a +6.8), Methyl Red (pK_a +4.8) and 4-(phenylazo)diphenylamine (pK_a +1.5). They were also positive(purple) to phenolphthalein for the Lewis acid. The footprint catalytic sites are evidently Lewis-acid sites, as previously discussed, since if the catalytic sites were Brønsted-acid sites, they should lose their catalytic activities by neutralization with 2,6-dimethylpyridine instead of pyridine.[3] However, Lewis-acid sites were still active because they cannot form acid-base complexes with 2,6-dimethylpyridine through a steric hindrance to two methyl groups. Their findings were also observed with the modified catalysts. Therefore, in order to obtain the molarity of the catalytic sites, the kinetic titrations of the catalytic sites by pyridine poisoning pro-

cedures were carried out (Fig. 2); the intercepts on the abscissa directly give the titers. The catalytic-site molarities per 1 gram catalysts were 2.38×10^{-5} mol for the $\text{Control}_{\text{mod.}}$ catalyst, 2.37×10^{-5} mol for $\{\mathbf{1}\}_{\text{mod.}}$, 2.38×10^{-5} mol for $\{\mathbf{2}\}_{\text{mod.}}$ and 2.62×10^{-5} mol for $\{\mathbf{3}\}_{\text{mod.}}$. Nearly identical titers were observed within the experimental error. This suggested that the amounts of native Lewis-acid sites on a Al^{3+} ion-doped gel preparation were not very much affected by the modification with HMDS. These findings evidently proved that the modified catalysts retained both quality and quantity of the Lewis-acid sites on the untreated footprint catalysts nearly intact. Therefore, the net structures of silica gel might be also retained, except for its surface structures.

Cavity Areas Percentage. Since the footprints might be shallow cavities, the cavity areas should be equal to the projection areas of the template molecules used in imprinting. The projection areas could be roughly estimated by a projection of Stuart models of the template molecules on a plane (Table 1). The coverage percentages of the cavities were calculated using the observed catalytic site molarities, the projection areas of template molecules, and the assumed surface area of Kieselgel 60 (500 m^2 per gram) (Table 1). These values of the coverage percentages might be lower limits, because the surface area (500 m^2), determined by BET adsorption isotherms using nitrogen gas,[19] might contain an inner area of narrow pores. The template molecules never enter into such pores to form cavities. Thus, a large value could be expected for cavities on the working outer surface of a catalyst, though the majority part of a surface would still be outside of the cavities.

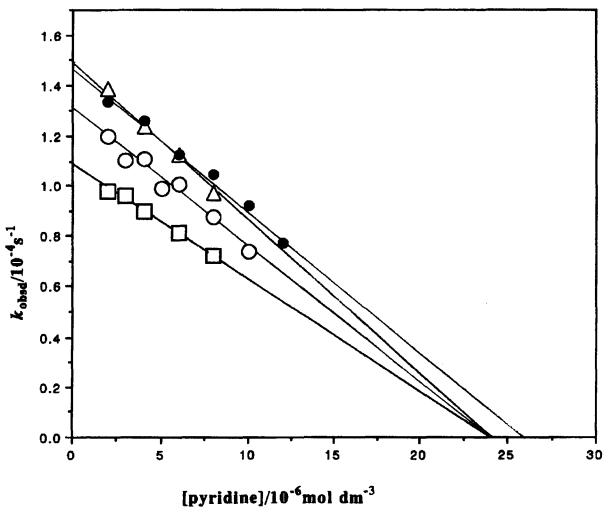


Fig. 2. Catalytic sites titration of the modified control and three footprint catalysts by pyridine poisoning in 2,4-dinitrophenolysis of benzoic anhydride. [benzoic anhydride]: 3.0×10^{-3} mol dm^{-3}, Catalyst: 50 mg in 50 cm^3 of pyridine–acetonitrile solution. □: The $\text{Control}_{\text{mod.}}$ catalyst, titre; 2.38×10^{-5} mol per 1 gram catalyst. △: The imprinted catalyst, $\{\mathbf{1}\}_{\text{mod.}}$ titre; 2.37×10^{-5} mol per 1 gram catalyst. ○: The imprinted catalyst, $\{\mathbf{2}\}_{\text{mod.}}$ titre; 2.38×10^{-5} mol per 1 gram catalyst. ●: The imprinted catalyst, $\{\mathbf{3}\}_{\text{mod.}}$ titre; 2.62×10^{-5} mol per 1 gram catalyst.

