Figure 2. Autoradiogram of 25% denaturing polyacrylamide gel: lane 1, 5' end-labeled (*) RNA only; lane 2, 5'* RNase U2 digest (A specific); lane 3, 5'* hydroxide cleavage ladder; lane 4, 5'* Cys 116 mutant digest; lane 5, 5'* hybrid enzyme digest; lane 6, 3'* Cys 116 mutant digest; lane 7, 3'* hybrid enzyme digest; lane 8, 5'* RNA only; lane 9, 5'* hybrid enzyme digest (excess enzyme). Hybrid enzyme cleavage conditions: To a 100 nM solution of either 5' or 3' end-labeled RNA in 50 mM Tris·HCl, 50 mM NaCl, 0.1 mM EDTA, pH 7.0, was added the hybrid nuclease (10 nM to 1 μM) to give a final volume of 9 μL. Hybridization was carried out by heating the mixture to 65 °C for 90 s and then cooling at 15 °C for 2 min. The cleavage reaction was initiated by the addition of 1 μL of 100 mM CaCl₂ at 15 °C and quenched after 1 s by the addition of 10 μL of formamide containing 10 mM pTp.

Figure 3. Histogram indicating the cleavage pattern of (a) the hybrid enzyme and (b) the underivatized Cys 116 mutant (from lane 4, Figure 2). The heights of the arrows indicate relative cleavage intensities at the indicated bases.

Cleavage reactions were performed by first incubating the hybrid enzyme with the RNA to form the DNA·RNA hybrid and then activating the nuclease by the addition of Ca²⁺ (the enzyme is completely dependent on Ca²⁺ for activity).[8] Cleavage of the end-labeled RNA by the hybrid enzyme was analyzed by high resolution denaturing polyacrylamide gel electrophoresis[16] (Figure 2).

Inspection of the histogram of the cleavage patterns produced by the hybrid enzyme (Figure 3) reveals that the oligonucleotide binding domain site selectively delivers the hydrolytic activity of staphylococcal nuclease to the RNA. Cleavage occurs over a 3 to 5 nucleotide region directly adjacent to the hybridization site. In contrast, cleavage of the RNA by staphylococcal nuclease (K116 to C116), in the presence or absence of the 14-nucleotide oligomer, occurs relatively nonspecifically at A–U rich regions of the RNA and at a much slower rate. The fact that the hybrid enzyme cleaves a number of phosphodiester bonds adjacent to the binding site may result from the flexibility of the tether linking the oligonucleotide to the enzyme. Cleavage of only one phos-

phodiester bond may result with a shorter or more rigid tether. Greater than 90% selective conversion of the RNA has been achieved with an excess of enzyme relative to substrate (lanes 8 and 9, Figure 2).[17] Cleavage of RNA without hybridization of the enzyme prior to the addition of Ca²⁺ also results in selective cleavage, suggesting that nonselective cleavage by the hybrid enzyme is relatively slow. At longer reaction times or elevated temperatures, the cleavage specificity decreases, possibly due to autolysis of the hybrid enzyme.

In conclusion, this work illustrates an approach for rationally modifying enzyme specificity which may not only lead to a class of selective ribonucleases but may also find application in designing additional selective catalysts.

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(17) Determined by densitometry of the autoradiogram in Figure 2.

Difference in the Methyl Group Conformational Preferences of T₁ and S₁ Acetophenone. A Probe of the Orbital Character of Excited Electronic States

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Ethane exists, in its electronic ground state, primarily in the staggered form because of the mutual repulsion between the filled π_{CH_3} orbitals of the two methyl groups in the higher energy eclipsed form. The same considerations dictate the more stable conformation of a methyl rotor attached to an R(-C)=X group, as in propene (R = H, X = CH₂), acetaldehyde (R = H, X = O), and acetophenone (R = φ, X = O), all of which have a C–H bond eclipsed (**1**) rather than staggered (**2**) with the double bond in

the ground state (S₀). But, because of the sensitivity of these nonbonding interactions to the orbital occupancies of neighboring groups, the situation might be expected to be different in electronically excited states.[1] We report here on a realization of this expectation, show that a *difference* exists in the conformational preferences of the methyl group in the lowest excited triplet (T₁) and singlet (S₁) states of acetophenone, *probably because of a difference in their orbital characters*, and comment briefly on the dynamic implications of this result.[2]

