Table I. Intramolecular Benzannulation Reactions of Siloxycarbene Complexes[a]

entry	complex	R^1	R^2	R^3	n	quinone	yield (%)[b]
1	**1a**	H	H	H	0	**2a**	30
2	**1b**	H	H	Me	0	**2b**	75
3	**1c**	H	H	Et	0	**2c**	51
4	**1d**	H	H	Ph	0	**2d**	60
5	**1e**	Me	H	H	0	**2e**	20[c]
6	**1f**	Me	H	Me	0	**2f**	75
7	**1g**	Me	H	Et	0	**2g**	79
8	**1h**	OMe	H	Me	0	**2h**	48
9	**1i**	Me	H	$SiMe_3$	0	**2i**	32[d]
10	**1j**	Me	Et	Me	0	**2j**	51[e]
11	**1k**	Me	H	Me	1	**2k**	56[e]
12	**1l**	Me	H	H	1	**2l**	22
13	**1m**	Me	H	H	2	**2m**	23

[a] Substituent positions refer to numbering scheme in eq 1. [b] Isolated yields by flash chromatography. [c] Acetylation in situ affords a 45% yield of the quinone acetate. [d] Compound **2i** is unstable toward silica gel chromatography, giving diminished yields. [e] Values represent combined yields of hydroxyquinone and carbonyl product from overoxidation in workup.

carbene complexes, such as indenoid or cyclobutenone compounds,[2] are formed.

In every case in which the carbene aryl group is substituted (complexes **1e–m**), a single isomer of the corresponding quinone (**2e–k**) is produced.[9] Analogous regiocontrol in benzannulation reactions has previously been reported for tethered reactions of chromium alkoxycarbene complexes which provide tricyclic products.[8] The present method extends the intramolecular strategy by providing bicyclic products with a convenient functional group for further elaboration.

Reaction conditions were optimized for complex **1f** and include the following variables: (1) irradiation with a 150-W Xe arc lamp was found to be superior to Hg arc sources; (2) a glass filter with half-maximum absorption at 345 nm was found to protect the product from photochemical degradation[10] and to insure selective irradiation of the lowest-energy electronic band of the siloxycarbene complexes at $\lambda_{max} \approx 360$ nm; and (3) the presence of 5 equiv of diphenylacetylene was found to roughly double the yield of naphthoquinone, without incorporation of the external alkyne. In all cases, diphenylacetylene can be recovered quantitatively. Carbon monoxide (1 atm), styrene, triphenylphosphine, dimethylacetylenedicarboxylate, and 2 equiv of diphenylacetylene were each found to be ineffective additives. Wulff and co-workers have shown the presence of an excess of alkyne to alter the course of benzannulation reactions of chromium, presumably by coordination to one or more intermediates in the multistep process.[11] Consistent with this rationale is our observation that the beneficial effect of 5 equiv of PhCCPh was eliminated in the presence of 1 atm of CO, but use of ^{13}CO resulted in *no* incorporation of label in the product quinone.

The intramolecular nature of the reaction was demonstrated by a crossover experiment in which an equimolar mixture of complexes **1b** and **1g** gave only naphthoquinones **2b** and **2g** in 78% overall yield. In contrast to all benzannulation reactions of chromium and the intermolecular reaction of a manganese titanoxy system,[4] the intramolecular reactions of **1** require irradiation and could not be effected by heating.[12]

Thus, the efficient and regioselective photochemical conversion of acetylenic alcohols to naphthoquinones has been accomplished, representing the first high-yield benzannulation chemistry of manganese carbene compounds. Studies concerning the scope, mechanism, and applications of this process will be reported in due course.

Acknowledgment is made to the National Science Foundation (CHE-9109000), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the Thomas F. and Kate Miller Jeffress Memorial Trust for the support of this research. We thank Dr. Michal Sabat for X-ray crystallographic analysis of the ketone derived from **2c**, and Mr. Michael Gross for assistance in the regiochemical assignment of compound **2l**.

Supplementary Material Available: Full details of experimental procedures and characterization of all compounds (15 pages). Ordering information is given on any current masthead page.