Modification Effects on Properties of Gel Particles. The introduction of trimethylsilyl groups into such an outside area of the cavities would make a silica-gel surface (Z-value; 80[19]) more hydrophobic. It would be less polar than the solvent acetonitrile (Z-value; 71.3[20]). Therefore, trimethylsilylation should affect the adsorption properties of a catalyst. The increased hydrophobicity of the modified surface should diminish any nonspecific adsorption of polar 2,4-dinitrophenolate anions on the outside area of the catalytic cavities. Such adsorption was usually observed to cause concave upwards in a semi-log plot (log O.D. vs. sec) within the initial 7—8 min. Figure 3 clearly shows that such a concave shape was eliminated by a surface modification to give a finely linear plot. Additionally, the gel particles with the modified surface resisted mechanical pulverization during a kinetic measurement. Usually vigorous stirring with a magnetic stirrer-bar obviously pulverized the gel catalyst particles. It caused a concave downwards shape in a semi-log plot with prolonged reaction period (over 1 h). No pulverization, however, was observed with a modified catalyst particle, which gave a linear plot without any concave downwards shape (Fig. 4). Therefore, the surface modification by trimethylsilylation could provide a precise and reliable kinetic determination.

Modification Effects on Molecular Recognition of Cavities. Three modified footprint catalysts imprinted with **1**, **2**, and **3** ($\{\mathbf{1}\}_{\text{mod.}}$, $\{\mathbf{2}\}_{\text{mod.}}$, $\{\mathbf{3}\}_{\text{mod.}}$) and one modified control catalyst were examine in order to confirm that trimethylsilylation with HMDS modified only silanol groups outside the cavities. Their catalyses and molecular recognition of subsite structures were investigated. Their kinetic parameters (K_{m}s,

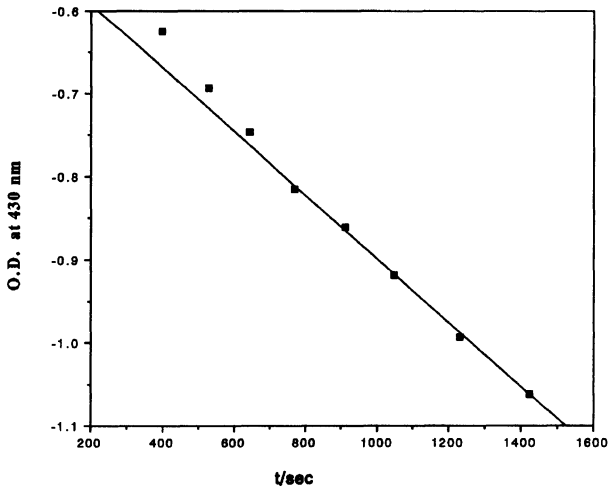


Fig. 3. Time scan of the catalyzed 2,4-dinitrophenolysis over unmodified footprint catalyst $\{\mathbf{1}\}$.

Table 1. An Estimation by the Projection of Stuart Models of the Template Molecules on a Plane

Footprint catalyst	Molarity per 1 kilogram of footprint catalyst		Projection areas template molecules	Coverage percentages of the cavities
	$mol\,kg^{-1}$	number/10^4 Å^2	Å^2	%
{**1**}	2.37×10^{-2}	2.85	104.3	2.97
{**2**}	2.38×10^{-2}	2.87	123.3	3.54
{**3**}	2.62×10^{-2}	3.16	79.7	2.52

Table 2. Kinetic Parameters of the Catalyzed Reactions by Modified Footprint Catalysts

