of *direct* exchange between the U and S sites is negligible compared with those of the other exchange processes. The proposed mechanism is

$$U \underset{k_u}{\overset{3k_u}{\rightleftharpoons}} E \underset{k_s}{\overset{k_s}{\rightleftharpoons}} S$$

where $k_u \equiv k_{-1}^{U,E}$ and $k_S \equiv k_1^{S,E}$ in Scheme I and the rate constants refer to magnetization transfer by chemical exchange. The $3k_u$ to k_u ratio for the first step arises from the 3:1 ratio of integrated equilibrium magnetization observed (as it must be) for E:U. Similarly, the 1:1 k_s:k_s ratio of rates for the second step reflects the 1:1 ratio of integrated equilibrium magnetization observed for E:S and is a consequence of the concentrations of phenolate and salt used in the experiment.

The differential equations are

$$\frac{dU}{dt} = -3k_uU + k_uE \qquad \frac{dE}{dt} = 3k_uU - (k_u + k_s)E + k_sS \qquad \frac{dS}{dt} = k_sE - k_sS \tag{7}$$

The matrix **K** is in this case

$$\mathbf{K} = \begin{bmatrix} -3k_u & k_u & 0 \\ 3k_u & -(k_u + k_s) & k_s \\ 0 & k_s & -k_s \end{bmatrix} \tag{8}$$

The characteristic equation yields

$$-\lambda(7k_sk_u + 2k_s\lambda + 4k_u\lambda + \lambda^2) = 0$$

with eigenvalues

$$\lambda_1 = -2k_u - k_s - (4k_u^2 + k_s^2 - 3k_sk_u)^{1/2}$$
$$\lambda_2 = -2k_u - k_s + (4k_u^2 + k_s^2 - 3k_sk_u)^{1/2} \qquad \lambda_3 = 0 \tag{9}$$

Equation 5 gives the projection operators $\mathbf{P}_1$, $\mathbf{P}_2$, and $\mathbf{P}_3$

$$\mathbf{P}_1 = \frac{(\mathbf{K} - \lambda_2\mathbf{I})(\mathbf{K} - \lambda_3\mathbf{I})}{(\lambda_1 - \lambda_2)(\lambda_1 - \lambda_3)} \qquad \mathbf{P}_2 = \frac{(\mathbf{K} - \lambda_1\mathbf{I})(\mathbf{K} - \lambda_3\mathbf{I})}{(\lambda_2 - \lambda_1)(\lambda_2 - \lambda_3)}$$
$$\mathbf{P}_3 = \frac{(\mathbf{K} - \lambda_1\mathbf{I})(\mathbf{K} - \lambda_2\mathbf{I})}{(\lambda_3 - \lambda_1)(\lambda_3 - \lambda_2)} \tag{10}$$

Finally, eq 6 affords the time-dependent magnetization

$$\begin{bmatrix} U(t) \\ E(t) \\ S(t) \end{bmatrix} = \exp(\lambda_1 t)\mathbf{P}_1 \begin{bmatrix} U_o \\ E_o \\ S_o \end{bmatrix} + \exp(\lambda_2 t)\mathbf{P}_2 \begin{bmatrix} U_o \\ E_o \\ S_o \end{bmatrix} + \exp(\lambda_3 t)\mathbf{P}_3 \begin{bmatrix} U_o \\ E_o \\ S_o \end{bmatrix} \tag{11}$$

An Analysis of Small-Molecule Binding to Functionalized Synthetic Polymers by ^{13}C CP/MAS NMR and FT-IR Spectroscopy

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Abstract: A spectroscopic investigation of the binding of mono- and diketones to template-functionalized network copolymers of styrene–*m*-diisopropenylbenzene is reported. Quantitative analysis of the binding modes of a substrate molecule (1,3-diacetylbenzene) to a difunctional polymer site was obtained by empirical calibration of FT-IR and ^{13}C CP/MAS NMR data. The two spectroscopic techniques provide a consistent representation of the manner in which 1,3-diacetylbenzene binds to the difunctionalized polymer site. The analysis also provides an opportunity to quantify site isolation within the polymer and the fidelity with which the functionalized site is maintained by the network polymer. Time-dependent binding studies yield information that aids in the analysis of the method by which template-functionalized polymers affect their recognition properties and calls attention to fundamental differences that exist in binding phenomena between naturally occurring macromolecules (proteins) and synthetic high polymers.

Introduction

Molecular imprinting has been found to be an effective means of encoding information in bulk material on a molecular scale.[1] The procedure involves incorporation of small amounts of an imprinting molecule in the polymerization medium. The imprinting molecule is removed after polymerization, leaving a functionalized cavity in the macromolecular network. The focus of past research has been to define the fidelity with which the network polymer maintains site integrity.

The introduction of organic functional groups at templated sites is achieved by the use of a polyfunctional imprinting or template molecule (A) (Scheme I). Sites prepared in this manner have been evaluated for the ability of the imprinting (template) molecule to "control" the positioning of organic functional groups[2,3] (i.e., C) and influence the shape of the microenvironment.[4,5] In both cases, molecular recognition has been the diagnostic used to evaluate these phenomena, either in batch kinetic rebinding[6,7]

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(2) Shea, K. J.; Dougherty, T. K. *J. Am. Chem. Soc.* **1986**, *108*, 1091.
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(5) (a) Wulff, G.; Sarhan, A.; Zabrocki, K. *Tetrahedron Lett.* **1973**, 4329. (b) Wulff, G.; Kemmerer, R.; Vietmeier, J.; Pohl, H.-G. *Nouv. J. Chem.* **1982**, *6*, 681. (c) Sarhan, A.; Wulff, G. *Makromol. Chem.* **1982**, *183*, 1603. (d) Wulff, G.; Vesper, W.; Grobe-Einsler, R.; Sarhan, A. *Makromol. Chem.* **1977**, *178*, 2799. (e) Wulff, G.; Gimpel, J. *Makromol. Chem.* **1982**, *183*, 2469. (f) Lepisto, M.; Sellergren, B. *J. Org. Chem.* **1989**, *54*, 6010.
(6) (a) Shea, K. J.; Thompson, E. A. *J. Org. Chem.* **1978**, *43*, 4253. (b) Shea, K. J.; Thompson, E. A.; Pandey, S. D.; Beauchamp, P. *J. Am. Chem. Soc.* **1980**, *102*, 3149.

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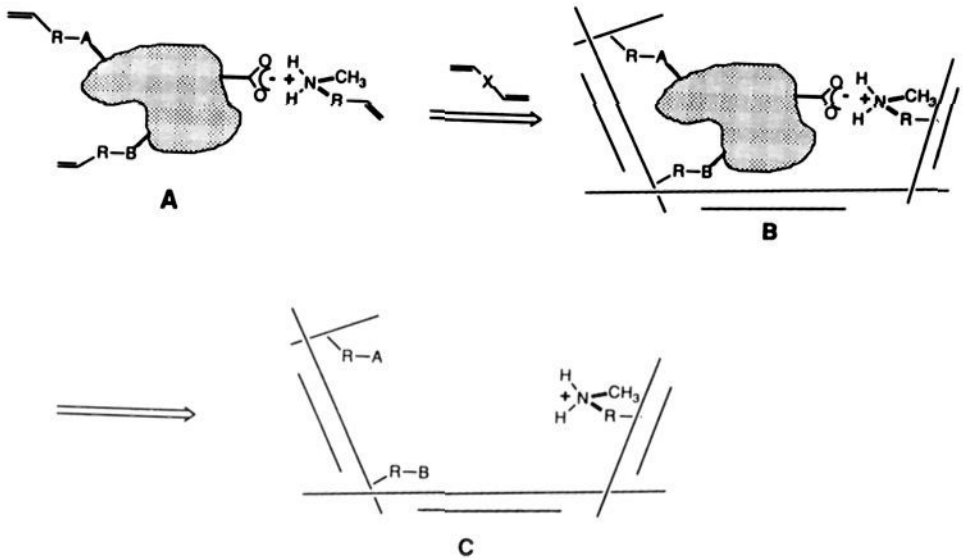
Scheme I



experiments or when the polymer is used in a chromatographic mode.[8,9]

These studies clearly establish that the template or imprinting molecule exercises a degree of control over both the spatial positioning of functional groups and the three-dimensional microenvironment shape. However, in situations thus far examined, little information is available regarding the molecular level details of the rebinding step. The absence of such detail prevents a true understanding of the origins of selectivity and, hence, impedes the design of second-generation materials with greater molecular selectivity and/or catalytic activity.

An analogy is often drawn between template-functionalized network macromolecules and biological receptors and enzymes.[1] This analogy may have some validity; however, synthetic network polymers have a distinctly different character than that of proteins. The materials used in molecular imprinting are amorphous, heterogeneous substances with time scales for segmented polymer chain motions[10] that can be different from those of proteins.[11,12] There may be a variety of factors, therefore, that are unique to rebinding events on synthetic polymers, and comparisons with biological receptors may not be entirely valid.

For these reasons, we have undertaken an investigation of the molecular level details of small-molecule binding to amorphous network polymers functionalized by molecular imprinting. The materials used as scaffolding for these studies are highly cross-linked and, thus, insoluble in all solvents. In addition, the presence of a wide distribution of pore sizes results in opacity. The spectroscopic techniques available for these studies, therefore, are somewhat limited. We have utilized two techniques for this study, FT-IR and solid-state ^{13}C CP/MAS NMR spectroscopies. Both methods give a consistent representation of events taking place and provide general protocols for the study of binding of small molecules to amorphous synthetic polymer solids.

Results

The systems chosen to study small-molecule binding to synthetic polymers is outlined in Scheme II.

Functionalized sites containing either one or two 1,3-diol binding groups are prepared by copolymerization of small amounts (ca. 1 mol %) of isomerically pure mono- or bisketal templates **1**, **4**, or **7** with styrene–*m*-diisopropenylbenzene (1:1). The resulting materials, after crushing, extraction to remove unreacted monomer, drying, and then sizing, are hydrolyzed ($MeOH/H_2O/H_2SO_4$) to liberate either acetophenone, 1,3-diacetylbenzene, or 1,3-diacetylpyrene.[2,4,13] The number of functional sites created in this step is quantified by evaluating the amount of acetophenone or 1,3-diacetylaryl liberated by hydrolysis. The hydrolyzed polymers **3**, **6**, and **9**, containing approximately 80 μmol sites/g polymer, were then subjected to rebinding conditions (ketone, benzene, *p*-toluenesulfonic acid(cat.); reflux). An analysis of rebinding selectivity to the difunctional sites has been reported elsewhere.[2,4] After sufficient reaction times, the template molecules acetophenone, 1,3-diacetylbenzene, and 1,3-diacetylpyrene can reoccupy the sites in near quantitative yield. The current study was designed to examine the molecular level details of rebinding to the functional sites. Rebinding of *difunctional* molecules to *monofunctional sites* (**3**) provides an opportunity for quantifying the extent of *isolation* of the polymer-bound functional groups **10**/**11** (Scheme III). Two possibilities are envisioned for rebinding to the *difunctional sites* (**6**, **9**) (Scheme IV). In the first, the diketone molecule can rebind to reform two new ketal linkages in a manner similar to its original situation (**12**).[6] Alternatively, the diketone can rebind to form only one ketal linkage, giving rise to a residual carbonyl functional group (**13**). The possibility of noncovalent binding by dicarbonyl compounds has been rigorously excluded (vide infra). Quantification of site isolation and a distinction between the two modes of rebinding were accomplished by a combination of FT-IR and ^{13}C CP/MAS NMR spectroscopy.

