# Equilibrium Studies of Water and Thiol Addition to Ketones: Substituent and Solvent Effects for Methyl Ketones

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**Abstract:** Equilibrium constants for addition of water and 3-mercaptopropanoic acid to $RCOCH_3$ (R = H, $CH_3$, $CH_2OCH_3$, $CH_2F$, $CHCl_2$, $COO^-$, COOH, $COOCH_3$, $COCH_3$) were determined in $D_2O$ at 34 °C. Water addition was sensitive to electronic effects but not steric effects ($\log K_{D_2O} = 1.61\sigma^* + 0.07E_s - 4.55$, $r = 0.996$, $n = 7$) whereas thiol addition was sensitive to both factors ($\log K_{RSD} = 1.55\sigma^* + 0.49E_s - 2.14$, $r = 0.996$, $n = 7$), the steric effect being ascribed to the R group in RSD. For $D_2O$ addition to fluoroacetone $\Delta H = -5.0$ kcal mol$^{-1}$ and $\Delta S = -29$ cal mol$^{-1}$ deg$^{-1}$; values for thiol addition were $\Delta H = -7.7$ kcal mol$^{-1}$ and $\Delta S = -27$ cal mol$^{-1}$ deg$^{-1}$. For fluoroacetone $K_{H_2O}/K_{D_2O} = 0.85 \pm 0.15$ and $K_{RSH}/K_{RSD} = 0.52 \pm 0.10$. Thiol addition was 2- to 9-fold less favorable in dioxane and 7- to 90-fold less favorable in benzene than in water whereas water addition was slightly more favorable in dioxane than in water. The potential biological importance of thiol addition to ketones is considered.

Animal and fungal steroid hormones possess carbonyl and $\alpha,\beta$-unsaturated carbonyl groups which may, in principle, serve as sites for reaction with biological thiol.[1] Studies of thiol addition to progesterone and testosterone indicated that the reactivity of the A-ring $\alpha,\beta$-unsaturated carbonyl group is not sufficient for addition of glutathione, the main thiol in most eucaryotic cells, to be of importance under physiological conditions, but suggested that thiol addition could be a significant factor in the binding of these and related steroids to receptor proteins having thiol groups at their binding sites.[2] In the present study we extend these studies to measurement of equilibria for thiol addition to the carbonyl group of simple ketones in order to provide a basis for assessing the potential importance of such reactions with the keto groups of steroids and other biologically important compounds.

It has been generally considered that simple ketones do not react with thiols whereas aldehydes undergo facile reaction. Addition of thiols to aldehydes is known to be important in several biochemical processes.[3-5] and equilibria for thiol addition to a variety of aldehydes have been studied by Jencks and co-workers[6-8] and Kanchuger and Byers.[9] Thiol addition to aldehydes is thermodynamically more favorable than the corresponding hydration reaction by at least 4 kcal/mol.[9] Assuming that this factor is also applicable for ketones and taking the value of $2.5 \times 10^{-5}$ M$^{-1}$ found by Hine and Redding[10] for hydration of acetone, we can estimate that the equilibrium constant for thiol addition to acetone is $\sim 0.03$ M$^{-1}$. Although this constitutes an unfavorable reaction, the equilibrium should be measurable with available techniques. As will be seen below, the assumption underlying this calculation is not reliable, but it was nevertheless possible to measure equilibria for addition of thiols to acetone and to a variety of more reactive methyl ketones.

## Results

Several thiols were tested in preliminary studies and 3-mercaptopropionic acid (3-MPA) was finally selected for use because of its miscibility with water and organic solvents. In the presence of water, hydration is a competing reaction and the equilibrium constant for water addition, $K_{H_2O}$ (or $K_{D_2O}$), was determined along with the equilibrium constant for thiol addition, $K_{RSH}$ (or $K_{RSD}$). Equilibrium concentrations were determined by NMR spectroscopy as illustrated in Figure 1 for the addition to acetone in dioxane as solvent. When 3-MPA and acetone were reacted in water two signals appeared immediately at about 0.6 and 0.7 ppm upfield from the acetone methyl signal. Assignment of these two reasonances to the methyl groups of the hemithioketal and the diol, respectively, is consistent with (1) the absence of these signals when only one reactant (thiol, ketone, or water) was present, (2) the direct dependence of the respective signal areas upon thiol or water concentration, (3) the direct dependence of both signal intensities upon acetone concentration, and (4) the reversible decrease of both signal intensities with an increase in temperature.