	$K_m\times10^4$	k_{cat}	$K_{i,1}$[b] $\times10^6$	$-\Delta G°_1$	$K_m/K_{i,1}$	$K_{i,2}\times10^6$
	M[a]	$M^{-1}\,s^{-1}$	M	$kJ\,mol^{-1}$		M
{**1**}$_{mod.}$[c]	22.58	221	3.06	31.99	738	5.04
{**2**}$_{mod.}$	42.64	233	15.33	27.93	278	6.48
{**3**}$_{mod.}$	37.49	184	64.79	24.30	58	14.69
Control$_{mod.}$[e]	38.31	207	No inhibition	—	—	No inhibition
	$-\Delta G°_2$	$K_m/K_{i,2}$	$K_{i,3}\times10^6$	$-\Delta G°_3$	$K_m/K_{i,3}$	$k_{cat}/K_m\times10^{-4}$
	$kJ\,mol^{-1}$		M	$kJ\,mol^{-1}$		$M^{-2}\,s^{-1}$
{**1**}$_{mod.}$[c]	30.73	448	8.76	29.34	258	9.79
{**2**}$_{mod.}$	30.09	658	15.74	27.86	270	5.46
{**3**}$_{mod.}$	28.03	255	4.21	31.18	890	4.91
Control$_{mod.}$[e]	—	—	No inhibition	—	—	5.40
	$K_m\times10^4$	k_{cat}	$K_{i,1}$[b] $\times10^6$	$-\Delta G°_1$	$K_m/K_{i,1}$	$K_{i,2}\times10^6$