FT-IR Studies. Calibration of Data. Blank, nonfunctionalized copolymers of styrene–*m*-diisopropenylbenzene (1:1) doped with monoketal **14** were prepared. The loadings of **14** range from 0 to 160 μmol/g polymer. Each of the FT-IR spectra of the doped polymers (KBr dispersion) was subjected to the same base line correction, and residual H_2O absorption was removed by spectral subtraction. A waterfall display of the resulting IR spectra is shown in Figure 1. The net peak area[14,15] of the carbonyl group

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(9) (a) Sellergren, B.; Ekberg, B.; Mosbach, K. *J. Chromatogr.* **1985**, *347*, 1. (b) Sellergren, B.; Lepisto, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853. (c) Glad, M.; Norrlow, O.; Sellergren, B.; Siegbahn, N.; Mosbach, K. *J. Chromatogr.* **1985**, *347*, 11. (d) Arshady, R.; Mosbach, K. *Makromol. Chem.* **1981**, *182*, 687.

(10) DeGennes, P.-G. *Introduction to Polymer Dynamics*; Cambridge University Press: Cambridge, UK, 1990.

(11) McCammon, J. A.; Harvey, S. C. *Dynamics of Proteins and Nucleic Acids*; Cambridge University Press: Cambridge, UK, 1987.

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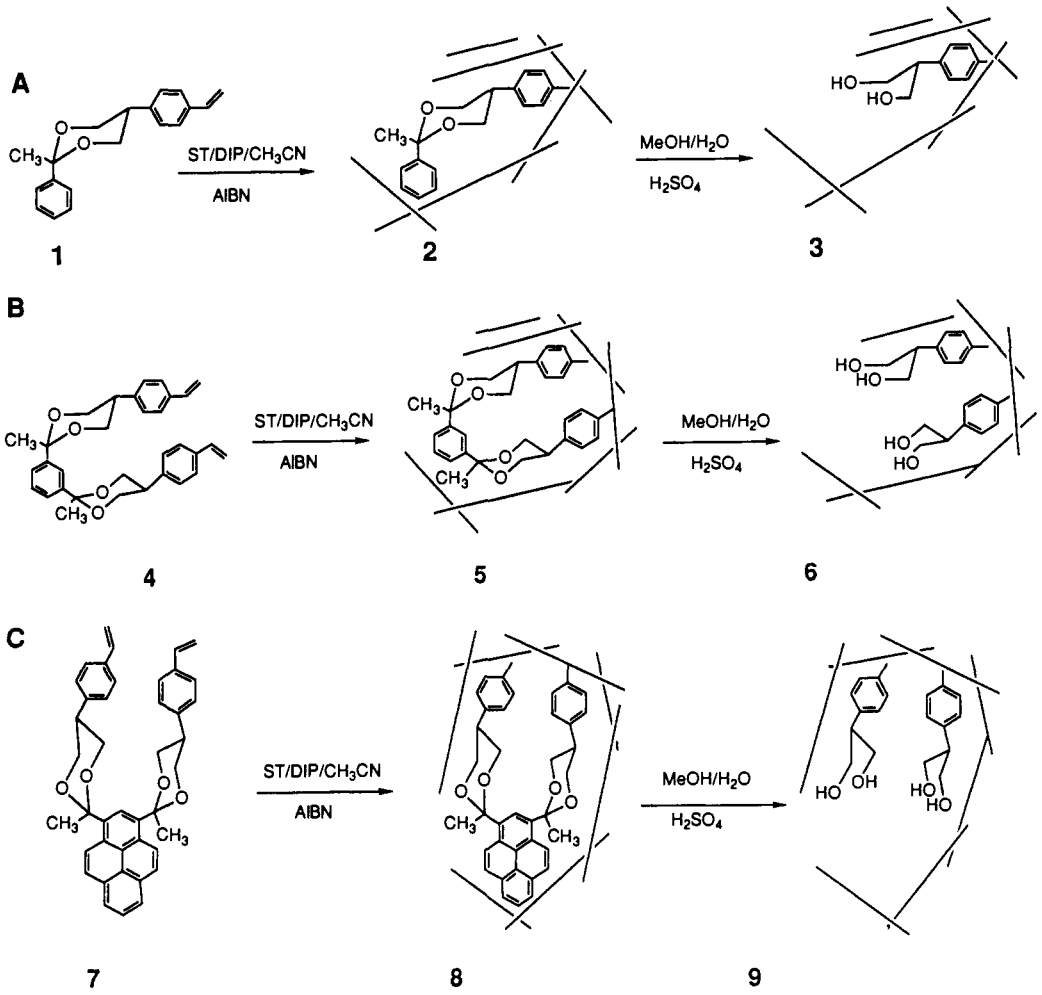
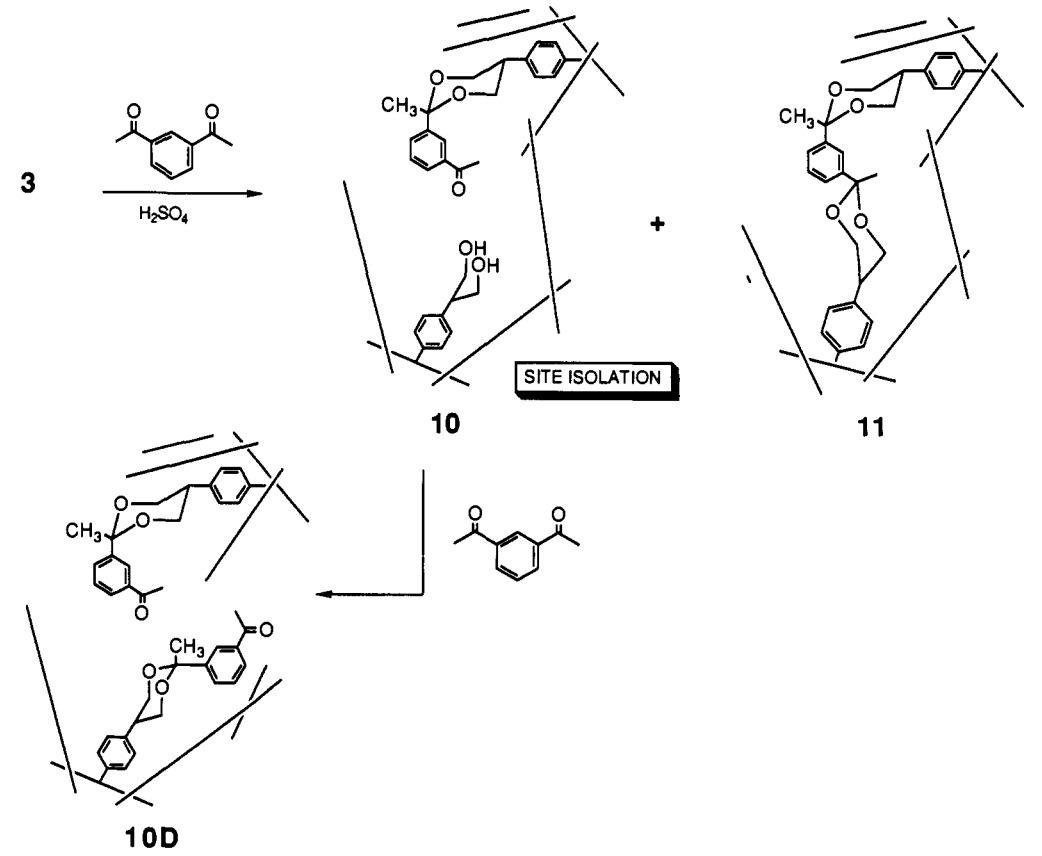
(13) Shea, K. J.; Sasaki, D. Y.; Stoddard, G. J. *Macromolecules* **1989**, *21*, 1722.

(14) Net peak area integrates the peak between the integration limits *from the spectral base line*. Total peak area integrates the peak between the integration limits *from zero absorbance*.

Scheme II



Scheme III



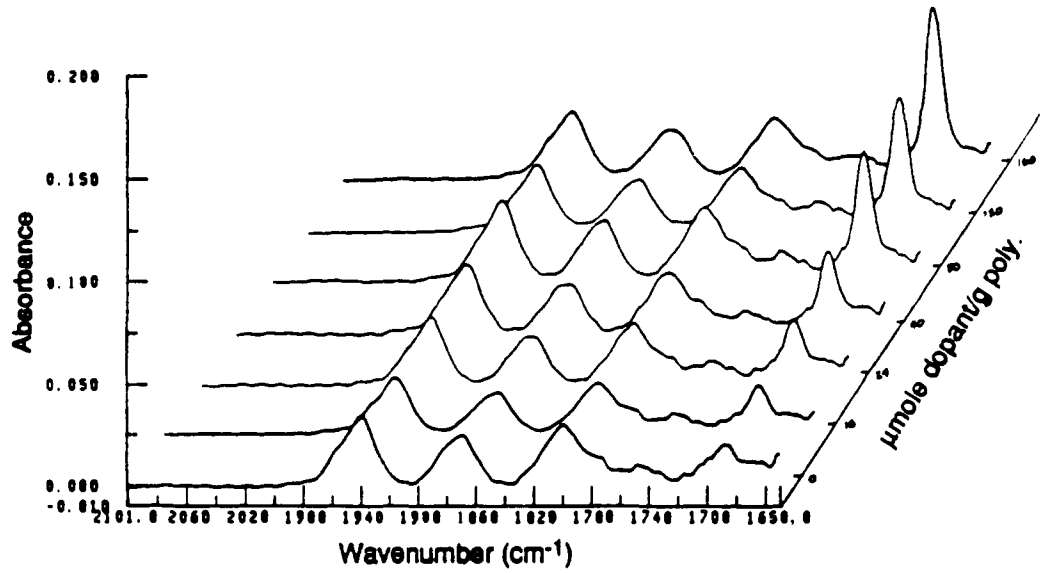
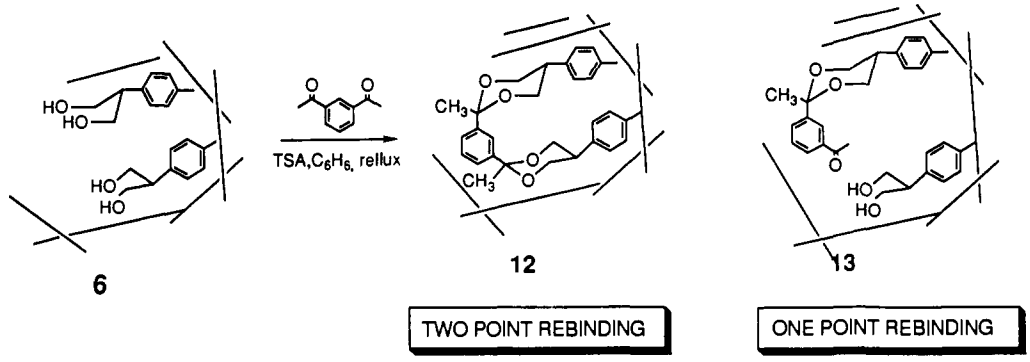


Figure 1. Waterfall display of FT-IR spectra of styrene–diisopropenylbenzene copolymer doped with **14**. The carbonyl peak intensity was used in the preparation of the calibration curve. The absorption of interest is in the carbonyl region (1691 cm^{-1}).[13]

Scheme IV



was determined in the absorbance mode between the integration limits of 1720.2 and 1670.1 cm^{-1}. The area was normalized against the net reference absorption at 1800 cm^{-1} with integration limits between 1839.8 and 1766.5 cm^{-1}. The normalized carbonyl peak area was then plotted against the micromoles of monoketal **14** per gram of polymer (Figure 2).