For some of the methyl ketones studied, changes in the NMR spectra with time indicated the occurrence of slower competing side reactions. These included thioketal formation with acetone and methoxyacetone and nucleophilic substitution reactions with fluoroacetone and dichloroacetone. Evidence for thioacetal formation from acetaldehyde was also obtained. In these cases equilibrium measurements were made as soon as equilibrium was achieved and account taken of the effect of side reaction upon the stoichiometry. Equilibrium constants were calculated from the expression

$$K_{RSD} = R_1[C_T - R_2C_K(1 + 2R_3)]^{-1} \quad (1)$$

where $C_T$ and $C_K$ are the stoichiometric concentrations of thiol and carbonyl compound, respectively, $R_1$ is the ratio of the methyl proton signal of hemithioketal to that of ketone, $R_2$ is the ratio of the methyl proton signal of hemithioketal to the sum of those for ketone, hemithioketal and products of competing reactions, and $R_3$ is the ratio of the methyl proton signal of the thioketal to that of the hemithioketal. Values for $K_{D_2O}$ were determined both in the absence and in the presence of added thiol from the ratio of peak areas for diol and ketone, and the averaged values are reported. Values for $K_{D_2O}$ and $K_{RSD}$ were independent of ketone concentration.

Results obtained for addition equilibria studied in water at 34 °C are presented in Table I. The effect of temperature was studied for most of the compounds listed in Table I and both thiol and water addition were found to decrease with increasing temperature in every case. Detailed results for fluoroacetone are given in Table II. These yield values for the enthalpy and entropy of hydration of -5.0 kcal mol$^{-1}$ and -29 cal mol$^{-1}$ deg$^{-1}$ and corresponding values for thiol addition of -7.7 kcal mol$^{-1}$ and -27 cal mol$^{-1}$ deg$^{-1}$, respectively. Since the reaction is catalyzed by only

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Table I. Equilibrium Constants for 3-MPA and Water Addition to Methyl Ketones and Acetaldehyde at 34 °C in Water

| | | | $10^3K$, $M^{-1}$ | | | |
|---|---|---|---|---|---|---|
| compd | [ketone], M | [3-MPA], M | $n^a$ | RSD | $n^a$ | $D_2O$ |
| $CH_3COCH_3$ | 0.3-1.4 | 0.3-1.2 | 9 | 5.2 ± 0.7 | 3 | 0.023 ± 0.002 |
| $CH_3COCH_2OCH_3$ | 0.4-0.8 | 0.5-2.3 | 5 | 54 ± 9 | 2 | 0.22 ± 0.02 |
| $CH_3COCH_2F$ | 0.69 | 0.12-1.2 | 4 | 330 ± 40 | 5 | 2.0 ± 0.2 |
| | 0.69 | 0.12-1.2 | 5 | 170 ± 10[b] | 5 | 1.7 ± 0.1[b] |
| $CH_3COCHCl_2$ | 0.24 | 0.12-1.2 | 8 | 1150 ± 140 | 7 | 26 ± 3 |
| $CH_3COCOO^-$ | 0.09-0.7 | 0.12-0.54 | 7 | 4200 ± 1000 | 10 | 1.3 ± 0.1 |
| $CH_3CHO$ | 0.04-0.4 | 0.06-0.6 | 5 | 54 000 ± 5000 | 6 | 16 ± 2 |
| $CH_3COCOOH$ | 0.09-0.2 | 0.03-0.2 | 4 | 58 000 ± 2000 | 4 | 30.5 ± 0.5 |
| $CH_3COCOOCH_3$ | 0.06-0.3 | 0.03-0.6 | 6 | 71 000 ± 7000 | 7 | 45 ± 5 |
| $CH_3COCOCH_3$ | 0.06-0.3 | 0.06-0.3 | 4 | 90 000 ± 5000 | 4 | 32 ± 3 |

[a] Number of determinations. [b] In $H_2O$.