(9) Quantitative capillary GLC analysis of the crude reaction mixtures showed a single quinone in every case. Stereochemical assignments were made for representative cases by comparison with the isomeric products produced in the reaction of $(CO)_5CrC(Ar)(OMe)$ with protected terminal alkynols, by observation of long-range $^1H-^{13}C$ NMR coupling, and by X-ray crystallography (supplementary material).

(10) Both the isolated naphthoquinones and their organometallic precursors in the benzannulation reaction mixture were found to be unstable toward irradiation in the 300–345-nm range.

(11) Bos, M. E.; Wulff, W. D.; Miller, R. A.; Chamberlin, S.; Brandvold, T. A. *J. Am. Chem. Soc.* **1991**, *113*, 9293–9319.

(12) Attempts to convert complex **1f** to **2f** by refluxing in CH_2Cl_2, THF, and toluene resulted in decomposition of the carbene complex to give no tractable organic products.

(13) Fischer, E. O.; Maasböl, A. *Chem. Ber.* **1967**, *100*, 2445–2456.

A Sequence-Specific Molecular Light Switch: Tethering of an Oligonucleotide to a Dipyridophenazine Complex of Ruthenium(II)

Yonchu Jenkins and Jacqueline K. Barton*

Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California 91125

Received July 7, 1992

There has been considerable interest in the development of new chemical methods to distinguish and detect nucleic acids with sequence specificity.[1] One focus of our laboratory has been on the application of ruthenium complexes[2,3] as spectroscopic probes

* Author to whom correspondence should be addressed.

(1) (a) Reid, T.; Baldini, A.; Rand, T. C.; Ward, D. C. *Proc. Natl. Acad. Sci. U.S.A* **1992**, *89*, 1388. (b) Lichter, P.; Ward, D. C. *Nature* **1990**, *345*, 93. (c) Duvalvalentin, G.; Thuong, N. T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 504. (d) Sun, J. S.; Francois, J. C.; Montenaygarestier, T.; Saisonbehmoaras, T.; Roig, V. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9198. (e) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645. (f) Strobel, S. A.; Doucettestamm, L. A.; Riba, L.; Housman, D. E.; Dervan, P. B. *Science* **1991**, *254*, 1639. (g) Tizard, R.; Cate, R. L.; Ramachandran, K. L.; Wysk, M.; Voyta, J. C.; Murphy, O. J.; Bronstein, I. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4514. (h) Murakami, A.; Tada, J.; Yamagata, K.; Takano, J. *Nucleic Acids Res.* **1989**, *17*, 5587. (i) Pollard-Knight, D.; Read, C. A.; Downes, M. J.; Howard, L. A.; Leadbetter, M. R.; Pheby, S. A.; McNaughton, E.; Syms, A.; Brady, M. A. W. *Anal. Biochem.* **1990**, *185*, 84.

(2) (a) Barton, J. K.; Goldberg, J. M.; Kumar, C. V.; Turro, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 2081. (b) Kumar, C. V.; Barton, J. K.; Turro, N. J. *J. Am. Chem. Soc.* **1985**, *107*, 5518. (c) Barton, J. K.; Basile, L. A.; Danishefsky, A.; Alexandrescu, A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1961. (d) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051. (e) Turro, N. J.; Barton, J. K.; Tomalia, D. M. *Acc. Chem. Res.* **1991**, *24*, 332.

(3) (a) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 4960. (b) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res.* **1991**, *19*, 2595. (c) Hartshorn, R. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 5919. (d) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry*, in press.