Our experiments were performed in the collision-free environment of a seeded supersonic jet of helium, by using a Nd³⁺:YAG pumped and doubled dye laser as the excitation source. Acetophenone was directly excited to the T₁ (or S₁) state, and the T₁ ← S₀ (or S₁ ← S₀) excitation spectrum was recorded by monitoring the total phosphorescence (or fluorescence) intensity

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Communications to the Editor

0^0_0

$T_1 \longleftarrow S_0$

45^1_0

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0        100        200        cm⁻¹
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$S_1 \longleftarrow S_0$

45^1_0

0^0_0

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0        100        200        cm⁻¹
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Figure 1. Comparison of the low-energy portions of the phosphorescence excitation ($T_1 \leftarrow S_0$) and fluorescence excitation ($S_1 \leftarrow S_0$) spectra of acetophenone in a supersonic jet. The two spectra have been displaced so that their electronic origins (at 25 794 and 27 286 cm⁻¹, respectively) coincide.

as a function of laser wavelength.[3,4] Figure 1 shows the results obtained; the two spectra have been displaced in energy so that their 0^0_0 bands coincide. Only the first 250 cm⁻¹ of excess energy above the respective origins are shown. Immediately apparent is the fact that the two spectra differ significantly in their vibrational activity. The 0^0_0 band is the strongest band in the $T_1 \leftarrow S_0$ spectrum but is relatively weak in the $S_1 \leftarrow S_0$ spectrum.[5] In addition, the $S_1 \leftarrow S_0$ spectrum exhibits a number of low-frequency bands not seen in the $T_1 \leftarrow S_0$ spectrum, which appear as progressions built on the origin and the 45^1_0 (acetyl torsion) band. This demonstrates, according to the Franck-Condon (FC) principle, that whereas the equilibrium geometry of the lowest excited triplet state must be similar to that of the ground state, the geometry of the lowest excited singlet state must be different. This difference may be associated with a change in the conformation of the methyl group in the S_1 state.

The two spectra may be fit by assuming a potential function for the hindered methyl rotor of the form[4] $V_3/2[1 - \cos 3 (\phi - \theta)]$, where V_3 is the barrier height, ϕ is the torsional angle, and θ is a phase factor that describes the relative orientation of the methyl group in the ground and excited (T_1 or S_1) states. The ground-state barrier in acetophenone is unknown, but a value in excess of 300 cm⁻¹ seems reasonable in view of the measured barriers for similar molecules.[6] In that event, only the lowest

(and nearly degenerate) A_1 and E levels of S_0 will be populated in the jet. Assuming, further, an internal rotation constant of 5.2 cm⁻¹ for all states and using a fitting procedure described elsewhere (which properly accounts for statistical weights),[4] we obtain calculated spectra that are in excellent agreement with experiment in both cases, with respect to both line positions (±2 cm⁻¹) and intensities (±20%). The parameters describing these fits are, for T_1, $V_3 = 110 \pm 5$ cm⁻¹ and $\theta = 0 \pm 10°$, and for S_1, $V_3 = 70 \pm 5$ cm⁻¹ and $\theta = 60 \pm 5°$, confirming that only S_1 acetophenone undergoes a conformational change on electronic excitation from the ground state.