The data in Figure 2 exhibit good linearity with a slope of 7.24×10^{-3}, y-intercept at 0.292, and a correlation coefficient of 0.998. When a peak height or total peak area[14] was used, less satisfactory correlations were obtained due to inconsistencies in base line curve reproduction. The preceding correlation allows for the calculation of the amount of monoketal present on polymers by the use of eq 1.

$$(\text{normalized C=O peak area} - 0.292)/(7.24 \times 10^{-3}) = \mu\text{mol monoketal/g polymer} \quad (1)$$

FT-IR Studies. Analysis of Rebinding Mode. Template-functionalized styrene–*m*-diisopropenylbenzene copolymers were subjected to hydrolysis conditions, and the amount of acetophenone and 1,3-diacetylbenzene liberated was quantified by gas chromatographic analysis. This value was used to quantify the number of "vacant" sites on each polymer. Each polymer was then subjected to the rebinding conditions. The amount of acetophenone or 1,3-diacetylbenzene taken up by the polymer was determined by GC analysis. The rebinding data are summarized in Table I. The majority of data reflect experiments performed in triplicate, with the results reported as the median value. Within a data set, the one-point binding percentage varied, on the average, no more than 10%. The data includes the calculated percentages of one-point and two-point binding based on the measured IR carbonyl intensities and the total amount of rebound template as determined by gas chromatographic analysis. Support for the absence of *adsorbed* substrate on the polymer under the rebinding

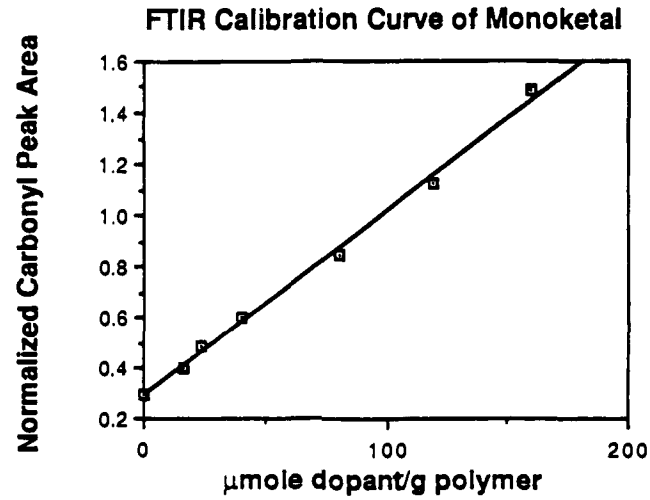


Figure 2. Calibration curve of normalized carbonyl peak area vs micromoles of **14** per gram of polymer). Linear fit by least squares.

(15) The carbonyl absorption for compound **14** shifted from 1680 cm^{-1} in its pure form to 1691 cm^{-1} when adsorbed onto blank polymer and when 1,3-DAB is ketalized to the polymer. This may be due to either the refractive index of the polymer or chemical or physical interaction of the substrate functionality with the polymer. For further discussions, see: (a) Allara, D. L. *Appl. Spectrosc.* **1979**, *33*, 358. (b) Jasse, B. In *Development in Polymer Characterization-4*; Dawkins, J. V., Ed.; Applied Science Publishers: New York, 1983; Chapter 3. (c) Coleman, M. M.; Zarian, J. *J. Polym. Sci., Polym. Phys. Ed.* **1979**, *17*, 837. (d) Coleman, M. M.; Varnell, D. F. *Ibid*, **1980**, *18*, 1403.

Table I. FT-IR/GC Analysis of the Binding of Acetophenone and 1,3-Diacetylbenzene to Templated Polymers

entry	polymerization template	available sites (μmol/g polymer) (%)	rebinding substrate	μmol substrate rebound g polymer (%)[b]	normalized C=O peak area	calcd μmol monoketal/ g polymer	rebinding (%) 1-pt	rebinding (%) 2-pt
1	acetophenone (A)	69 (86)			0.269			
2		69 (86)	acetophenone (24 h)	60 (86)	0.273			
3		69 (86)	1,3-diacetylbenzene (24 h)	43	0.544	35	81	19
4	1,3-diacetylbenzene (B)	65 (81)			0.245			
5		65 (81)	1,3-diacetylbenzene (13 h)	40 (61)	0.423	18	45	55
6		65 (81)	1,3-diacetylbenzene (38 h)	60 (92)	0.608	44	73	27
7		65 (81)	1,3-diacetylbenzene[a]	58 (89)	0.461	23	40	60

[a] 1,3-Diacetylbenzene was rebound for 24 h (58 μmol 1,3-diacetylbenzene/g polymer), separated from free 1,3-diacetylbenzene, and then subjected to refluxing with Soxhlet extraction of H_2O in fresh, dry, acidic benzene for an additional 24 h. [b] Rebindings were carried out by refluxing hydrolyzed polymer in benzene containing a catalytic amount of *p*-toluenesulfonic acid in the presence of a 3-fold excess of substrate to theoretical sites. The amount of substrate bound to the polymer was determined by GC analysis.

Table II. ^{13}C CP/MAS NMR Data of Binding of ^{13}C-Labeled 1,3-DAB to Templated Polymers

entry	polymerization template	available sites (μmol/ g polymer)	rebinding time[b] (h)	μmol 1,3-diacetylbenzene rebound/g polymer NMR	μmol 1,3-diacetylbenzene rebound/g polymer GC	μmol ketone/g polymer	μmol ketal/g polymer	rebinding (%) 1-pt	rebinding (%) 2-pt
1	acetophenone (A)	69	24	50	32	36	63	73	27
2		69	88	55	54	50	59	92	8
3	DAB (B)	65	24	64	46	23	104	36	64
4		65	88	81	68	34	128	42	58
5	DAP (C)	56	24	64	43	33	95	52	48
6		56	88	66	66	32	99	49	51

[a] Rebindings were carried out by refluxing hydrolyzed polymer in acidic benzene in the presence of a 3-fold excess of ^{13}C-labeled 1,3-DAB substrate for the indicated times.

conditions is given in entry 2. The data are from a sample of hydrolyzed polymer templated with acetophenone (**3**) and then rebound with acetophenone. This sample reveals no adsorbed acetophenone on the polymer (absence of carbonyl absorption) since acetophenone on blank polymer absorbs approximately in the same region and with an extinction coefficient similar to that of the 1,3-diacetylbenzene monoketal adsorbed on polymer. All the acetophenone bound to the polymer (60 μmol/g polymer, 86% rebinding), therefore, must be in the ketalized form **2**. The workup procedure and rebinding conditions leave no adsorbed substrate to interfere with the analysis.

The second column in Table II indicates the molecule used to imprint the polymer. The amount of sites produced upon hydrolysis was calculated on the basis of an initial 80 μmol template/g polymer loading and is given in column 3. In the fourth column, the substrate used for rebinding is given, with the rebinding time indicated in parentheses. The amount of substrate rebound (μmol/g polymer) is given in column 5. The FT-IR carbonyl integration data are in column 6 and by using this data and eq 1 column 7 is produced. The binding percentages (last two columns) are then calculated from the data of columns 5 and 7.

It should be noted that the template hydrolysis did not result in *complete* removal of ketone. However, the hydrolyzed acetophenone-templated polymer **3** and 1,3-diacetylbenzene-templated polymer **6** were free from ketone absorption. Thus, under the rebinding conditions, the template remaining (14–20%) on the polymer should not interfere with the spectroscopic study; the residual templates remaining after hydrolysis can be considered "masked".

For polymer **6**, conditions for rebinding with 1,3-diacetylbenzene were varied in an effort to determine if the mode of rebinding exhibited a kinetic dependence. In entry 5, the standard rebinding reaction was performed for 13 h, resulting in the rebinding of 40 μmol 1,3-diacetylbenzene/g polymer (GC analysis). IR analysis revealed a one-point binding (**13**) amounting to 45% and two-point binding (**12**) of 55% (Scheme IIB). When the rebinding reaction was allowed to proceed for 38 h, the amount of 1,3-DAB bound to the polymer increased to 60 μmol/g polymer (GC) and the IR analysis is revealed two-point binding decreased to 27% (entry 6). In contrast, allowing polymer **6** that had been rebound with 1,3-diacetylbenzene at 58 μmol/g polymer to undergo additional refluxing in the absence of 1,3-diacetylbenzene in acidic benzene with removal of H_2O resulted in an *increase* in the amount of two-point rebinding to 60%.

^{13}C CP/MAS NMR Studies. Calibration of Data. Despite the importance of ^{13}C CP/MAS NMR in the characterization of solid polymers, there were uncertainties regarding its application to the current problem. The dispersity of chemical shifts due to sample heterogeneity, the potential for significant differences in relaxation times resulting from a broad range of functional group mobilities, and lack of sensitivity due to low levels of functionality were all potential problems.[16,17] As the following data reveal, these difficulties can be overcome and ^{13}C CP/MAS NMR is shown to be an extraordinary valuable technique for the examination and quantification of molecular level detail of small-molecule binding to solid polymers. The templated polymer systems chosen for study were the same as those used for the IR work. Importantly, solid-state ^{13}C CP/MAS NMR has the potential for detection of both ketone and ketal functionality in the substrate-rebound polymers. Quantifying both ketone and ketal groups permits the direct determination of one-point and two-point rebinding. To quantify the amount of ketone and ketal functionality present in the polymer, we developed an empirical calibration of the relevant NMR signals. Since the amount of substrate rebound to the polymer is less than 1 mol % of the total number of monomers (20–130 μmol template/g polymer), sensitivity was of particular concern. Unlike the FT-IR experiment where the carbonyl extinction coefficient is large compared with other absorptions, the **14**-^{13}C ketal and ketone absorptions are of the same

(16) (a) Axelson, D. E.; Russell, K. E. *Prog. Polym. Sci.* **1985**, *11*, 221. (b) Yannoni, C. S. *Acc. Chem. Res.* **1982**, *15*, 201. (c) Maciel, G. E. *Science (Washington, D.C.)* **1984**, *226*, 282. (d) Mehring, M. In *High Resolution NMR Spectroscopy in Solids*; Diehl, P., Fluck, E., Kosfeld, R., Eds.; Springer-Verlag Berlin: Heidelberg, Germany, 1976. (e) Schaefer, J.; Stejskal, E. O. In *Topics in Carbon-13 NMR Spectroscopy*; Levy, G. C., Ed.; Wiley-Interscience: New York, 1979; Chapter 4. (f) Andreis, M.; Koenig, J. L. *Adv. Polym. Sci.* **1989**, *89*, 69.