0002-7863/92/1514-8736$03.00/0 © 1992 American Chemical Society

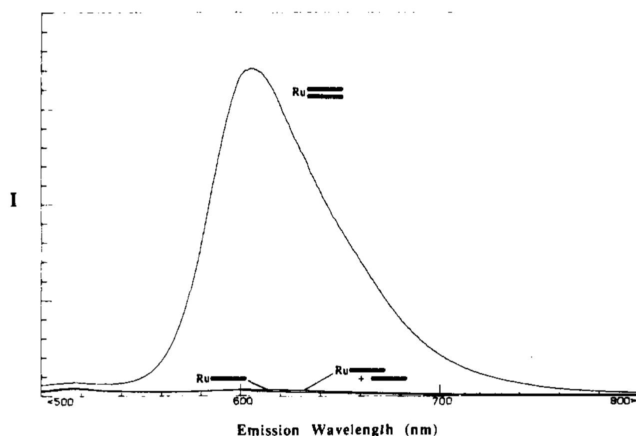
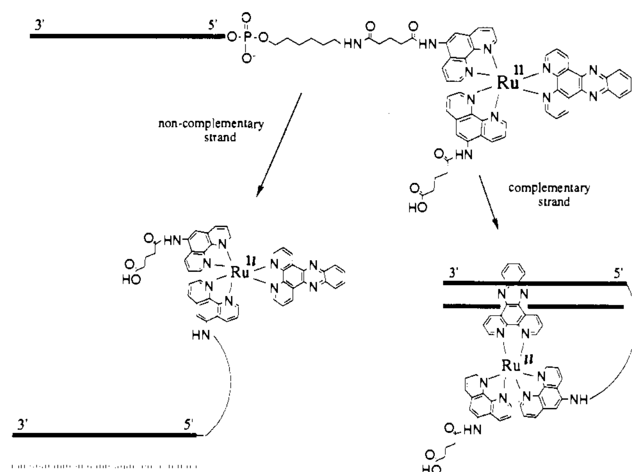


Figure 1. Emission spectra of the single-stranded metalated complex in the presence of its complementary strand (Ru=), in the absence of added oligonucleotide (Ru—), and in the presence of a noncomplementary strand (Ru— + —). Excitation is at 440 nm. Intense luminescence is observed only in the presence of the complementary strand. For Ru=, $\Phi = 0.0071$, while $Ru(phen)_2dppz^{2+}$ noncovalently bound to the oligomer gives $\Phi = 0.0063$.

Scheme I



for nucleic acid conformations. Recently, we have found[3] that $Ru(bpy)_2dppz^{2+}$ (dppz = dipyrido[3,2:*a*-2',3':*c*]phenazine) and $Ru(phen)_2dppz^{2+}$ may serve as molecular light switches for double-helical DNA. These complexes and their derivatives, which bind DNA avidly through intercalation, show no luminescence in aqueous solution, but with intercalation into the helix and the concomitant protection of the phenazine ring from quenching by interaction with water, intense luminescence is apparent. We report here the design of a sequence-specific light switch to target single-stranded DNAs through tethering a dppz complex of ruthenium(II) to an oligonucleotide (Scheme I).

An oligonucleotide containing the ruthenium complex tethered to its terminus was prepared[4,5] by the coupling of a 15-mer functionalized with a hexylamine at its 5'-terminus to $Ru(phen')_2dppz^{2+}$ (phen' = 5-(glutaric acid monoamide)-1,10-phenanthroline).[6,7] Figure 1 displays the emission spectra of the single-strand-tethered metal complex in the absence and presence of DNAs. Little luminescence is apparent for the single-strand complex. Addition of the complementary strand, however, yields intense luminescence. In contrast, addition of a noncomplementary strand produces no detectable emission. The oligonucleotide-tethered complex therefore can function as a sequence-specific molecular light switch. Time-resolved luminescence studies of the tethered metal complex hybridized to its complementary strand indicate a biexponential decay in emission with excited-state lifetimes of 500 (60%) and 110 (40%) ns (λ_{ex} = 480 nm, λ_{em} = 598 nm).[8] As with $Ru(phen)_2dppz^{2+}$, a biexponential decay in emission is observed on DNA binding.

Intercalation of the dppz derivative into the double helix is consistent with the luminescence; without protection of the phenazine ring from water through stacking, no luminescence would be evident, and rigid intercalation further increases the luminescence. That the intercalation is intramolecular is apparent in dilution experiments; at ≤5 μM duplex, the luminescence is linear with concentration. Addition of unmodified duplex to the covalently bound duplex yields a ≤5% change in the luminescence. Addition of unmetalated duplex to the metalated single strand does, however, lead to some luminescence and intermolecular intercalation.