	M[a]	$M^{-1}\,s^{-1}$	M	$kJ\,mol^{-1}$		M
{**1**}	3.78	321	2.22	32.79	170	6.28
{**2**}	5.88	316	3.23	31.85	182	2.25
{**3**}	15.77	429	n.d.	n.d.	n.d.	n.d.
Control[e]	18.00	640	No inhibition	—	—	No inhibition
	$-\Delta G°_2$	$K_m/K_{i,2}$	$K_{i,3}\times10^6$	$-\Delta G°_3$	$K_m/K_{i,3}$	$k_{cat}/K_m\times10^{-4}$
	$kJ\,mol^{-1}$		M	$kJ\,mol^{-1}$		$M^{-2}\,s^{-1}$
{**1**}	30.17	60.2	n.d.[d]	n.d.	n.d.	95.5
{**2**}	32.76	261	4.68	30.92	126	53.7
{**3**}	n.d.	n.d.	3.98	31.32	396	27.2
Control[e]	—	—	No inhibition	—	—	35.6

a) M; $mol\,dm^{-3}$. b) $K_{i,1}$; inhibition by **1**. c) { }$_{mod.}$; modified footprint catalyst. d) n.d.; not determined. e) Control and Control$_{mod.}$; catalysts without footprint cavity.

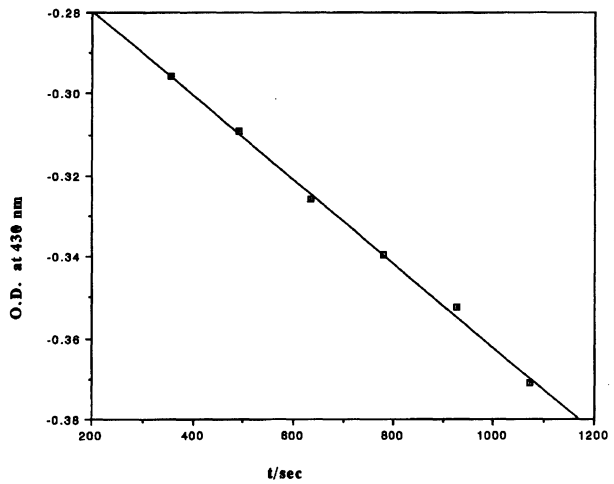
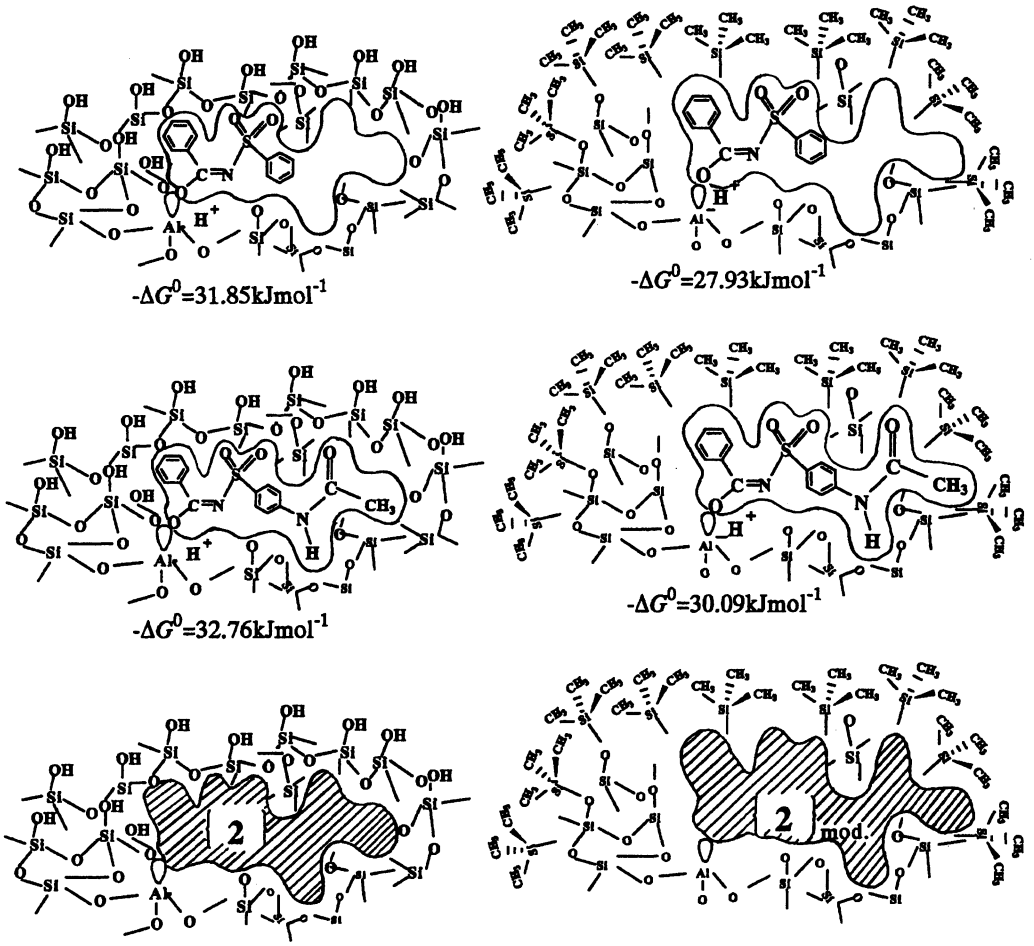