Table II. Temperature Dependence of 3-MPA[a] and Water Addition to Fluoroacetone[b] in $D_2O$

| $T$, K | $K_{RSD}$, $M^{-1}$ | $K_{D_2O}$, $M^{-1}$ |
|---|---|---|
| 274 | 1.2 | 0.0047 |
| 292 | 0.73 | 0.0027 |
| 307 | 0.33 | 0.0020 |
| 319 | 0.21 | 0.0012 |
| 342 | 0.083 | |
| 346 | 0.066 | |

[a] 1.15 M. [b] 0.693 M.

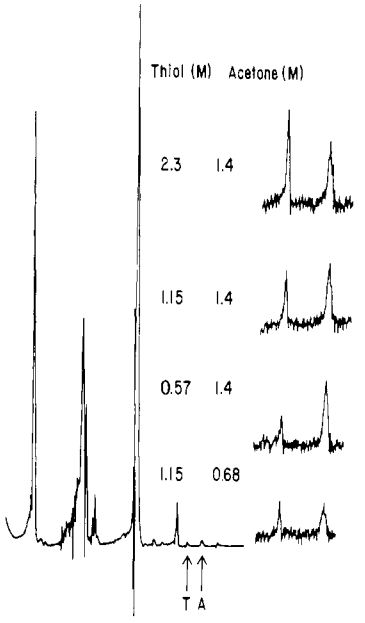


**Figure 1.** Proton NMR spectrum (1–3 δ) of a solution of 3-MPA and acetone in anhydrous dioxane. Acetone C-13 satellite and hemithioketal resonances for the methyl group are labeled A and T, respectively. Inserts show expanded scale traces of these resonances as a function of thiol and acetone concentration.

trace amounts of base, these parameters refer to equilibration among unionized species and contain no significant contribution from the ionized forms.

Attempts were made to measure equilibria for water and 3-MPA addition to α,α,α-trifluoroacetone, but in this case it was not possible to detect the free ketone and only the ratio $K_{RSD}/K_{D_2O}$ = 31 could be obtained.

Hydration and thiol addition were also examined in dioxane as solvent, but in this case attainment of equilibrium was slow unless base was added to catalyze the reaction. Results obtained for addition of 3-MPA in dioxane are given in Table III. Values of $K_{RSD}$ were unaffected by base (up to 2% of thiol concentration) and were also independent of the RSD concentration over the range studied. Comparison of Tables I and III reveals that $K_{RSD}$ decreases upon going from water to dioxane solvent by a factor

Table III. Equilibrium Constants for Base-Catalyzed 3-MPA Addition to Methyl Ketones and Acetaldehyde at at 34 °C in Dioxane

| compd | [ketone], M | [3-MPA], M | $n^a$ | $K_{RSD}$, $M^{-1}$ |
|---|---|---|---|---|
| $CH_3COCH_3$ | 0.7-1.4 | 0.6-2.3 | 8 | 0.0027 ± 0.0004 |
| $CH_3COCH_2F$ | 0.69 | 0.12-1.2 | 4 | 0.19 ± 0.02 |
| | 0.69-1.4 | 0.57-2.2 | 3 | 0.083 ± 0.009[b] |
| $CH_3COCHCl_2$ | 0.24-0.48 | 0.12-1.2 | 5 | 0.208 ± 0.014 |
| $CH_3CHO$ | 0.09-0.18 | 0.057-0.59 | 4 | 10.4 ± 1.3 |
| $CH_3COCOOCH_3$ | 0.057-0.23 | 0.058-0.58 | 5 | 7.8 ± 1.0 |
| $CH_3COCOCH_3$ | 0.29 | 0.12-1.2 | 4 | 7.2 ± 0.8 |
| $CH_3COCF_3$ | 0.13-0.028 | 0.012-0.057 | 10 | 58 ± 10 |

[a] $n$ is the number of determinations. [b] RSH.

Table IV. Equilibrium Constants for Base-Catalyzed Water Addition to Methyl Ketones and Acetaldehyde at 34 °C in Dioxane

| | | $10K_{D_2O}$, $M^{-1}$ | |
|---|---|---|---|
| compd | [ketone], M | $[D_2O]$ = 5.5 M | $[D_2O]$ = "0" M[a] |
| $CH_3COCH_2F$ | 0.69 | 4.4 | 7.9 |
| | 0.69 | 4.3[b] | |
| $CH_3COCHCl_2$ | 0.24 | 33 | 50 |
| $CH_3CHO$ | 0.45 | 19 | 32 |
| $CH_3COCOOCH_3$ | 0.57 | 45 | 60 |

[a] Extrapolated to zero $D_2O$ concentration. [b] In $H_2O$.