(17) (a) Lindberg, J. J.; Hortling, B. *Adv. Polym. Sci.* **1985**, *66*, 1. (b) Patterson, D. J.; Koenig, J. L. In *Characterization of Highly Crosslinked Polymers*; Labana, S. S., Dickie, R. A., Eds.; ACS Symposium Series 243; American Chemical Society: Washington, DC, 1984; Chapter 12, p 205. (c) Harris, R. K. *Analyst* **1985**, *110*, 649.

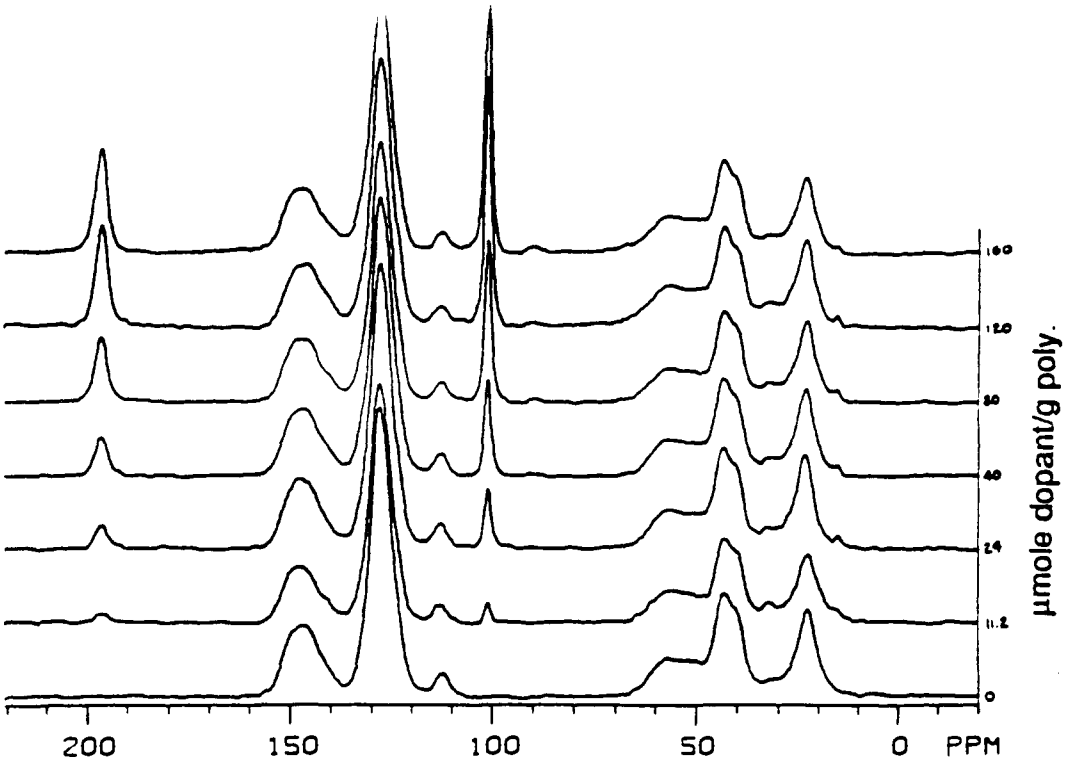
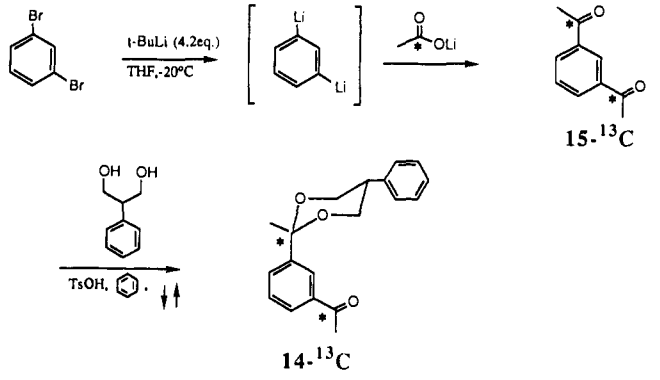


Figure 3. Waterfall display of ^{13}C CP/MAS NMR spectra used in the production of the calibration curves in Figure 5 and 6. The signal at δ 198 ppm is the ketone carbon absorption, and the one at δ 100 ppm is from the ketal carbon absorption arising from doping blank styrene–diisopropenylbenzene with **14**-^{13}C.

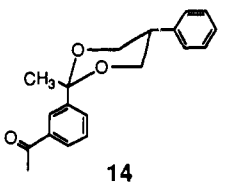
Scheme V



intensity as the aryl and alkyl carbons. To circumvent this problem, $^{13}C_{C=O}$-labeled 1,3-diacetylbenzene (99% enrichment) was used in the rebinding studies and **14**-^{13}C as the dopant for the calibration studies. The heavy-atom ^{13}C isotope effect is not expected to influence the chemical events in the present study.[19]

Synthesis of the doubly labeled 1,3-diacetylbenzene (**15**-^{13}C) was achieved by coupling of 1,3-dilithiobenzene and sodium acetate-*1*-^{13}C. The dilithio compound was in turn prepared by lithiation of 1,3-dibromobenzene with *t*-BuLi (Scheme V).

The ^{13}C NMR absorptions of **14** for the ketone and ketal carbons are at δ 198 and 100 ppm, respectively. These peaks were well-resolved from the sp^2 and sp^3 carbon resonances arising from the bulk styrene–*m*-diisopropenylbenzene copolymer. The doped polymers used for calibration were prepared by adsorbing known amounts of monoketal **14**-^{13}C onto blank polymer. Their peak areas were then measured from their ^{13}C CP/MAS NMR spectra (Figure 3).



14

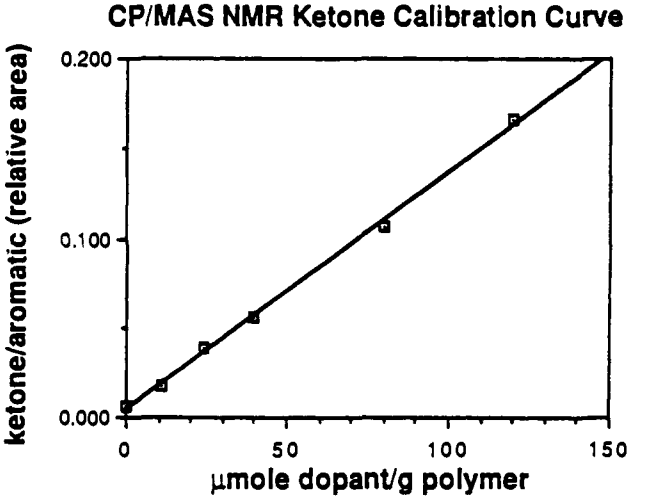


Figure 4. Calibration curve of normalized ketone carbon peak area vs micromoles of **14**-^{13}C per gram of polymer. The linear fit was determined by least squares, which showed a correlation coefficient of 0.9991.

The area of the carbonyl resonance (198 ppm) was normalized with respect to the aromatic region (δ 159.5–106.2 ppm) of the cross-linked polymer and then plotted against the micromoles of dopant **14**-^{13}C per gram of polymer to produce the calibration plot for the ketone functional group (Figure 4). The same treatment was used for the ketal carbon absorption (100 ppm), resulting in a calibration curve for the ketal functionality shown in Figure 5.

Both calibration plots are linear over the concentration range studied, and their *y*-intercepts are very close to zero. Equations 2 and 3, derived from the NMR data, can be used to determine amounts of ^{13}C-labeled ketone and ketal groups resulting from rebinding of ketone substrates to the polymer.

$$(\text{normalized ketone area} - 2.15 \times 10^{-3})/(1.36 \times 10^{-3}) = \mu\text{mol ketone groups/g polymer} \quad (2)$$

$$(\text{normalized ketal area} - 5.39 \times 10^{-3})/(1.77 \times 10^{-3}) = \mu\text{mol ketal groups/g polymer} \quad (3)$$

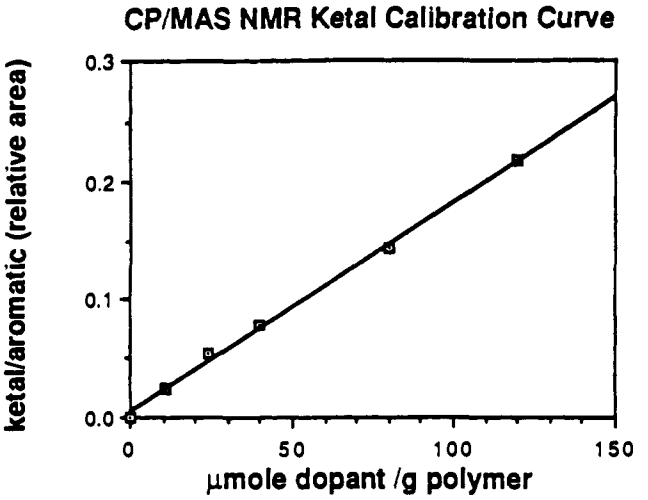


Figure 5. Calibration curve of normalized ketal carbon peak area vs micromoles of **14**-^{13}C per gram of polymer. The linear fit was determined by least squares with a correlation coefficient of 0.9986.

^{13}C CP/MAS NMR Studies. Analysis of Rebinding Mode. Figure 6 shows a ^{13}C CP/MAS NMR of a typical rebinding experiment employing 99% ^{13}C-enriched 1,3-diacetylbenzene (**15**) as the substrate. The carbonyl and ketal resonances (*) are clearly resolved from the aromatic and aliphatic resonances of the polymer, and the robust signal intensity provides for accurate integration of the signals. Also of importance is the finding that the carbonyl and ketal peak widths of 5 and 3 ppm, respectively, are surprisingly narrow considering the heterogeneous nature of the materials. The distribution of polymer microenvironments does not produce significant line broadening. This finding suggests that CP/MAS NMR can be of general utility in providing molecular level details of small-molecule binding to solid amorphous materials.