That such a change occurs is not surprising. Unlike the eclipsed (1) ground states, the singlet $n\pi^*$ states of acetaldehyde,[7] acetone,[7] methylglyoxal,[8] biacetyl,[8] and toluquinone[9] and the triplet $n\pi^*$ states of methylglyoxal,[4] biacetyl,[4] and toluquinone[9] all exhibit methyl group conformations in which a C–H bond is staggered (2) with respect to the C=O bond. This reversal in the relative energies of the two conformers occurs in excited states because of the addition of an attractive term to the hindering potential,[9] a term produced by the overlap of partially occupied *antibonding* orbitals.[10,11] The relevant orbitals in the $n\pi^*$ states of planar α-methylated carbonyls are the empty $\pi^*_{CH_3}$ orbital and the half-filled π^*_{CO} orbital. The degree of stabilization of the staggered form depends on the π^*_{CO}–$\pi^*_{CH_3}$ overlap, their energy separation, and the occupation and polarization of the π^*_{CO} orbital.[11] Clearly, this stabilization is large in the case of S_1 acetophenone, leading to conformational change and the conclusion that the lowest excited singlet state has considerable π^*_{CO} character.

What *is* surprising is that a similar conformational change does *not* occur on excitation of the lowest triplet state. Conventional wisdom would seem to dictate that S_1 and T_1 have the same orbital character.[12] Both excitations presumably involve a delocalized π^* orbital, with relatively small orbital coefficients on the carbonyl group.[13] *But there must be some difference in the magnitudes of these coefficients in the two states.* T_1 excitation results in a substantial reduction of the methyl torsional barrier but no change in its sign. From this we conclude that the lowest triplet state has less π^*_{CO} character, with smaller orbital coefficients on the carbonyl group. In valence bond terms, this means that there must be a larger contribution to the S_1 wave function of structures of the type $\dot\phi$ = C–Ö. Indeed, we find (Figure 1) that the acetyl torsional frequency of S_1 is significantly larger than that of T_1, providing strong supporting evidence for this interpretation.

We appear to have discovered a unique and subtle probe of the orbital populations of the electronically excited states of isolated molecules, one that should prove especially valuable in studies of species possessing two chromophores and thus closely spaced states of mixed electronic character. Especially interesting to us is the new insight that such studies will provide into the dynamical behavior of such states, free of environmental perturbations. The efficient $S_1 \sim> T_1$ intersystem crossing of acetophenone[14] is easily understood, for example, since the spin-orbit matrix elements

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connecting two states of differing orbital character should be large. These matrix elements should not be diminished by small FC factors owing to geometry differences between the T_1 and S_1 states. Conformational changes on electronic excitation may also accelerate other radiationless transitions, such as internal conversion and intramolecular vibrational relaxation.[15] The photochemical behavior of T_1 and S_1 acetophenone might also be quite different. Some of these issues are now being explored in our laboratories.

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Synthesis of Cyclopentenyl Sulfones via the [3 + 2] Cyclization–Elimination Reaction of (Phenylsulfonyl)allene

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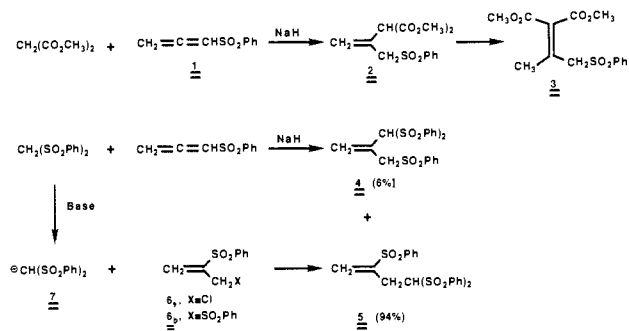*Department of Chemistry, Emory University
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Approaches to carbocyclic systems wherein a five-membered ring is generated by means of a [3 + 2]-anionic cycloaddition reaction remain the focus of intense synthetic efforts.[1,2] As part of a program concerned with the chemistry of unsaturated sulfones,[3] we sought to develop a general strategy for five-ring construction which would allow direct entry into a variety of cyclopentenyl-substituted sulfones. In this communication we describe the stepwise reaction of (phenylsulfonyl)allene with activated olefins by using catalytic quantities of a nucleophilic reagent as a method for producing five-membered rings in high yield (eq 1).
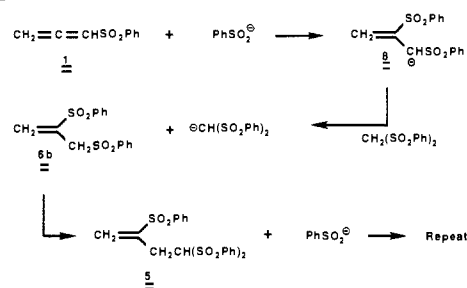