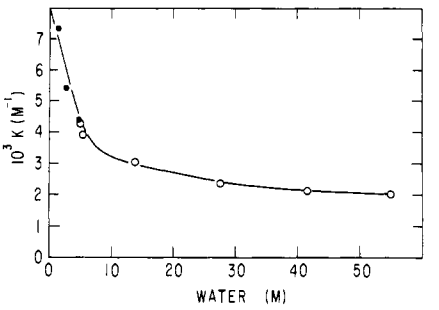


**Figure 2.** Equilibrium constant for addition of water to fluoroacetone in dioxane at 34 °C as a function of $H_2O$ concentration (○) and $D_2O$ concentration (●).

ranging from 2 to 13 depending upon the ketone.

The value of $K_{D_2O}$ in dioxane increases as the water concentration decreases at low water concentration (see Figure 2). A similar dependence was found by Bell and McDougall[11] for hydration of chloroacetone in dioxane. Values for pure dioxane were obtained by linear extrapolation to zero water concentration of the data obtained below a water concentration of 5 M; the results are given in Table IV. The values may be somewhat low as there

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Table V. Equilibrium Constants for Base-Catalyzed 3-MPA Addition to Methyl Ketones and Acetaldehyde at 34 °C in Benzene

| compd | [ketone], M | [3-MPA], M | $n^a$ | $K_{RSD}$, $M^{-1}$ |
|---|---|---|---|---|
| $CH_3COCH_2F$ | 0.33-1.4 | 0.29-1.2 | 4 | 0.047 ± 0.003 |
| $CH_3COCHCl_2$ | 0.49-0.9 | 0.28-1.1 | 5 | 0.0126 ± 0.0024[b] |
| $CH_3COCF_3$ | 0.56 | 0.12-1.2 | 5 | 6.5 ± 0.6 |
| $CH_3CHO$ | 0.18-0.4 | 0.12-1.2 | 4 | 3.3 ± 0.3 |
| $CH_3COCOOCH_3$ | 0.29-1.1 | 0.12-2.3 | 6 | 4.7 ± 0.5 |

[a] $n$ is the number of determinations. [b] Values extrapolated to zero thiol concentration.

appears to be upward curvature of the data points at low water concentration. Acetone could not be accurately measured below 11 M $D_2O$, where the value of $K_{D_2O}$ was found to be $3-4 \times 10^{-5}$ $M^{-1}$.

Equilibrium constants for addition of 3-MPA to some of the more reactive methyl ketones were also determined in benzene (Table V). Here also the reaction was slow unless catalyzed by base. In benzene the hemithioketal methyl resonance was found 0.1–0.3 ppm upfield of the ketone methyl signal.

The data presented above allow evaluation of the deuterium isotope effect for several of the equilibria studied. For 3-MPA addition to fluoroacetone $K_{RSH}/K_{RSD} = 0.52 \pm 0.10$ in water and $0.44 \pm 0.10$ in dioxane (Tables I and III). For hydration of fluoroacetone $K_{H_2O}/K_{D_2O} = 0.85 \pm 0.15$ in water and $1.0 \pm 0.1$ in dioxane containing 5.5 M water (Tables I and IV).

The equilibria measured in water were examined to ascertain the degree to which linear free energy structure–reactivity correlations can be made with the data. For this purpose values of $\sigma^* = 1.83$ and $E_s = 2.02$ were calculated for the $COOCH_3$ group from hydrolysis data for dimethyl oxalate;[12] these values were also assumed for the COOH group. All other $\sigma^*$ and $E_s$ values were taken from Taft;[13] acetaldehyde and pyruvate were excluded from the correlations. A good correlation was obtained for hydration ($\log K_{D_2O} = 1.66\sigma^* - 4.57$, $r = 0.995$, $n = 7$) but not for 3-MPA addition ($\log K_{RSD} = 1.94\sigma^* - 2.27$, $r = 0.893$, $n = 7$). Inclusion of steric effects gave no significant improvement for hydration ($\log K_{D_2O} = 1.61\sigma^* + 0.07E_s - 4.55$, $r = 0.996$, $n = 7$) but a significantly better correlation for thiol addition ($\log K_{RSD} = 1.55\sigma^* + 0.49E_s - 2.14$, $r = 0.996$, $n = 7$). Similar results were obtained by using the data measured in dioxane and benzene, but the smaller number of data points make the correlations less significant.

