The proposed approach, based on the 3D NMR concept, presents all the advantages of the Fourier transform over any filter-based experiment (simplicity, acquisition time saving, improved resolution, compensation for phase imperfections, and oversampling capabilities[7]) and benefits from the recent developments of 3D NMR software.[8] Its wide-range applicability is demonstrated through the protein NMR experiments here described. Moreover, it generalizes the construction of 3D NMR experiments, the third variable parameter being no longer restricted to a time.

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(8) The Gifa NMR software used throughout this work has been developed in this laboratory and is available from the authors.

Conjugated Ketenes: Cyclopropyl, Alkenyl, Alkynyl, and Acyl Substituents

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The chemistry of ketenes has been receiving increasing attention,[1-3] but the way in which substituents affect the stability and reactivity of ketenes is not well understood. Conjugating substituents are of particular interest,[3a] and reports[3] of the first preparations of alkynylketenes[3e] have recently appeared, although these species were not directly observed. There has been continued study of alkenyl-,[1e,2b,4] cyano-,[5] and acylketenes.[6] Despite longstanding interest in cyclopropyl conjugation with alkenes,[7] the only cyclopropylketenes previously studied were reactive intermediates.[8] However, there has been no systematic effort to understand the way in which these conjugating substituents affect the properties of ketenes, so we have initiated theoretical studies of this series. We also report here the first isolation and direct observation of cyclopropylketenes, the simple generation and trapping of hydrocarbon-substituted alkynylketenes, and kinetic measurements of the reactivity of an acylketene.

Table I. Rates of Hydration in H_2O/CH_3CN of c-PrCPhC=O (**4**) and c-Pr_2C=C=O (**6**) at 25 °C

H_2O		k_{obsd}, s^{-1}		[HCl],[a] M	
vol. %	[H_2O], M	**4**	**6**	(20% H_2O)	k_{obsd}, s^{-1}
10	5.56	0.0930	0.0102	0.806 (**4**)	0.138
20	11.1	0.297[b]	0.0486[c]	1.21 (**4**)	0.400
30	16.7	0.514	0.112	0.0102 (**6**)[d]	0.0638
40	22.2	0.728	0.200[c]	0.0204 (**6**)	0.0799
50	27.8		0.406[c]	0.0307 (**6**)	0.0978
				0.0409 (**6**)	0.110
				0.0511 (**6**)	0.123

[a] μ = 0.05 (NaCl). [b] For *i*-PrCPh=C=O,[2b] $k(H_2O)$ = 0.712 × 10^{-3} s^{-1}. [c] For Et_2C=C=O,[2d] $k(H_2O)$ = 0.0492, 0.196, and 0.357 s^{-1} in 20, 40, and 50% H_2O/CH_3CN, respectively. [d] k_{obsd} = 1.46(M^{-1} s^{-1})[H^+] + 0.0502; for Et_2C=C=O,[2d] k_{H^+} = 30.1 M^{-1} s^{-1}.

Ab initio molecular orbital calculations of the isodesmic reaction

$$RCH{=}C{=}O + CH_3CH{=}CH_2 \rightarrow CH_3CH{=}C{=}O + RCH{=}CH_2$$

were carried out to compare the effect of various conjugating substituents relative to CH_3 in stabilizing the alkenyl bond of ketene compared to that of ethylene. At the 3-21G//3-21G level with optimized geometries values of ΔE (kcal/mol) for different R groups for this reaction are −1.9 (c-Pr), −0.1 (HC≡C), −0.2 (CH_2=CH), and 3.3 (O=CH). Thus formyl is relatively more stabilizing for ketene, whereas the cyclopropyl substituent is favored as a substituent on ethylene. Higher level calculations are in progress, but the 3-21G results suggest that there are no extraordinary effects of substituents on ketene stability. Our calculations agree with related work[6h] which appeared since submission of this manuscript.

All four of these substituents are σ-electron withdrawing, formyl is also a strong π acceptor, and C_β of ketene is negatively charged,[2a,f] so the relative stabilizing effect of formyl may be due to conjugation. The net substituent effect on ketene reactivity will also depend on how the substituents affect the different transition states for reaction, but on the basis of the ground-state influences, the conjugated ketenes appear likely to be comparable in accessibility to the well-studied alkylketenes.

For the preparation of ethynylketenes, 2-alkyl-4-phenylbutynoic acids (**1**, eq 1)[9] were converted to acid chlorides, which reacted readily even at −78 °C with triethylamine to give yellow colors presumably due to the alkynylketenes **2** (eq 2), but after warming to 25 °C IR measurements did not reveal any residual ketenes. When **2** were generated in the presence of cyclopentadiene, the cycloaddition products **3** formed as single stereoisomers (eq 3) with the larger alkyl group assigned to the endo positions, as established for ketene cycloadditions with cyclopentadiene[4h,10a] and vinyl ethers.[10b]

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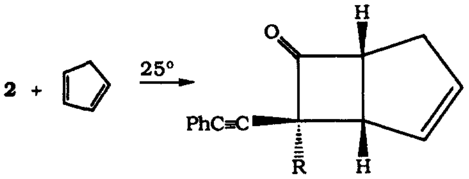
$$\mathrm{PhC{\equiv}CCHOHR} \xrightarrow[\text{3. CH}_2\text{O; 4. CrO}_3]{\text{1. PBr}_3\text{; 2. Zn}} \mathrm{PhC{\equiv}CCHRCO_2H} \quad (1)$$