Fig. 4. Time scan of the catalyzed 2,4-dinitrophenolysis over modified footprint catalyst {**1**}$_{mod.}$.

k_{cat}s) and their competitive inhibition constants (K_is) by the effects of **1**, **2**, and **3** were determined (Table 2). Figure 5 displays Lineweaver–Burk plots for the catalyzed reactions of a modified control catalyst under the presence of **1**, **2**, and **3** as an inhibitor, respectively. These almost overlapping plots clearly showed that the catalytic sites of the modified control catalyst, being independent of the inhibitors, lacked any molecular-recognition capability to these inhibitors, as expected. Additionally, since trimethylsilylation with HMDS hardly seemed to affect the Lewis-acid sites, they apparently retained their function of acid catalysis. Figures 6, 7, and 8 are Lineweaver–Burk plots of the catalyzed reactions on {**1**}$_{mod.}$, {**2**}$_{mod.}$, and {**3**}$_{mod.}$ with and without inhibitors **1**, **2**, and **3**, respectively. In contrast to Fig. 5, they obviously displayed typical competitive inhibition plots, their intercepts on the vertical axis being identical, which meant that these inhibitors bind



Scheme 2.

onto the same catalytic cavities, respectively. The observed coincidences of the intercepts provided competitive inhibition constants (K_is) by the usual way using a Michaelis–Menten equation under the presence of competitive inhibitors. The K_is constants enabled a quantification of the molecular-recognition capabilities of the cavities, defined as $-\Delta G^\circ = RT \ln (K^{-1})$, as previously reported.[2,5] As shown in Table 2, $\{\mathbf{1}\}_{\text{mod.}}$ revealed preferential affinities (molecular recognition) in the order $\mathbf{1}>\mathbf{2}>\mathbf{3}$ to the inhibitors; $\{\mathbf{2}\}_{\text{mod.}}$ did those of $\mathbf{2}>\mathbf{1}=\mathbf{3}$; $\{\mathbf{3}\}_{\text{mod.}}$ did those of order $\mathbf{3}>\mathbf{2}>\mathbf{1}$. That is, they possessed the largest affinities to their original template molecules used in imprinting, respectively, the same as the unmodified cavities.[2,4,5] The "whole" molecular recognition is the sum of the "partial" molecular recognition due to subsites of a cavity.[2] That of $\{\mathbf{2}\}_{\text{mod.}}$ to **2** could be divided into their partial molecular recognition by subtractive treatments of the observed $-\Delta G^\circ$s.[2] Scheme 2 displays the partial molecular recognition of $\{\mathbf{2}\}_{\text{mod.}}$ and that of $\{\mathbf{2}\}$ for a comparison. A possible slight introduction of trimethyl groups into the footprint $\{\mathbf{2}\}$ during the modification procedure brought about a smaller affinity value on $\{\mathbf{2}\}_{\text{mod.}}$ to **1** and **2** compared with the unmodified catalyst $\{\mathbf{2}\}$. Nevertheless a near similarity was obviously revealed between them. These findings strongly suggested that detailed molecular-recognition capabilities of the cavities remained nearly intact throughout the modification by the effective protection with template molecules. The above-mentioned molecular-recognition implied positive affinities due to "inclusion effects" of the cavities.[2] Whereas, negative affinities due to "exclusion effects" of the cavities, i.e., incorrect fitting and steric repulsion, were also known.[2] The introduction of bulky trimethylsilyl groups onto the vicinity of the cavities, especially into their rims, could provide some exclusion effects. These possible exclusion effects were examined as follows. The inhibition constant (K_i) of **2** on $\{\mathbf{1}\}_{\text{mod.}}$ and that of **2** on $\{\mathbf{1}\}$ (Table 2) were compared. The cavity $\{\mathbf{1}\}$ excluded the acetamido substituent group of **2** by repulsion, which caused less affinity (30.17 kJ) than that of $\{\mathbf{1}\}$ to **1** (32.79 kJ). Such negative affinity ($-\Delta\Delta G^\circ = 30.17 - 32.79 = -2.62$ kJ) might be enhanced if bulky trimethylsilyl groups were introduced into the place where the acetamido substituent group should exist. Table 2 shows that the affinity of $\{\mathbf{1}\}_{\text{mod.}}$ to **2** was 30.73 kJ and that to **1** was 31.99 kJ ($-\Delta\Delta G^\circ = 30.73 - 31.99 = -1.26$ kJ). No remarkable enhancement, but small reduction, in the negative affinity to **2** could be observed. Since mutual interactions of the

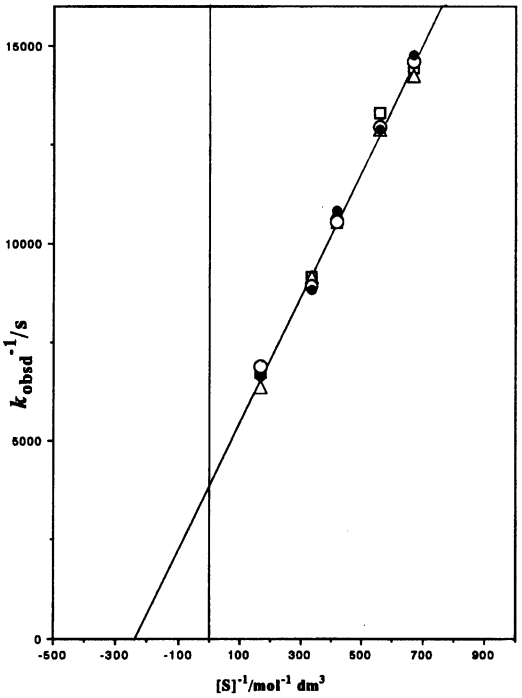


Fig. 5. Competitive inhibition of a Control$_{mod.}$ catalyst by **1**, **2**, **3**. [S]: benzoic anhydride concentration. □ [I]: 0, △ [I]: 2.0×10^{-6} mol dm^{-3} (**1**), ○ [I]: 2.0×10^{-6} mol dm^{-3} (**2**), ● [I]: 2.0×10^{-6} mol dm^{-3} (**3**).