## Discussion

The assumption that the ratio of thiol to water addition to ketones would be generally comparable to that for aldehydes did not hold up, the values in water as solvent ranging from a low value of $K_{RSD}/K_{D_2O} = 31$ for trifluoroacetone to a high value of about 1600 for methyl pyruvate. The reason for the variation resides in the greater sensitivity of thiol addition than water addition to steric effects, the more sterically hindered ketones exhibiting the lowest values of $K_{RSD}/K_{D_2O}$. These values decrease further when measured in dioxane owing to the differing effect of solvent on thiol and water addition, a low value of $K_{RSD}/K_{D_2O}$ of about 4 being obtained for $\alpha,\alpha$-dichloroacetone in dioxane. We consider now the underlying reasons for the various differences between water and thiol additions to aldehydes and ketones.

We begin with the deuterium isotope effects. The values of $K_{RSH}/K_{RSD}$ (0.52 in water and 0.44 in dioxane) for addition to fluoroacetone are similar to the value (0.44) obtained by Lienhard and Jencks[6] for addition of 2-methoxyethanethiol to acetaldehyde in water. As pointed out by these authors, this is the expected isotope effect for a reaction in which a weaker bond, the SH bond, is converted to a stronger bond, the OH bond of the hemithioacetal or hemithioketal. A smaller isotope effect would be expected for the hydration reaction, where an OH bond of water is converted to another OH bond in the diol, and this is what is found. The value of $K_{H_2O}/K_{D_2O} = 0.85$ found here for fluoroacetone in water as solvent is almost the same as that found for acetaldehyde (0.86) by Lienhard and Jencks[6] and (0.84) by Gruen and McTigue.[14] Thus, deuterium isotope effects for both thiol and water addition are essentially the same for ketones as found for aldehydes.

The difference in solvent effects observed for the thiol addition and the hydration reactions can also be traced to differences in properties of the SH and OH bonds. Thus, thiols form weak hydrogen bonds at best[15] while the OH group is a good hydrogen-bond donor and acceptor. As a consequence, stabilization of the hemithioketal by hydrogen bonding causes thiol addition to be favored in water, where hydrogen bonding is strong, relative to dioxane and benzene, which cannot serve as hydrogen-bond donors. For hydration, however, hydrogen bonding is important in both reactant and product, and solvent effects upon hydrogen bonding would be expected to be smaller. In fact, hydration is slighly favored in dioxane relative to water, suggesting that hydrogen bonding is somewhat more important for water than for the product diol.

Hydration and thiol addition equilibria exhibit similar responses to inductive effects in the methyl ketone series, and the $\rho^*$ values found are only about 0.1 unit lower than found previously[9,16] for hydration and thiol addition to aldehydes. Steric factors were not found to be important in addition of thiol or water to aldehydes[9] and are shown here to be of little importance in the hydration of methyl ketones.

Steric factors are important, however, in the addition of 3-MPA to methyl ketones and the reason for this deviation merits consideration. If we examine the conformation about the C–S bond in the thiol adduct, it is apparent that all possible conformations lead to interactions in which the R group of RSH is gauche to two non-hydrogen substituents ($CH_3$, OH, or the R′ group of $R'COCH_3$). For addition to an aldehyde, however, two conformations place the R group adjacent to the sterically nondemanding H atom. Thus, replacement of the H in an aldehyde by a methyl group logically introduces additional gauche interactions which can account for the observed steric effect in thiol addition to methyl ketones. The absence of steric effects in the hydration reaction likely results from the absence of a bulky group attached to oxygen. If this analysis is correct, then hydrogen sulfide addition should not exhibit sensitvity to steric effects, and alcohol addition should be sensitive to such effects. We have confirmed the latter prediction through studies of alcohol addition to acetaldehyde and $\alpha,\alpha$-dichloroacetone; the results will be presented elsewhere. It can also be noted that Wiberg and Squires[17] have shown that equilibria for ketal formation from methyl ketones and methanol are quite sensitive to steric effects.