Table II summarizes data from rebinding studies of three different templated polymers. The templates used for the synthesis were acetophenone (polymer **3**), 1,3-diacetylbenzene (polymer **6**), and 1,3-diacetylpyrene (polymer **9**). The conditions used for rebinding the mono- and dicarbonyl substrates were the same as that used in the FT-IR studies although slightly different rebinding times were used. In all cases, $^{13}C_{C=O}$-labeled (99%) 1,3-diacetylbenzene (**15**) was used for the rebinding.

With use of eqs 2 and 3, the micromoles of ketone per gram of polymer and micromoles of ketal per gram of g polymer were calculated from the observed NMR peak intensities at 100 and 198 ppm. The one-point and two-point binding percentages were calculated from the following:

$$\mu\text{mol ketone groups/g polymer} = \mu\text{mol monoketal units/g polymer}$$

and

$$(\mu\text{mol ketal groups/g polymer}) - (\mu\text{mol monoketal units/g polymer}) = 2(\mu\text{mol bisketal units/g polymer})$$

The percent of 1-point rebinding is calculated as follows:

$$100 \times \mu\text{mol monoketal units}/(\mu\text{mol monoketal units} + \mu\text{mol bisketal units}) = \%\ \text{1-point rebinding}$$

Also included in Table II are the NMR and gas chromatographic values for the amount of ^{13}C-labeled substrate rebound to the polymer as determined by the two analytical techniques. In general, the *absolute* amounts of 1,3-diacetylbenzene-^{13}C rebound to the polymers is best quantified by the GC data since the absolute values of the NMR data were considerably more sensitive to the choice of spectroscopic parameters and data manipulation. The agreement between the two methods in some cases is only fair. The ratios of ketal to ketone, however, when appropriate comparisons can be made are in good agreement.

Discussion

Copolymerization of small amounts of ketal template assemblies such as **1**, **4**, and **7** with cross-linking monomers in the presence of an inert diluent (porogen) results in formation of a macroporous network copolymer.[2] The materials have a high internal surface area (100 m^2/g) and an average pore radius of 50 nm. The polymer is hydrolyzed (acid catalysis), liberating the ketone template molecule acetophenone, 1,3-diacetylbenzene, or 1,3-diacetylpyrene. The number of functionalized sites produced is

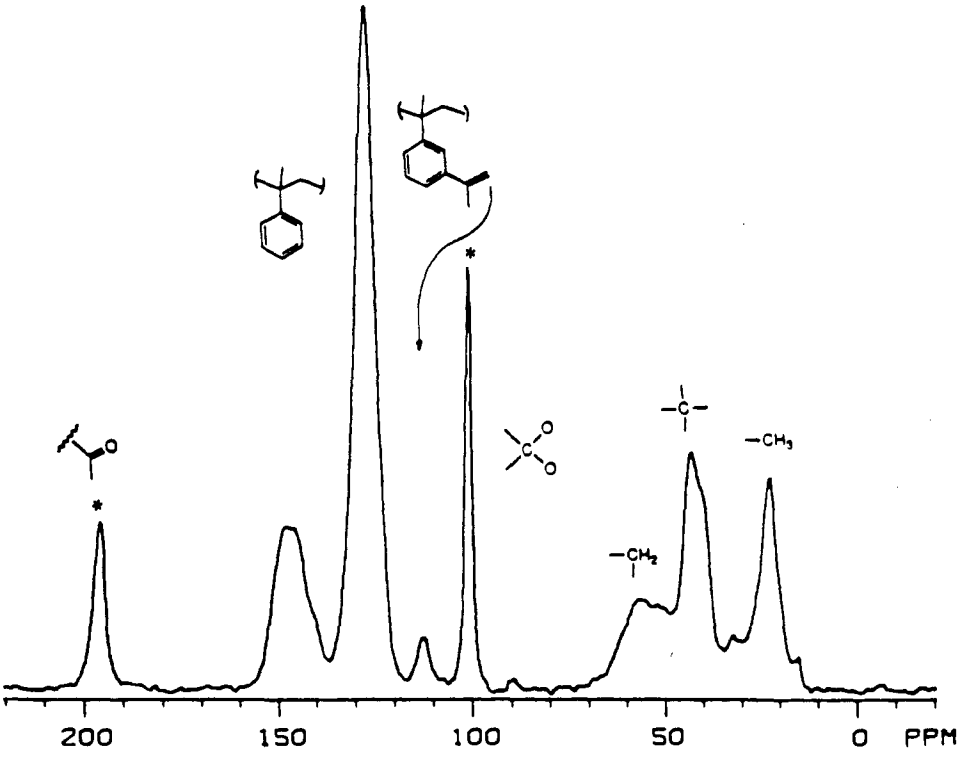


Figure 6. ^{13}C CP/MAS NMR spectrum of a typical rebinding experiment with substrate **15** and polymer **6**. All carbons of the sample are shown with their associate absorptions.

quantified from the amount of ketone liberated. The hydrolysis results in formation of polymer-bound 1,3-diol groups. Two types of sites (monofunctional and difunctional) have been generated by this technique (**3**, **6**, and **9**). Rebinding studies by our group and related studies by Wulff,[3,5] Mosbach,[7,9] and Neckers[7a,b] have demonstrated that polyfunctional sites prepared by template polymerization exhibit a "memory" for the original template molecule. The aforementioned studies were carried out under conditions of both kinetic and thermodynamic control. Furthermore, these polyfunctional sites exhibit a distinctive behavior that is dependent upon the relative positioning of the functional groups at each site, indicating an as yet unspecified *cooperative* phenomena between the functional groups during rebinding. Finally, in situations where the separation of templated functional groups are held constant, the *shape* of the template has been found to be an important determinant in creating the microenvironment shape of the functionalized site.[1,4] This manifests itself in rebinding selectivities of molecules with different shapes.

The preceding findings were obtained from studies of substrate uptake and from competition rebinding and chromatographic investigations. A deeper understanding of the origins of the rebinding selectivity would be significantly enhanced by a detailed molecular level analysis of the events taking place during rebinding. Such information can provide insight into the origins of selectivity and can point the way to the design of second-generation binding sites. This study is concerned with three facets of ketone rebinding to templated sites. First, we wish to determine to what extent the sites are truly isolated from one another. Second we want to know how difunctional molecules rebind to difunctional sites (i.e., whether one or two new ketal linkages are formed). Third, we wish to determine if the manner of diketone rebinding changes both with time of reaction and/or as a function of the number of sites reoccupied. Experiments were designed so that FT-IR and CP/MAS NMR spectroscopies could quantitate a very similar problem. A comparison of the two spectroscopic studies would not only add credibility to the results, but would also provide useful guidelines for the choice of analytical technique for studying molecular level phenomena on amorphous solid materials.

Site Isolation.[18] When monofunctional template **1** is incorporated into the polymeric matrix and then hydrolyzed in the presence of a catalytic amount of toluenesulfonic acid (TSA), *monofunctional sites* are produced (**3**). By starting with a polymer containing 80 μmol template **1**/g polymer, hydrolysis affords 86% of the theoretical amount of acetophenone. This corresponds to 69 μmol sites/g polymer. The rebinding of acetophenone proceeded to 87% completion (60 μmol) by GC analysis. There were no carbonyl groups in the IR confirming the absence of adsorbed ketone under the rebinding conditions. Under the same conditions with 1,3-diacetylbenzene as substrate, 62% rebinding to **3** was observed (43 μmol DAB/g polymer). Analysis of the carbonyl intensity of this rebound polymer by FT-IR permits one to compute the presence of 35 μmol C=O/g polymer. This value corresponds to 80% *one-point attachment* of 1,3-diacetylbenzene, which implies to 80% site isolation (Scheme III) (vide infra).

A related series of experiments using ^{13}C CP/MAS NMR were performed with use of bis-^{13}C-labeled 1,3-diacetylbenzene as the substrate for *monofunctional* sites (**3**). The amount of one-point rebinding calculated after 24 h (46% coverage) in refluxing benzene is 73% (Table II, entry 1). A second experiment (Table II, entry 2), analyzed after rebinding had taken place for 88 h (78% coverage), indicated 92% one-point attachment. The results of these two similar experiments (73%, 92%) bracket the FT-IR findings and reveal that close to the theoretical amount of carbonyl groups are observed when an excess of a difunctional reagent is allowed to rebind with 1,3-diol groups that are randomly bound to the polymer.

To summarize, at functional group loadings of 60–70 μmol/g polymer on materials with dry surface areas of approximately 100 m^2/g, the NMR and IR methods give a consistent representation of the degree of site isolation—the sites may be considered to be 80 ± 10% isolated.

It should be noted, however, that estimation of the degree of site isolation may be complicated by the relative rates of reaction of singly bound difunctional molecules with a second "surface" or site functional group (i.e., **10** → **11**) versus the rate of reaction of that same surface functional group with unbound (free) diketone in bulk solution (i.e., **10** → **10D**, Scheme III). If the quasi intramolecular reaction is not considerably faster than the bimolecular reaction, factors such as the concentration of "free" diketone and the extent of covering of the surface functionality (the instantaneous concentration of available surface functionality) can influence the apparent site isolation values. For simplicity, our analysis assumes that the intramolecular reaction is relatively fast. Some justification for this is necessary in view of the results of entry 7 (Table I) that show that, in the absence of external ketone, the distribution of rebound monoketal and bisketal groups changes with time. This, of course, implies that reaction of the second functional group can be relatively slow on the laboratory time scale! The explanation for this apparent contradiction is that pairwise introduction of functional groups by template polymerization produces "sites" of functionality that are prone to interact in a *cooperative* manner and not as isolated functional groups that are introduced individually. Evidence for this comes in part from rebinding studies with small, highly reactive substrate molecules reacting at difunctionalized sites—both functional groups at a surface site can be reacted. However, as the *size* of the substrate reagent is increased, the number of functional groups that react converges toward one per site; thus, reaction of one of the functional groups with a bulky reagent blocks access to the second functional group at the site.[6] This, of course, results from the proximity of the two functional groups. Functionality introduced *singly* at low loadings is not expected to exhibit this special *cooperative behavior*. Rebinding at monofunctional sites, therefore, can be used as a guide to site isolation.

Rebinding of Difunctional Molecules to Difunctional Sites. Incorporation of templates **4** and **7** followed by hydrolysis results in formation of *difunctional sites* containing two 1,3-diol groups (**6**, **9**). Rebinding of a diketone to the difunctional site can take place by one of two rebinding modes, by formation of either a monoketal or a bisketal (Scheme IV). This analysis has been simplified by excluding hemiketals as the product. However, it should be noted that the two spectroscopic techniques do *not* rigorously rule out their possible involvement.