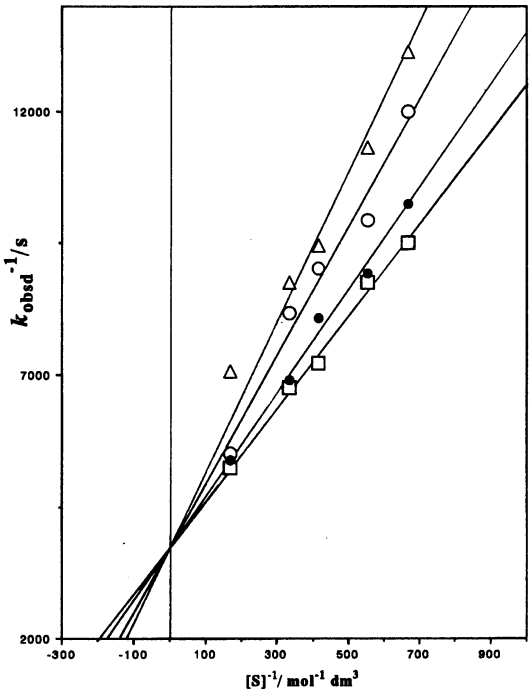
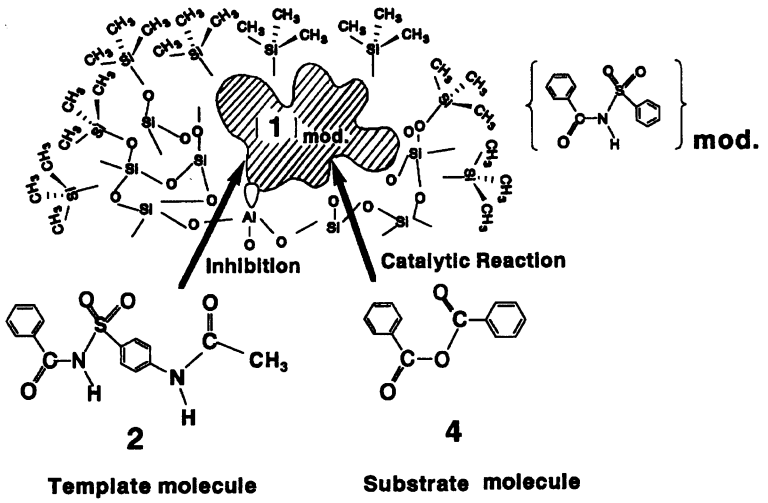


Fig. 6. Competitive inhibition of a footprint catalyst {**1**}$_{mod.}$ by **1**, **2**, **3**. [S]: benzoic anhydride concentration. □ [I]: 0, △ [I]: 2.0×10^{-6} mol dm^{-3} (**1**), ○ [I]: 2.0×10^{-6} mol dm^{-3} (**2**), ● [I]: 2.0×10^{-6} mol dm^{-3} (**3**).

excluded acetamido group with the surface of modified catalyst were assumedly different from that of an unmodified catalyst, a precise comparison of the exclusion effect might be difficult. However, the modified catalyst apparently retained the molecular-recognition capabilities on the whole.

Modification Effects on Catalytic Behavior. The k_{cat}, K_m, and k_{cat}/K_m values of the modified cavities and a modified control were compared with those of the unmodified cavities and unmodified control to clarify effects of modification on catalysis. As shown in Table 2, {**1**}$_{mod.}$, {**2**}$_{mod.}$, {**3**}$_{mod.}$ and Control$_{mod.}$ showed closely dispersed (2.26—3.75) K_ms of 10^{-3} order, and also dispersed (184—207 dm^3 mol^{-1} s^{-1}) k_{cat}s. However, {**1**}, {**2**}, {**3**}, and unmodified control showed broadly dispersed (3.36—18.00 mol dm^{-3}) K_ms of 10^{-4} order and also dispersed (316—640 dm^3 mol^{-1} s^{-1}) k_{cat}s. Their catalytic efficiency (k_{cat}/K_m) was diminished by the modification, depending on their K_m values. These differences in K_ms and k_{cat}s between the modified and unmodified cavities demonstrated that the modification apparently reduced the catalytic activities of the cavities with a slight levelling effect. The trimethylsilylation on the outside area, apart from the cavities, would not directly affect the catalytic reactivities, and it would be independent of the Lewis-acid strength. Therefore, the observed modification effects on catalyses might be caused by the steric effects of bulky trimethylsilyl groups introduced in the vicinity of, or not apart from, the cavities, especially around the subsite for a benzoyl group (Scheme 3). They might make a barrier to the cavities; they seemingly might deepen the cavities. The catalytic ability of a footprint cavity was essentially based on the molecular-recognition capabilities of the cavity. The molecular recognition toward substrate molecules should be similar to that toward template molecules. A Stuart-model examination, however, showed that the molecular shape of a substrate molecule was not finely identical with that of a template molecule. A precise complementarity of the footprint cavities to the template did not mean the best fitting for the substrate molecules (Scheme 3). Thus, the precise complementarity could generate a "strain" to the binding substrate molecules, as illustrated in Scheme 3. Compared to the "unmodified" footprint catalysts, the "deepend" cavities by the trimethylsilylation could enhance this "strain" to hinder the binding step of catalysis, which would result in manifest effects on the K_m values.