We consider next the implications of the present results with regard to addition of thiols to ketones in biological systems. Glutathione is present in most eucaryotic cells at concentrations in the range 1–10 mM.[18] Taking the upper limit to this range and assuming that the equilibrium constants for hemithioketal formation with glutathione and 3-MPA are comparable,[19] it follows that in order to have 10% or more of the ketone in the hemithioketal form at equilibrium requires that $K_{RSH}$ be greater than about 10 $M^{-1}$. In addition, hemithioketal formation involving

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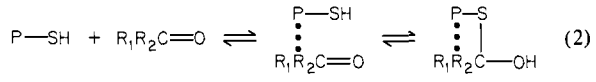
(18) Kosower, N. S.; Kosower, E. M. *Int. Rev. Cytol.* **1978**, *54*, 109–160.

(19) Barnett and Jencks[8] have shown that there is little variation in $K_{RSH}$ for addition to acetaldehyde for thiols differing in acidity by as much as 7 $pK_a$ units but having similar steric requirements. On the basis of preliminary results obtained in this laboratory, this insensitivity to electronic factors in the thiol holds for addition to methyl ketones as well.

glutathione will not compete effectively with hydration unless $K_{RSH}/K_{ROH}$ is greater than about 500. The first five compounds of Table I fail to meet these requirements. Acetone itself is predicted to form hemithioketal with glutathione to at most 0.002% and to be hydrated to the extent of 0.1%. The most reactive of these five, pyruvate, is predicted to exist as the hemithioketal to the extent of about 2% and as the diol to the extent of about 6%. Although these species constitute minor forms, they might conceivably have significance as reactive intermediates.

The last four compounds of Table I (including acetaldehyde) are all predicted to form substantial amounts of hemithioketal (9–15%) and of diol (38–61%) at equilibrium. $\alpha,\alpha$-Dichloroacetone is expected to be substantially hydrated (55%) but to form only minute amounts (0.2%) of hemithioketal as a consequence of steric hinderance to glutathione addition. Thus, reaction with glutathione should be important only when steric crowding in the hemithioketal is not severe, as is the case in the $\beta$-dicarbonyl compounds of Table I.

Although simple ketones are not expected to react to a substantial extent with intracellular glutathione, the possibility exists that they may react with protein thiol groups if certain conditions are met. Consider the case of a compound containing a keto group ($R_1R_2C{=}O$) which binds with low affinity to the site of an enzyme or receptor protein having a thiol group favorably oriented for reaction (P—SH) as illustrated in eq 2. We may now ask how



(2)

much the thiol addition process can contribute to the stability of the complex. Here the thiol addition is an intramolecular process and should be substantially more favorable than the intermolecular reaction with simple thiols where a low affinity binding does not occur. Intramolecular reactions can be favored over intermolecular reactions by factors of $10^3$–$10^8$ M, depending upon the extent to which optimal orientation is achieved and conformational degrees of freedom are reduced.[20] If we simply assume that the negative entropy (−27 cal $mol^{-1}$ $deg^{-1}$) observed with fluoroacetone is typical and is reduced to zero in the intramolecular process, a factor of $\sim 10^6$ M is obtained for the ratio of intramolecular to intermolecular equilibrium constant. This estimate indicates that thiol addition to a keto carbonyl group could make the difference between low affinity and high affinity binding, even in the case of a keto group as unreactive as that in acetone. Bulky groups adjacent to the carbonyl will disfavor such stabilization. The degree of stabilization provided will also depend upon the presence or absence in the binding site of suitably oriented groups which can stabilize the hemithioketal by hydrogen bonding.

Although the foregoing arguments indicate that reversible thiol addition to a simple ketone carbonyl group can contribute significantly to the stability of complexes between small molecules and proteins, there is as yet no case where substantial evidence has been accumulated to indicate that this actually occurs. Perhaps the most promising case is the progesterone receptor where binding via hemithioketal formation at the C-20 carbonyl has been suggested by O'Malley and co-workers[21] on the basis of the observation that a 21-fluoro substituent increases binding.