Table I contains luminescence data for the metalated oligonucleotide annealed to a series of 15-mers containing mismatches near or far from the intercalation site. Luminescence is decreased with a mismatch near the 5'-terminus of the metalated strand, where intercalation is likely, but is decreased to a substantially greater extent with a mismatch at the 3'-terminus; helix desta-

Table I. Luminescence Data for Metalated Oligonucleotide Annealed to a Series of 15-mers Containing Various Base Mismatches (Indicated by Arrows)

sample[a]	no. of base mismatches	RI[b,c]
ACGTTCGAACCGTGA TGCAAGCTTGGCACT + Ru	0	1
ACGTTCGAACCGTGA-$(CH_2)_6$-NH_2—(Ru) TGCAAGCTTGGCACT	0	1.16
ACGTTCGAACCGTGA-$(CH_2)_6$-NH_2—(Ru) TGCAAGCTTGGCCCT	1	0.68
ACGTTCGAACCGTGA-$(CH_2)_6$-NH_2—(Ru) TGCCAGCTTGGCACT	1	0.37
ACGTTCGAACCGTGA-$(CH_2)_6$-NH_2—(Ru) TGCCAGCTTGACACT	2	0.26
ACGTTCGAACCGTGA-$(CH_2)_6$-NH_2—(Ru) TACCCGCCTGACACT	5	0.11[d]

[a] The melting temperature of 2 μM duplex in the absence of ruthenium is 56 °C in 50 mM NaCl/5 mM Tris buffer. The absorption spectrum of the ruthenium complex makes the determination of melting temperatures in the presence of ruthenium (covalently or noncovalently bound) extremely uncertain. [b] Samples used for luminescence measurements contained 5 μM duplex. For instrumentation, see ref 3b. [c] Relative emission intensities (RI) were determined as a ratio of emission relative to the emission of 5 μM noncovalent complex bound to 5 μM 15-mer. Error estimated to be ±10%. [d] This value is comparable to the luminescence of the single-stranded ruthenium complex.

(4) $[Ru(phen')_2dppz](PF_6)_2$ was prepared analogously to the method of Amouyal, E.; Homsi, A.; Chambron, J.-C.; Sauvage, J.-P. *J. Chem. Soc., Dalton Trans.* **1990**, 1841.

(5) Dicyclohexylcarbodiimide and 1-hydroxybenzotriazole were added to $[Ru(phen')_2dppz](PF_6)_2$ (C_1 isomer) in 1:1 DMF/dioxane. Amino-linked oligonucleotide 5'-$H_2N(CH_2)_6$AGTGCCAAGCTTGCA-3' was then added as a suspension followed by LiOH. After shaking at 37 °C, the reaction was quenched with water. Product was isolated on a Hewlett-Packard 1050 HPLC system (C_{18} column using a triethylamine-acetic acid/acetonitrile gradient). Product analysis was conducted by ultraviolet-visible (ϵ(440 nm) = 2.1 × 10^4 M^{-1} cm^{-1}) and atomic absorption spectroscopy and colorimetry for phosphate (Lindberg, O.; Ernsten, L. *Methods of Biochemical Analysis*; Glick, D., Ed.; Interscience: New York, 1954; Vol. 3). Circular dichroism of the tethered oligomer in the presence of the complementary strand is also identical to $Ru(phen)_2dppz^{2+}$ noncovalently bound to DNA.

(6) (a) Nagai, K.; Hecht, S. M. *J. Biol. Chem.* **1991**, *266*, 3994. (b) Francois, J. C.; Saisonbehmoaras, T.; Barbier, C.; Chassignol, M.; Thuong, N. T. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9702. (c) Chen, C. H. B.; Sigman, D. S. *J. Am. Chem. Soc.* **1988**, *110*, 6570. (d) Haralambidis, J.; Duncan, L.; Angus, K.; Tregear, G. W. *Nucleic Acids Res.* **1990**, *18*, 493. (e) Telser, J.; Cruickshank, K. A.; Schanze, K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221. (f) Le Doan, T.; Perrouault, L.; Chassignol, M.; Thuong, N. T.; Helene, C. *Nucleic Acids Res.* **1987**, *15*, 8643.

(7) $Ru(dppz)_2(phen')^{2+}$ luminesces in the absence of DNA (Friedman, A. E., unpublished results in our laboratory).

(8) The excited-state lifetimes of $Ru(phen)_2dppz^{2+}$ bound noncovalently to the oligomer are 420 (35%) and 90 (65%) ns.

blization with a mismatch near the 5′-terminus may be balanced by intramolecular intercalation, but a similar effect would not be available at the 3′-end. The mismatch results, therefore, are consistent with intramolecular intercalation by the dppz complex tethered to the 5′-terminus.