$$CH_2=C=CHSO_2Ph \quad + \quad A=B \xrightarrow{Nu^\ominus} \quad (1)$$
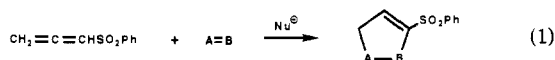
(Phenylsulfonyl)allene is highly activated toward nucleophilic addition because of its markedly lowered LUMO energy level compared with allene.[4] While the reactions with heteronucleophiles have been well investigated,[5] much less attention has been paid to the carbon–carbon bond-forming reactions of **1** with carbon nucleophiles.[6,7] We have studied the reaction of **1** with dimethyl malonate in the presence of a trace of sodium hydride and found that the expected Michael-type adduct **2** was isolated as the
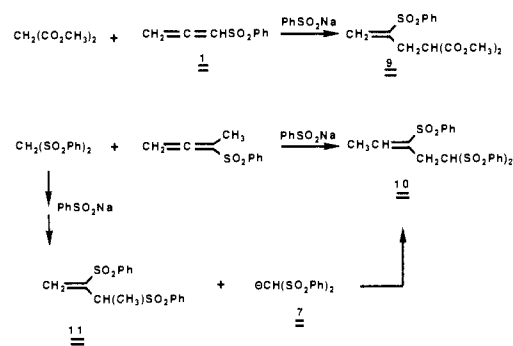
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Scheme I

Scheme II

Scheme III

exclusive product (90%) (see Scheme I). This material readily rearranged to the thermodynamically more stable isomer **3** by stirring with potassium *tert*-butoxide in THF for longer periods of time. In marked contrast to this result, the reaction of **1** with bis(phenylsulfonyl)methane in the presence of a trace of base afforded the expected Michael-type adduct **4** in only 6% yield. Interestingly, the major product obtained (94%) corresponded to structure **5**.[8] All attempts to isomerize **4** to **5** under a variety of basic and thermal conditions failed. Structure **5** was independently synthesized by treating the anion derived from bis(phenylsulfonyl)methane (**7**) with either 3-chloro-2-(phenylsulfonyl)-1-propene (**6a**) or 2,3-bis(phenylsulfonyl)-1-propene (**6b**).

A mechanism which rationalizes the formation of **5** and which is consistent with all the data (vide infra) is outlined in Scheme II. Carbanion **8** is the probable key intermediate in this novel chain process. Proton transfer of **8** with bis(phenylsulfonyl)methane followed by an SN'_2 reaction of the resulting carbanion with **6b** generates **5** and an additional quantity of benzenesulfinate anion. This material undergoes a subsequent nucleophilic addition with allene **1** and regenerates **7**. Presumably a trace of benzenesulfinate anion was present in the reaction mixture and served as the initiator for the chain reaction.[9]

Supporting evidence for the proposed mechanism is provided by the observation that the reaction of dimethyl malonate with allene **1** in the presence of added sodium benzenesulfinate afforded

(8) **5**: NMR (CDCl₃, 300 MHz) δ 3.21 (d, 2 H, J = 6.0 Hz), 5.57 (t, 1 H, J = 6.0 Hz), 6.07 (s, 1 H), 6.49 (s, 1 H), and 7.5–7.8 (m, 15 H).

(9) Even when a highly purified sample of bis(phenylsulfonyl)methane was used, the reaction still produced compound **5** in high yield. More than likely, the phenyl sulfinate anion is derived from some equilibrium between the bis-sulfone and the base.