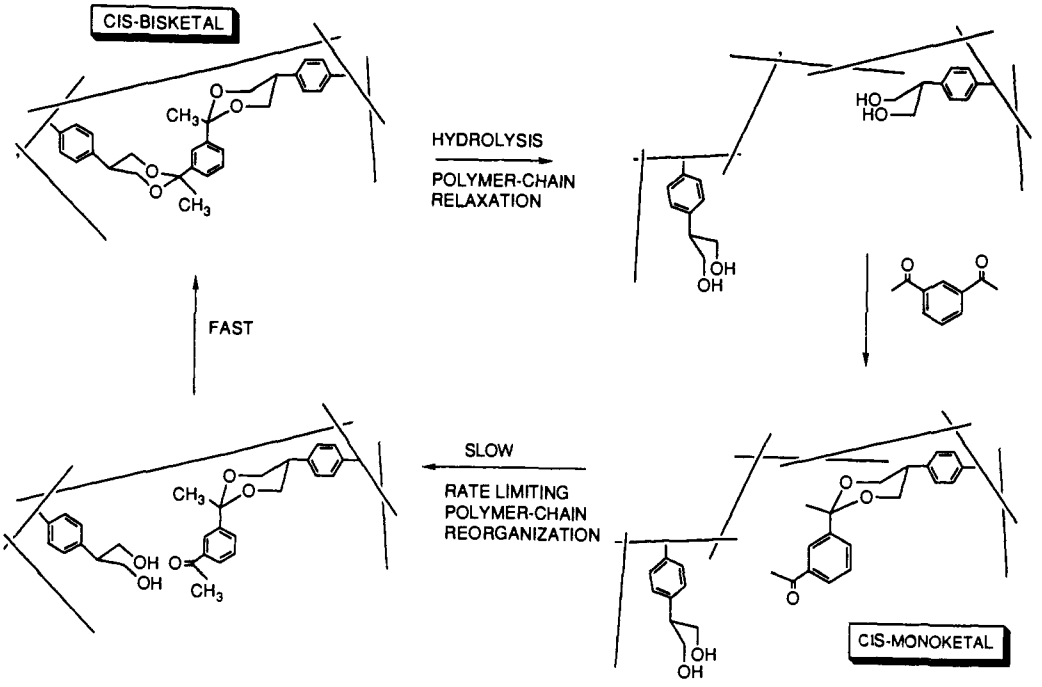
FT-IR Analysis. When diketones are rebound at difunctional sites (Scheme IV), the amount of residual carbonyl groups permit quantification of how the site is reconstituted. Reketalization of functionalized polymers containing 65 μmol sites/g polymer with a 3-fold molar excess of substrate (1,3-diacetylbenzene, TSA(cat.)) for 13 h results in 62% reoccupancy of sites. Analysis of the carbonyl region by FT-IR permits one to calculate that rebinding occurs with 45% monoketal formation and 55% bisketal formation. Furthermore, if the ketalization time is increased to 38 h total, 92% of the sites are reoccupied but now 73% are rebound as monoketals and 27% as bisketals. Interestingly, this result indicates that a significant amount of all late-binding 1,3-diacetylbenzene does so as the monoketal.

It was of interest to examine if, after a certain amount of rebinding, the distribution of monoketal and bisketal groups undergoes a change *upon further exposure to the ketalization conditions*. For this experiment, difunctional sites prepared with bisketal **4** (65 μmol sites/g polymer) were rebound with 1,3-diacetylbenzene (24 h, 58 μmol 1,3-diacetylbenzene/g polymer, 89% coverage). The percent coverage for this rebound polymer is almost identical with that of entry 6 (92%). The shorter rebinding time (24 vs 38 h) permits estimation of a minimum of 73% monoketal formed at this stage. The ketalization was interrupted

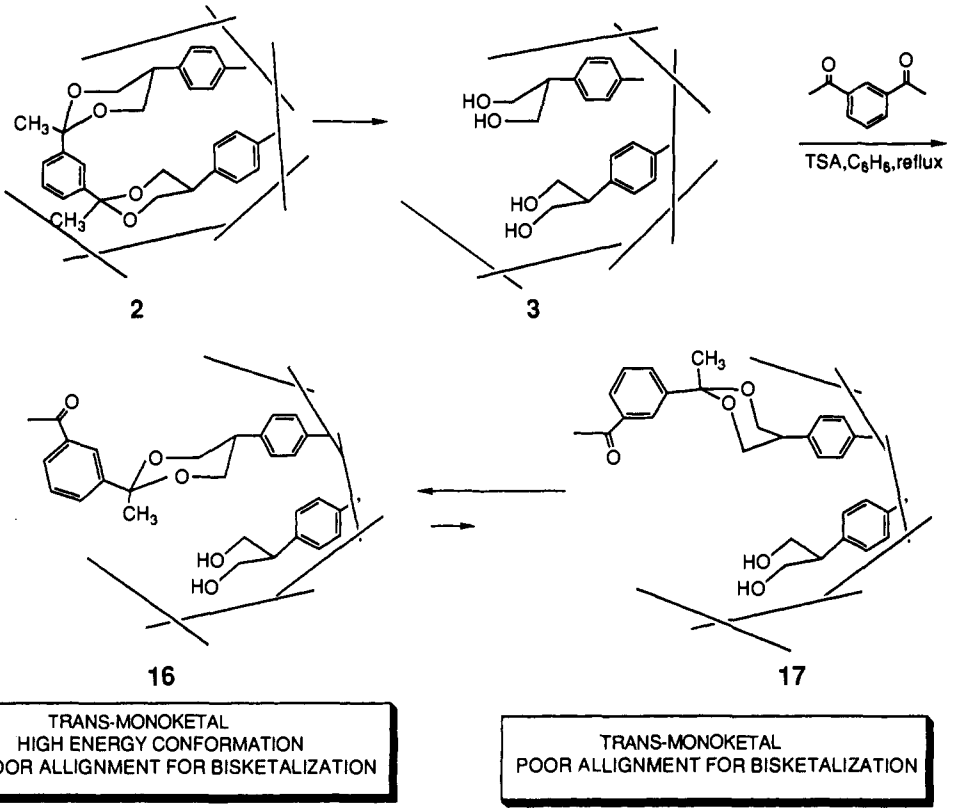
(18) (a) Ford, W. T. In *Polymeric Reagents and Catalysts*; Ford, W. T., Ed.; ACS Symposium Series 308; American Chemical Society: Washington DC, 1986; Chapter 11. (b) Shea, K. J.; Thompson, E. A. *Macromolecules* **1985**, *18*, 814. (c) Lochmuller, C. H.; Hill, W. B., Jr. *Anal. Chim. Acta* **1984**, *157*, 65. (d) Crowley, J. I.; Rapoport, H. *Acc. Chem. Res.* **1976**, *9*, 135.

(19) (a) Stothers, J. B.; Bourns, A. N. *Can. J. Chem.* **1962**, *40*, 2007. (b) Melander, L.; Saunders, W. H., Jr. *Reaction Rates of Isotopic Molecules*; Wiley-Interscience: New York, 1980.

Scheme VI. Rebinding Mode Dependent upon Rate-Limiting Polymer Chain Reorganization



Scheme VII



and the polymer separated from free 1,3-diacetylbenzene and then subjected to refluxing with Soxlet extraction of H_2O in benzene containing catalytic TSA for an additional 24 h. IR analysis of the carbonyl region reveals that 60% of the bound 1,3-diacetylbenzene is now present as the *bisketal*.

The results indicate that extensive ketalization can take place after the first carbonyl binds. The effect is most pronounced at slow- or late-binding difunctional sites. This finding is best understood in terms of a slow second ketalization step between the remaining carbonyl group of 1,3-diacetylbenzene monoketal and the second 1,3-diol group at the site. The fact that the second intramolecular ketalization at these late-binding sites is relatively slow may be attributed to several factors. Upon hydrolysis of the template 1,3-diacetylbenzene, the resulting 1,3-diol groups may separate by a considerable distance from each other (Scheme VI). Cross-linked network polymers can exhibit rates of molecular motions that vary over a very large time scale.[10] If motions at the longer end of this time scale are involved with the movement of these functional groups, *segmented chain motion will now be the rate-determining step for bisketal formation* (Scheme VI). The observed slow rates of ketalization for the second (intramolecular) step are then reasonable. A second interesting possibility arises from what may be both chemical and conformational restrictions to bisketal formation. A single isomeric bisketal is used for template **4** (cis, cis). There is a very strong bias for this isomer that arises from a preference to avoid placing a methyl

group in an axial position in the 1,3-dioxalane.[20] Under kinetically controlled rebinding conditions, both cis and trans isomers may be produced in the first ketalization step. Formation of a trans isomer (**16**) is likely to result in a conformational change of the 1,3-dioxolane ring that allows the methyl group to occupy an equatorial position (**17**) (Scheme VII). This conformational change could then result in positioning the second carbonyl group in a location too distant from the remaining 1,3-diol group for ketalization. The issue of conformational mobility within the binding site is not well-understood but if mobility is restricted, this may seriously impact the distribution of mono- and bisketal formation under these kinetically controlled rebinding conditions. *Both the slow time scale for polymer chain motion and restricted mobility in the binding site may influence the manner by which substrate molecules rebind to the functionalized polymer sites.* These factors, particularly the former, distinguish the binding of small molecules to synthetic polymers from binding to proteins (enzymes).

^{13}C CP/MAS Analysis. Rebinding of ^{13}C-enriched (99%) 1,3-diacetylbenzene to difunctional sites (**6**) (24 h, 71% reoccupancy), followed by ^{13}C CP/MAS analysis, reveals 64% bisketal and 36% monoketal. If the ketalization is run for 88 h, 105% of the sites are reoccupied and distribution of bisketal to monoketal is 58:42. These results are in good agreement with related experiments using analysis of the carbonyl absorption via FT-IR. Their correspondence lends further credibility to the analysis and also marks the great potential for ^{13}C CP/MAS (or other nuclei) spectroscopy since the use of isotopically enriched substrate molecules allows for the selective analysis of a wide variety of chemical events. Although the preference for monoketal formation during the later stages of rebinding at slow or late binding sites is not quite as dramatic as revealed by FT-IR, both techniques give consistent representation of rebinding to difunctional sites at the late stages of rebinding.[22]

Several polymer-rebinding experiments were undertaken with materials prepared with 1,3-diacetylpyrene as template (**7**), a molecule with carbonyl spacing identical with that of 1,3-diacetylbenzene but with a larger "tail". On the basis of earlier rebinding studies, we noted that 1,3-diacetylpyrene creates larger cavities (binding sites) when used as a templating molecule.[4] Rebinding of 1,3-diacetylbenzene-^{13}C to this difunctional site (**9**) permits a comparison of the rebinding to this modified site with that of the 1,3-diacetylbenzene-templated site. This comparison reveals that, after 24 h, 1,3-diacetylbenzene rebinds to site **9** (77% reoccupation of sites) with approximately equal amounts of monoketal and bisketal formation (52:48). When the rebinding experiment was run for 88 h, 118% of the sites had been reoccupied with virtually no change in the ratio of mono- and bisketal (49:51). The manner of rebinding to sites generated by 1,3-diacetylpyrene exhibits far less dependence with time than those from 1,3-diacetylbenzene. This may reflect a larger, less congested binding site that has fewer kinetic constraints to rebinding than sites prepared with 1,3-diacetylbenzene as template.