The results taken together establish that an oligonucleotide functionalized with a dppz complex of ruthenium can be used to target single-stranded DNA in a sequence-specific fashion. This complex could be extremely valuable in the development of novel hybridization probes both for heterogeneous and homogeneous assays.

Acknowledgment. We are grateful to the NIH (GM33309 and an NRSA to Y.J.) for their financial support. We also thank N. Ghatlia and S. Bossmann for suggestions in the coupling procedure and C. J. Murphy for assistance in collection of the time-resolved spectroscopic data.

Multiple Forms of Thioacetyl Coenzyme A Binding to Citrate Synthase. Resonance Raman Evidence

P. J. Tonge,[†] P. R. Carey,*[,†] and V. E. Anderson[‡,§]

Institute for Biological Sciences
National Research Council[‖]
Ottawa, Ontario, K1A 0R6 Canada
Department of Chemistry
Brown University
Providence, Rhode Island 02912

Received May 27, 1992

Citrate synthase catalyzes the stereospecific condensation of the acetyl group from acetyl coenzyme A (acetyl-CoA) with oxaloacetate to generate citrate. The reaction occurs in a ternary complex wherein both acetyl-CoA and oxaloacetate are bound and is thought to proceed via generation of the enol form of acetyl-CoA, which then acts as a nucleophile to attack the C_2 carbonyl carbon of oxaloacetate.[1,2] A reactive ternary complex can be generated where acetyl-CoA is replaced by thioacetyl-CoA, a substrate analogue of acetyl-CoA in which the C=O group of the acetyl moiety is replaced by C=S. The enzyme catalyzes the exchange of the acetyl methyl protons of thioacetyl-CoA with solvent presumably through the required enol intermediate. While the exchange reaction is 250-fold faster for thioacetyl-CoA than for acetyl-CoA, k_{cat} for the condensation of thioacetyl-CoA with oxaloacetate is only ca. 0.0002% (pH 7.4) that of acetyl-CoA.[3] Thus a slowly-reacting ternary complex can be generated with an acetyl-CoA analogue which, because of its dithio ester moiety, is amenable to resonance Raman spectroscopic characterization.

In order to gain further insight into the details of acetyl-CoA binding, we have obtained resonance Raman (RR) spectra of thioacetyl-CoA bound in a ternary complex with oxaloacetate to citrate synthase. The dithio chromophore has an absorption band near 306 nm,[4] and excitation into this electronic transition gives rise to RR features associated with normal modes of the $H_3CC(=S)SCH_2CH_2NH$ moiety, analysis of which provides information on the rotational isomers involving this group. The data show that the bound form of thioacetyl-CoA is present as at least two rotational isomers.