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## Experimental Section

**Materials.** 3-Mercaptopropanoic acid was obtained from Aldrich, distilled under nitrogen or vacuum, sealed in glass vials under nitrogen, and stored at 4°C. Deuteration of 3-mercaptopropanoic acid (6 mL) was achieved by mixing with 30 mL of $D_2O$ (norell 99.85%) and distilling off water at less than 60 °C (130 mm). The process was repeated twice with the pot residue and the final product distilled at 108 °C (5 mm). Potassium *tert*-butoxide (Aldrich) and potassium carbonate (Mallinckrodt) were stored in a desiccator and used without further purification. Mallinckrodt reagent grade dioxane and benzene were distilled over calcium hydride prior to use.

Sodium pyruvate was purchased from Sigma and used without further purification. Acetone (Fischer) was purified by the $AgNO_3$ method.[22] 1,1-Dichloro-2-propanone was prepared by a procedure analogous to that of Bell and McDougall[11] and the final product further purified by preparative gas chromatography. All other carbonyl compounds were from Aldrich. Methoxyacetone was distilled just prior to use. Trifluoroacetone was distilled, sealed in glass vials, and stored at 4 °C. Acetaldehyde (20 mL) containing 6 drops of 3 N HCl was distilled, and the center fraction (10 mL) collected and stored in a desiccator at 4 °C. The remaining ketones were purified by preparative gas chromatography (Varian Aerograph: 4 ft × 0.25 in. 20% SE-52 in series with 8 ft × 0.25 in. 20% DEGS on Chromosorb P). The purity of all compounds was checked by NMR spectroscopy.

**NMR Spectra.** Measurements were made on a Varian EM-390 spectrometer fitted with a EM-3940 variable-temperature probe. Chemical shifts (ppm) were measured relative to the solvent signal assigned as follows: water (4.6), HDO (4.6), dioxane (3.6), and benzene (7.24). Probe temperature was routinely determined by measuring the chemical shift of a methanol standard containing 0.02% HCl. Sample temperature was determined by using a reference capillary held in the NMR tube by a Teflon sleeve, methanol being used as reference for low temperature and ethylene glycol for high temperature. For equilibration experiments, integration of signals was obtained as soon as possible after equilibrium was achieved so that corrections resulting from competing reactions could be minimized. For unfavorable equilibria the upfield carbon-13 satellite signal for the methyl ketone was utilized to determine ketone concentration.

**Equilibrium Studies.** Solutions were prepared in 1-mL volumetric flasks, transferred to NMR tubes, and equilibrated in water bath at the probe temperature. For base-catalyzed reactions, stock solutions were prepared containing potassium carbonate—11 mg per mL in 3-MPA and 7 mg per mL in $D_2O$. Solutions containing thiol plus base, deuterated thiols, acetaldehyde, or trifluoroacetone were prepared in a glovebag purged with nitrogen and exposed to fresh $P_2O_5$. Solutions containing acetaldehyde or trifluoroacetone were initially prepared in a cold room at 4 °C to control volatility losses, sealed with a septum cap, equilibrated to room temperature, and diluted to the mark. All volumes were corrected to room temperature.

Evidence for a slow nucleophilic substitution by $D_2O$ catalyzed by 3-MPA was obtained with fluoroacetone. Thus, fluoroacetone (0.6 M) was converted to a new compound having singlets at 2.3 (3 H) and 3.6 (2 H) ppm in the presence of 3-MPA (1.1 M) with a half-life of about 4 h. Only a catalytic amount of 3-MPA was necessary for complete conversion of fluoroacetone and the reaction did not occur in dioxane.

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**Registry No.** $CH_3COCH_3$, 67-64-1; $CH_3COCH_2OCH_3$, 5878-19-3; $CH_3COCH_2F$, 430-51-3; $CH_3COCHCl_2$, 513-88-2; $CH_3COCOO^-$, 57-60-3; $CH_3CHO$, 75-07-0; $CH_3COCOOH$, 127-17-3; $CH_3COCOOCH_3$, 600-22-6; $CH_3COCOCH_3$, 431-03-8; $D_2$, 7782-39-0; 3-MPA, 107-96-0.

(22) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. "Purification of Laboratory Chemicals"; Pergammon Pres: New York, 1966; pp 57.