Conclusion. The modification with HMDS significantly reduced the catalyses over footprint cavities. Probable steric hindrance effects of bulky trimethylsilyl groups around the cavities might cause increasing of K_m values. On the other hand, the trimethylsilylation afforded more rigid and stable gel catalyst particles with a more hydrophobic surface. The precise molecular-recognition capabilities of the cavities retained nearly



Scheme 3.

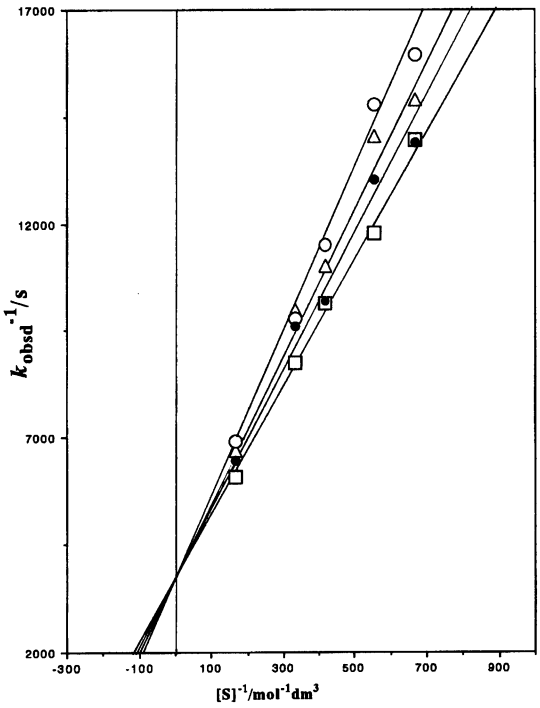


Fig. 7. Competitive inhibition of a footprint catalyst $\{\mathbf{2}\}_{mod.}$ by **1**, **2**, **3**. [S]: benzoic anhydride concentration. □ [I]: 0, △ [I]: 2.0×10^{-6} mol dm^{-3} (**1**), ○ [I]: 2.0×10^{-6} mol dm^{-3} (**2**), ● [I]: 2.0×10^{-6} mol dm^{-3} (**3**).

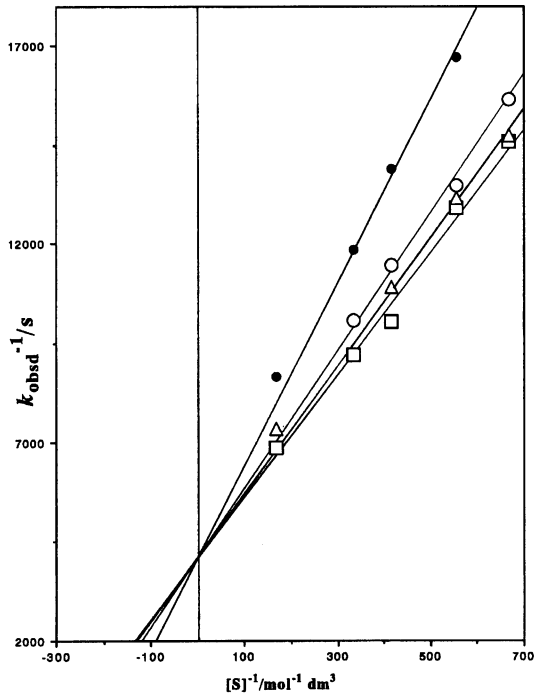


Fig. 8. Competitive inhibition of a footprint catalyst $\{\mathbf{3}\}_{mod.}$ by **1**, **2**, **3**. [S]: benzoic anhydride concentration. □ [I]: 0, △ [I]: 2.0×10^{-6} mol dm^{-3} (**1**), ○ [I]: 2.0×10^{-6} mol dm^{-3} (**2**), ● [I]: 2.0×10^{-6} mol dm^{-3} (**3**).

intact during the modification procedures. The modification effects would alter the practical usefulness of the footprint cavities. They would favor the cavities as receptor sites, such as adsorbents for chromatography, etc., but would not favor cavities as effective catalytic sites.

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