Summary

Although some differences exist between the rebinding conditions used for the FT-IR and CP/MAS NMR experiments (rebinding time), there is general agreement between the two techniques. Between 12 and 24 h of rebinding, 55% (IR) and 64% (NMR) bisketal rebinding is indicated. Upon prolonged reketalization, both techniques indicate that additional binding takes place but with a greater preference for one-point attachment. In the absence of competing substrate, polymer-bound monoketals will undergo a slow reorganization to form bisketals. Rate-limiting polymer chain motion is implicated.

Conclusion

The spectroscopic methods of FT-IR and ^{13}C CP/MAS NMR are shown to be effective as quantitative analytical techniques for small-molecule binding to amorphous polymer networks. The data reveal important insights into the method of binding of difunctional substrates to various templated polymers. *The dominant one-point binding of 1,3-diacetylbenzene in acetophenone-templated polymers indicates good site isolation in these materials. It is appropriate, therefore, to interpret subsequent rebinding at difunctional sites at the molecular level* of *detail.* The relatively large amount of bisketal formation of 1,3-diacetylbenzene- and 1,3-diacetylpyrene-templated polymers by the 1,3-diacetylbenzene substrate demonstrates substantial control of functional group arrangement in the binding site. The time-dependent element of substrate binding is one of the most important findings of this study. The greater percentage of monoketal formation at the latter stages of rebinding is suggestive of more poorly defined binding sites occurring in less accessible domains. These sites lack the organization of functional groups and complimentary shape of more well-defined sites. When intermolecular competition is excluded, a slow reorganization of the binding site is observed, which results in a higher proportion of bisketal formation. The time scale for these events is in contrast to the often rapid conformational events that influence substrate binding to protein receptors.[11,12,22] Poorly defined sites, with slow functional group mobility, will diminish rebinding selectivity and mark a fundamental difference between binding phenomena with proteins and synthetic network polymers.

As mentioned previously, templated sites containing two 1,3-diol groups have been shown to exhibit kinetic selectivity in competition rebinding experiments.[2] For example, when **6** is permitted to compete for equimolar quantities of 1,3-diacetylbenzene and 1,7-diacetylfluorene, a *kinetic* rebinding selectivity of 85:15 is observed in favor of the 1,3-diacetylbenzene. It is instructive to consider how the findings of the present study aid in understanding the possible origins of the rebinding selectivity.

The binding of difunctional molecules to difunctional sites occurs in a stepwise manner. Although the rate-determining step in the reaction is not known, analogy to homogeneous ketalizations[23] would suggest it occurs during formation of the hemiketal of the first carbonyl group. Factors that could influence kinetic selectivity involve noncovalent bonding interactions between the *second* carbonyl of the substrate and the second 1,3-diol group at the site as well as nonbonding interactions between the substrate and the polymer cavity. A cavity shape complimentary to the substrate would, of course, result in favorable van der Waals interactions, while the "wrong" substrate can produce repulsive nonbonded interactions. Once the monoketal with the "correct" configuration is formed, well-defined sites that contain a carbonyl and 1,3-diol group in proximate relationship will quickly undergo the second ketalization (Scheme VIII). As the more accessible, well-defined sites are occupied, rebinding to more poorly defined sites that contain carbonyl and 1,3-diol groups in ill-defined relationships and a binding cavity that is not properly shaped may impede access to the remaining 1,3-diol group. The result may be a monoketal site that can react no further. Under these circumstances, intermolecular reaction may dominate the competition for remaining 1,3-diol groups. These sites give rise to a greater amount of monoketal formation and a lower kinetic selectivity.

Finally, from binding studies that eliminated the intermolecular competition, it was possible to detect a slow reorganization of the functional groups at the binding site resulting in eventual bisketal formation (Scheme VI). These sites presumably contain 1,3-diol groups that are separated by a considerable distance (and kinetic barrier). These sites will contribute to lower selectivity in the following ways. First, they lack the proximity of the second 1,3-diol group to assist the selective binding of difunctional substrate molecules. Furthermore, the second 1,3-diol group may

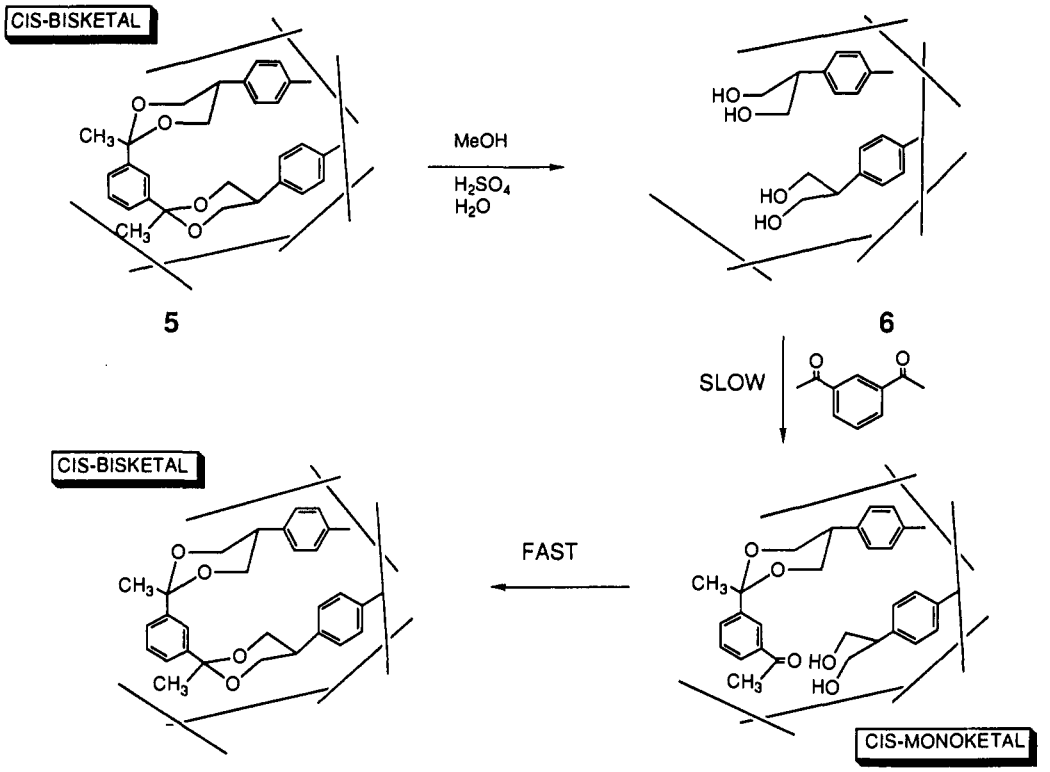
(20) Shea, K. J.; Dougherty, T. K. *J. Org. Chem.* **1985**, *50*, 4439.

(21) In a system with molecular level details quite unrelated to the present study, Sarhan and co-workers have also observed that the selectivity of rebinding to template-functionalized polymers is greatest at the early stages of reaction, that is, at the most accessible sites (Sarhan, A. *Makromol. Chem.* **1989**, *190*, 2031).

(22) Huber, R. *Angew., Chem. Int. Ed. Eng.* **1988**, *27*, 80.

(23) (a) Ogata, Y.; Kawasaki, A. In *The Chemistry of the Carbonyl Group*; Zabicky, J., Ed.; Wiley Interscience: London, 1970; Chapter 1. (b) Patai, S., Ed. *The Chemistry of the Ether Linkage*; John Wiley & Sons: New York, 1967; Chapter 7.

Scheme VIII



indiscriminately bind free diketone.

An early rate-determining step and the fact that a significant amount of monoketal is produced upon rebinding suggests that bisketal formation is not necessary for rebinding selectivity. At the probable rate-determining step, there will be (1) one covalent bond between substrate and polymer site, (2) possible weak noncovalent interactions between the remaining carbonyl and 1,3-diol groups, and (3) a microenvironment shape that is complimentary to the substrate. All of these factors can contribute to rebinding selectivity. In polymers prepared by template polymerization, the microenvironment shape is formed during the polymerization step as a result of nonbonded interactions between template and monomer molecules. It is possible to envision amplifying these interactions by building in addition secondary interactions between template and monomer in an effort to design better binding sites by template polymerization. Efforts along these lines are currently being pursued.

Experimental Section

General Procedures. Solution-phase ^1H NMR spectra were measured in $CDCl_3$ (unless otherwise specified) with either a 250-MHz (Bruker WM-250) or at 300-MHz (General Electric QE-300) instrument. ^{13}C NMR spectra were obtained at 75.4 MHz (QE-300). Chemical shifts are reported in δ units utilizing TMS as an internal reference for ^1H NMR and the absorption at δ 77.24 of $CDCl_3$ for ^{13}C NMR. ^{13}C CP/MAS spectra were recorded in a modified Bruker 37.7 mHz (^{13}C) instrument at the Colorado State University NMR Center. Infrared spectra were recorded on an Analect RFX-40 FT-IR spectrometer.

tert-Butylithium (Aldrich) was standardized with 2,5-dimethoxybenzyl alcohol. Anhydrous sodium acetate-*1*-^{13}C (Isotec Inc., 99% enrichment) was stored in a desiccator and used without further purification.

***cis*-2-(3-Acetylphen-1-yl)-2-methyl-5-phenyl-1,3-dioxane (14).** Benzene (50 mL) with *p*-toluenesulfonic acid monohydrate (60 mg, 0.315 mmol) was refluxed with a Dean–Stark trap for 30 min. 1,3-Diacetylbenzene (1.00 g, 6.17 mmol) and 2-phenyl-1,3-propanediol (0.937 g, 6.17 mmol) were added as solids to the cooled mixture, and the mixture was brought to reflux for 16 h. The cooled mixture was then dried (K_2CO_3) and concentrated to a volume of ~5 mL. The concentrate was flash column chromatographed on silica (20% Et_2O/hexanes (v/v), R_f = 0.19) to yield after concentration a clear, colorless oil that was crystallized from benzene/hexanes to give white crystals of **14** (764 mg, 41.9%; mp 73–74 °C): ^1H NMR δ 8.08 (s, 1 H, ArH), 7.96 (ddd, J = 7.7, 1.3, 1.5 Hz, 1 H, ArH), 7.71 (ddd, J = 7.8, 1.3, 1.5 Hz, 1 H, ArH), 7.55 (dd, J = 7.7, 7.7 Hz, 1 H, ArH), 7.22 (m, 3 H, ArH_f, ArH), 6.98 (dd, J = 5.9, 1.9 Hz, 2 H, ArH), 4.02 (dd, J = 11.7, 4.6 Hz, 2 H, OCH), 3.78 (dd, J = 11.7, 11.6 Hz, 2 H, OCH), 3.33 (m, 1 H, ArCH), 2.65 (s, 3 H, $COCH_3$), 1.60 (s, 3 H, CCH_3); ^{13}C NMR δ 198.22, 141.76, 138.18, 137.96, 131.83, 129.51, 128.83, 128.12, 127.66, 127.50, 126.93, 100.35, 66.64, 41.07, 32.54, 27.01; IR (KBr) 3057, 2976, 1680, 1601, 1358, 1223, 1186, 1043, 810, 702 cm^{-1}; high-resolution MS calcd for $C_{18}H_{17}O_3$ (M – CH_3) 281.1178, found (M – CH_3) 281.1179.