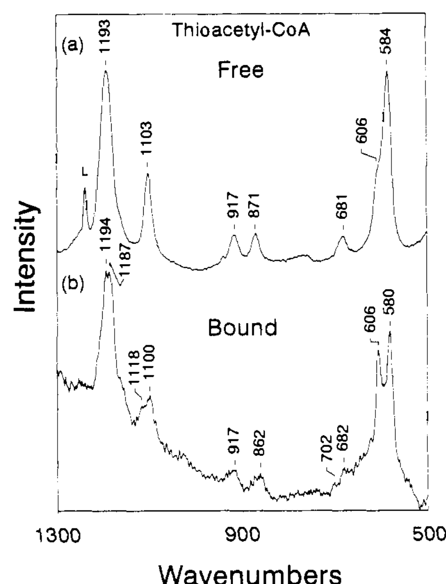


Figure 1. Resonance Raman (RR) spectra of free and bound thioacetyl-CoA were obtained at room temperature using 180° back-scattering geometry, 70-mW 324-nm Kr^+ laser excitation, 7-cm^{-1} spectral resolution, and published detection procedures.[11] Spectra were accumulated in memory blocks each 4 × 6 s, and identical data sets were co-added. (a) Resonance Raman spectrum of thioacetyl-CoA 0.9 mM in 0.1 M Tris pH 7.5; 48-s acquisition time. (b) Resonance Raman spectrum of thioacetyl-CoA bound to citrate synthase in 0.1 M Tris pH 7.5; 72-s acquisition time. For RR data collection the sample contained 0.65 mM citrate synthase, 2 mM oxaloacetate, and 0.2 mM thioacetyl-CoA. Using K_M = 60 μM for thioacetyl-CoA,[3] it is estimated that ca. 90% of the dithio ester is bound. A RR spectrum of enzyme/oxaloacetate in the absence of chromophore has been subtracted. Citrate synthase (EC 4.1.3.7) (lot numbers 77F-9560, 69F 9585) from Sigma Chemical Co. was purified by ion-exchange HPLC. Two milliliters of the enzyme suspension in ammonium sulfate was centrifuged in an Eppendorf microfuge. The pellet was dissolved in 1 mL of 15 mM Tris pH 8.0 and chromatographed on two (2 × 0.5 mL) Sephadex G-25 (fine) 5-mL "centrifuge" columns. Subsequently the protein (1 mL, 25 mg) was chromatographed on a Tosohaas DEAE 5PW column (21.5 mm × 15.0 cm) attached to a Gilson HPLC. The buffers used were 15 mM Tris-HCl pH 8.0 (A) and 15 mM Tris-HCl, 0.5 M KCl pH 8.0 (B). From t = 0–15 min, a gradient of 0–17% buffer B was run at 4 mL/min, the flow rate was reduced (over 1 min) to 2 mL/min, and the protein eluted isocratically with 17% B (retention time 50 min). The main fractions from the protein peak at 50 min were pooled and concentrated (Amicon Centricon-10). During concentration the protein was exchanged into 0.1 M Tris-HCl pH 7.5. Citrate synthase concentration was determined using ϵ_{280} = 70 000 M^{-1} cm^{-1} per active site. Immediately before the resonance Raman experiments, a 200 mM oxaloacetic acid solution was prepared in 0.1 M Tris-HCl at pH 7.5. Thioacetyl-CoA was dissolved in 0.1 M Tris-HCl pH 7.5 and its concentration determined using ϵ_{306} = 11 000 M^{-1} cm^{-1}.

In Figure 1 the 324-nm-excited RR spectrum of thioacetyl-CoA contains bands due solely to the (thioacetyl)thio group. The spectrum of the unbound ligand (Figure 1a) closely resembles the RR spectrum of ethyl dithioacetate reported by Teixeira-Dias et al.,[4] and band assignments can be made by analogy to their analysis. Most bands are due to complex motions, and simple group frequency assignments do not apply. One exception to this may be the band at 681 cm^{-1}, which has a high degree of S-CH_2 stretching character. Teixeira-Dias et al.[4] identified the intense feature at 1193 cm^{-1} as having a significant (but not predominant) $\nu_{C=S}$ contribution and the 1103-cm^{-1} feature as due to a mode with ν_{C-C} (CH_3—C(=S) bond) and ν_{C-S} (C(=S)—S bond) contributions, and the 917- and 871-cm^{-1} bands were assigned to CH_3—C(=S) rocking modes. The intense band at 584 cm^{-1} is highly mixed in character, with contributions from ν_{C-S}, $\nu_{C=S}$, and ν_{C-C} (CH_3—C(=S) bond). These assignments are supported by a 29-cm^{-1} shift to lower frequency in the 1193-cm^{-1} band and smaller shifts in the other bands in the [1-^{13}C]thioacetyl-CoA RR spectrum (unpublished work, this laboratory).

* Author to whom correspondence should be addressed.
† National Research Council.
‡ Brown University.
§ Present address: Department of Biochemistry, Case Western Reserve University Medical School, Cleveland, OH 44106.
‖ Issued as NRCC No. 34278.

(1) Alter, G. M.; Casazza, J. P.; Zhi, W.; Nemeth, P.; Srere, P. A.; Evans, C. T. *Biochemistry* **1990**, *29*, 7557–7563.
(2) Karpusas, M.; Branchaud, B.; Remington, S. J. *Biochemistry* **1990**, *29*, 2213–2219.
(3) Wlassics, I. D.; Stille, C.; Anderson, V. E. *Biochim. Biophys. Acta* **1988**, *952*, 269–276.
(4) Teixeira-Dias, J. J. C.; Jardim-Barreto, V. M.; Ozaki, Y.; Storer, A. C.; Carey, P. R. *Can. J. Chem.* **1982**, *60*, 174–189.

0002-7863/92/1514-8738$03.00/0 © 1992 American Chemical Society