R = Me, Et, *i*-Pr, *t*-Bu; product **1**

$$\mathbf{1} \xrightarrow[\text{2. Et}_3\text{N, THF, -78 °C}]{\text{1. SOCl}_2} \mathrm{PhC{\equiv}CCR{=}C{=}O}\ (\mathbf{2}) \quad (2)$$


(3)

3

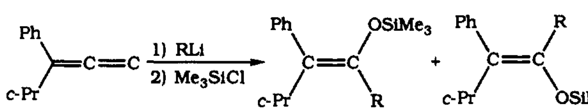
Cyclopropylketenes **4–6** prepared as shown in eqs 4 and 5 were stable enough for purification by distillation at room temperature and were characterized by their IR, NMR, UV, and mass spectra.

$$\text{c-PrCR=O} \xrightarrow[\text{2. H}_3\text{O}^+]{\text{1. CH}_3\text{OCHPPh}_3} \text{c-PrCHRCH=O} \quad (4)$$

$$\text{c-PrCHRCH=O} \xrightarrow[\text{3. Et}_3\text{N, THF}]{\text{1. PDC; 2. SOCl}_2} \text{c-PrCR=C=O} \quad (5)$$

R = Ph (**4**), *t*-Bu (**5**), c-Pr (**6**)

Reaction of **4** with *n*-BuLi followed by capture of the intermediate enolates with Me_3SiCl gave the stereoisomeric silyl enol ethers **7** with a 79/21 preference for formation of the *z* isomer resulting from nucleophilic attack syn to cyclopropyl, whereas the corresponding reaction of *t*-BuLi gave a 9/91 preference for attack anti to cyclopropyl (eq 6).



(6)

7-Z **7-E**

Results on the hydrolytic reactivity of cyclopropylketenes are summarized in Table I. The rate ratios for neutral hydrolysis $k(\text{c-Pr}_2\text{C=C=O})/k(\text{Et}_2\text{C=C=O}) = 1.2$ and $k(\text{PhC(c-Pr)=C=O})/k(\text{PhC}(i\text{-Pr})\text{=C=O}) = 500$ suggest that steric factors affect cyclopropylketene reactivity and that cyclopropyl is smaller than isopropyl. The rate ratio for acid hydrolysis $k(\text{Et}_2\text{C=C=O})/k(\text{c-Pr}_2\text{C=C=O}) = 21$ shows a rate-decelerating effect of cyclopropyl for protonation, by eq 7, as observed earlier for other alkenes.[11]

$$\text{c-Pr}_2\text{C=C=O} \xrightarrow{\text{H}^+} \text{c-Pr}_2\text{CHC}^+\text{=O} \xrightarrow{\text{H}_2\text{O}} \text{c-Pr}_2\text{CHCO}_2\text{H} \quad (7)$$

The persistent acylketene $t\text{-BuC(CO}_2\text{Et)=C=O}$[6g] reacted in H_2O to give $t\text{-BuCH(CO}_2\text{Et)CO}_2\text{H}$ with $k(\text{H}_2\text{O}) = 0.124\ \text{s}^{-1}$ and $k(\text{OH}^-) = 12.4\ \text{M}^{-1}\ \text{s}^{-1}$ in H_2O ($\mu = 0.1$, NaCl) at 25 °C. Thus this ketene is less reactive than $n\text{-BuCH=C=O}$,[2b,8] which has $k(\text{H}_2\text{O}) = 99.4\ \text{s}^{-1}$ and $k(\text{OH}^-) = 3.29 \times 10^4\ \text{M}^{-1}\ \text{s}^{-1}$.

In summary, molecular orbital calculations suggest that while conjugating substituents do not in general have a profound stabilizing effect on ketene, these species are also not prohibitively destabilized. The preparation and reactivity studies of alkynyl-, cyclopropyl-, and acylketenes are consistent with these conclusions.

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Inducible Alkylation of DNA Using an Oligonucleotide–Quinone Conjugate

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Messenger RNA has recently become a viable target for inhibiting the expression of a desired gene in vivo.[1] Compounds created for this purpose have drawn from the advances in site-specific modification of DNA[2,3] and the synthesis of metabolically stable oligonucleotides that can traverse cell membranes.[4] Although a large number of reactive appendages are available for related use in vitro,[3,5] only a limited set of these may be incorporated into protocols for in vivo study.[6] Naphthoquinones should neither impede the cellular uptake of appropriately modified nucleotides nor react with DNA indiscriminately; these compounds serve as the basis for our search of reactive components with a potential for in vivo application. Previously, methyl-1,4-naphthoquinone was shown to sensitize the selective oxidation of thymine.[7] We now report that related derivatives are also capable of alkylating DNA when held adjacent to a target sequence and subjected to UV irradiation.

5-Methyl-1,4-naphthoquinone[8] was condensed with 3-mercaptopropionic acid to provide a convenient method for attaching a sequence-directing oligonucleotide (Scheme I). The products of this reaction, two inseparable regioisomers (**1**),[9] were carried together throughout the following procedures. Treatment of the acid **1** with *N*-hydroxysuccinimide in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide yielded the activated ester **2**; this was subsequently used to acylate a hexamethyleneamino linking arm that was coupled to the 5′ terminus of an oligonucleotide 15 bases in length. Preparation of the oligonucleotide plus linker relied completely on standard proce-

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