1,3-Diacetylbenzene-7,9-^{13}C (15-^{13}C). 1,3-Dibromobenzene (1.02 mL, 2.00 g, 8.48 mmol) was dissolved in THF (20 mL) and cooled to −50 °C. *t*-BuLi (24.1 mL, 4.2 equiv) was syringed in dropwise, and the solution turned from colorless to dark red over 1 h. The solution was added to a suspension of sodium acetate-*1*-^{13}C (2.14 g, 25.5 mmol) in THF (50 mL) at −20 °C in a dropwise manner by cannula needle. The orange-red heterogeneous solution was let stir at −20 °C for 6 h and then allowed to come to room temperature and stirred for an additional 6 h. The reaction mixture was quenched with H_2O (16 mL), the layers were separated, and the aqueous layer was extracted with Et_2O (3 × 20 mL). Organics were combined and washed with saturated aqueous NaCl (20 mL) and then dried ($MgSO_4$). After filtration and concentration, the residual oil was flash column chromatographed on silica gel (30% Et_2O/hexanes (v/v)) to yield a yellowish oil (**2**) (40% Et_2O/hexanes, R_f = 0.22). The oil was further purified by Kugelrohr distillation (130–140 °C (3 mmHg)) to yield a clear, colorless oil that crystallized upon cooling (0.217 g, 16%; mp 35–36 °C): ^1H NMR δ 8.52 (m, 1 H, ArH), 8.16 (m, 2 H, ArH), 7.60 (dd, J = 7.7, 7.7 Hz, 1 H, ArH), 2.67 (d, J = 5.7 Hz, 6 H, $COCH_3$); ^{13}C NMR δ 197.48, 132.72, 129.26, 128.30, 27.20, 26.62; IR (KBr) 3078, 3005, 1647, 1591, 1425, 1354, 1273, 1207, 1182, 688 cm^{-1}. High-resolution MS calcd for $^{13}C_2{}^{12}C_8H_{10}O_2$ 164.0748, found 164.0732.

^{13}C-Labeled *cis*-2-(3-Acetylphen-1-yl)-2-methyl-5-phenyl-1,3-dioxane (14-^{13}C). Toluenesulfonic acid monohydrate (10 mg, 5.3 × 10^{-5} mol) in benzene (15 mL) was refluxed with a Soxhlet extractor charged with 3-Å molecular sieves for 1 h. 1,3-Diacetylbenzene-7,9-^{13}C (143 mg, 8.73 × 10^{-4} mol) and 2-phenyl-1,3-propanediol (133 mg, 8.75 × 10^{-4} mol) were added to the cooled solution, which was again brought to reflux. The molecular sieves were periodically changed throughout the 10-h reaction time. The solution was cooled and concentrated (~2 mL). The concentrate was flash column chromatographed on silica (75% benzene/hexanes (v/v), R_f = 0.11) to yield a colorless oil (**3**) that was crystallized in benzene/hexanes (0.104 mg, 40.0%; mp 73–74 °C): ^1H NMR δ 8.08 (m, 1 H, ArH), 7.96 (m, 1 H, ArH), 7.72 (m, 1 H, ArH), 7.56 (dd, J = 7.5, 7.5 Hz, 1 H, ArH), 7.21 (m, 3 H, ArH_f, ArH), 6.98 (dd, J = 7.6, 1.9 Hz, 2 H, ArH), 4.02 (m, 2 H, OCH), 3.79 (dd, J = 11.2, 11.2 Hz, 2 H, OCH), 3.33 (m, 1 H, ArCH), 2.64 (d, J = 6.0 Hz, 3 H, $COCH_3$), 1.60 (d, J = 4.7 Hz, 3 H, CCH_3); ^{13}C NMR δ 198.28, 137.95, 131.86, 129.56, 129.49, 128.85, 128.12, 127.68, 127.53, 126.94,

100.36, 66.65, 41.10, 32.23, 27.32; IR (neat) 3033, 2964, 1707, 1651, 1371, 1355, 1261, 1192, 1161, 1138 cm^{-1}; high-resolution MS calcd for $^{13}C_2{}^{12}C_{16}H_{17}O_3$ (M − CH_3) 283.1245, found (M − CH_3) 283.1230.

Polymer Synthesis. Styrene (Aldrich), *m*-diisopropenylbenzene (Goodyear Tire Co. and American Cyanamid), azobis(isobutyronitrile) (Aldrich), and acetonitrile (Fisher Scientific) were purified prior to use. Styrene and *m*-diisopropenylbenzene were washed twice with 10% NaOH and once with saturated NaCl solution and dried ($MgSO_4$). The monomers were filtered from solids and distilled. AIBN was recrystallized from methanol. Acetonitrile was dried by refluxing over CaH_2 for 24 h and then distilled.

All polymers were produced at a template loading of 80 μmol template/g of polymer, with acetonitrile as diluent at $f_m = 0.5$, and an AIBN initiator amount of 1% by weight of monomers. A typical polymer procedure is as follows.

Template monomer of **1** (0.221 g, 0.789 mmol), 1,3-diisopropenylbenzene (DIB) (4.93 g), styrene (4.93 g), AIBN (98.5 mg, 0.601 mmol), and acetonitrile (10.8 mL) were placed in a medium-walled tube. The vessel was freezed–thawed–degassed for three cycles and then frozen, degassed, and sealed. After the tube reached room temperature, it was placed in an 80 °C oil bath for 14 h, and then the temperature was brought to 120 °C for 12 h. The tube was cooled and opened, and the polymer was crushed and sized. The 60–200-mesh particles were Soxhlet extracted with toluene for 20 h, after which time they were dried at 50 °C at 5 mmHg for 8 h. The polymer was then further dried on high vacuum (5 μmHg) for 12 h. The hydrolysis and the substrate rebinding procedures have been previously described.[2,4,13]

Polymer Samples. Blank (nontemplated) copolymers of styrene–*m*-diisopropenylbenzene (1:1 (w/w)) were doped with either **1** or **3** to produce the calibration data for the curves in Figures 3, 5, and 6. The dopant was adsorbed onto the blank polymer by adding a weighed quantity of polymer to a solution of benzene containing a known quantity of the dopant and slowly removing solvent under vacuum. For example, preparation of a doped polymer (80 μmol **1**/g polymer) included the following: Dry blank polymer (50.0 mg) in benzene (3 mL) and 50 μL of an 80 μmol/mL solution of **1** in benzene was swirled for 1 min, and the solvent was slowly removed under rotary evaporation. The free-flowing polymer was then placed on high vacuum (8 μmHg) at room temperature for ~1 h.

Spectroscopic Analysis. FT-IR binding data was collected on an Analect RFX-40 FT-IR spectrometer. Polymer samples were prepared as KBr pellets as follows: Dried Polymer (~2 mg) was mixed with ~85 mg of dry KBr. The solid mixture was ground with a mortar and pestle until the solids were fine and of homogeneous quality and then pressed in a dye at ~1700 psi for 3–4 min. The resultant pellet was then either immediately placed in the spectrometer for analysis or stored over $CaSO_4$ for analysis at a later time. The sample chamber of the instrument was continuously purged with pure, dry N_2.

FT-IR Spectrometer Acquisition Parameters

	sample	background
gain	2.00	2.00
resolution	4.00 cm^{-1}	4.00 cm^{-1}
apodization	Norton-Beer (strong)	
detector	TGS	
scans	30	40
aperature	3.3 mm	

^{13}C CP/MAS NMR data acquisition and manipulation was performed by James S. Frye of Colorado State University NMR center. The instrument parameters were as follows: ^1H 90° pulse, 8.20 μs; contact time, 2.00 ms; decoupler on time (effective acquisition time), 52.00 ms; wait time between repetitions, 2.00 s; number of scans, 2800; acquisition time, 102.45 ms; analog/digital converter precision, 12 bits; sweep width, ±1000.00; dwell time, 50 μs; spectrometer frequency, 37.735 000; spin rate, 4000 Hz; approximate integration region for the peaks of interest, for the carbonyl δ 207.4–184.4, aromatics δ 159.5–106.2, and ketal δ 106.2–94.6.

Hydrolysis and Rebinding to Templated Polymers. Yields in the hydrolysis and rebinding experiments were determined by GC analysis. A Hewlett-Packard 5710A gas chromatograph was used with a packed column (8 ft × 1/8 in., packed with 10% SP-2100 on 100/120 Supelcoport) with a splitless injection port. Standards were used for the derivation of yields of sample compounds. Dodecane (Aldrich, Gold label) was used as the standard for **1** and **2** analysis and monoacetylpyrene (MAP) was used for **3**. Flame ionization detector (FID) response factors were determined for each compound with their respective standard. Standard solutions were prepared at 20 μmol/mL in dry benzene.

Hydrolysis Procedure. The templated polymer (1.000 g) was placed in 20 mL of MeOH and 2 mL of 10% H_2SO_4. The solution was stirred at reflux for the indicated amount of time. The solution was then filtered hot through a sintered glass funnel and washed with hot MeOH (2 × 20 mL) and hot benzene (3 × 20 mL). The filtrate was concentrated in vacuo to ~3 mL and taken up in 25 mL of benzene. The organic layer was washed (NaCl, $NaHCO_3$, and NaCl). The aqueous layer was washed twice with benzene (2 × 7 mL), and the organics were combined. The solution was dried (K_2CO_3), the was standard added, and the solution was then concentrated and analyzed by gas chromatography.

Rebinding Procedure. The dried hydrolyzed templated polymer (250 mg, dried on high vacuum for 12 h) was refluxed for 22 h in a solution of 80 mmol of the rebinding ketone (or diketone) in dry benzene (4 mL) and 1 mL of 0.005 M *p*-toluenesulfonic acid in benzene. The mixture was then filtered through a sintered glass filter funnel and washed with hot benzene (4 × 15 mL). The standard was then added, the solution concentrated to a desired volume, and GC analysis conducted.

Each value in Table II reported is an average of the two median values of several runs.

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Registry No. 1, 133401-78-2; **14**, 133401-81-7; **14**-^{13}C, 133401-79-3; **15**-^{13}C, 133401-80-6; DAP, 90814-79-2; AcC_6H_4-*m*-Ac, 6781-42-6; $HOCH_2CH(Ph)CH_2OH$, 1570-95-2; BrC_6H_4-*m*-Br, 108-36-1; acetophenone, 98-86-2; (styrene)(diisopropenylbenzene) (copolymer), 26124-82-3; sodium acetate-*1*-^{13}C, 23424-28-4.