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Supporting Information

Total Synthesis and DNA-Cleaving Properties of Thiarubrine C

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General Methods.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker Model WM-360 (360 MHz), a Varian Inova-400 (400 MHz), or a Varian Inova-500 (500 MHz) FT NMR spectrometer. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a Brucker Model WM-360 (90.6 MHz), or a Varian Inova-400 (100.6 MHz) FT NMR spectrometer. The solvent used for NMR spectroscopy was chloroform-d_1 ($CDCl_3$), acetone-d_6 (CD_3COCD_3), methyl sulfoxide-d_6 (d_6-DMSO), or acetonitrile-d_3 (CD_3CN) as indicated. Chemical shifts are reported in δ units with respect to tetramethylsilane (δ 0.00) as internal standard. Coupling constants (*J* values) are given in hertz (Hz). The following abbreviations are used to describe peak patterns: "s" for singlet, "d" for doublet, "t" for triplet, "q" for quartet, "m" for multiplet, "br" for broadened, and "ABq" for the two-spin AB system. Data are presented as follows: chemical shift (multiplicity, integrated intensity, and coupling constant). The multiplicity indicated for each ^{13}C NMR chemical shift represents the observed splitting pattern of the corresponding C-13 peak when run in an off-resonance decoupling mode.

Infrared (IR) spectra were recorded on a Nicolet Model 5-DX FT-IR spectrometer using sodium chloride plates (liquid) or potassium bromide pellets (solid). Data are reported in wave numbers (cm^{-1}). Ultraviolet (UV) spectra were recorded on a Shimadzu UV2101/3101 PC UV-Vis scanning spectrophotometer using a quartz cuvette (12.5 x 12.5 x 44 mm) with ethanol as the solvent. Data

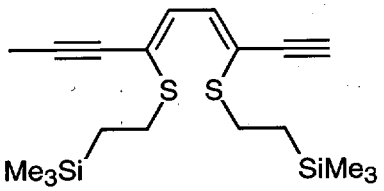
are report in wavelength (nm) and extinction coefficient. High resolution mass spectroscopic (HRMS) data were obtained using a VG Analitica 170-250S mass spectrometer.

Flash column chromatography was performed by the method of Still (Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925) using Merck 230-400 mesh silica gel. Analytical thin layer chromatography (TLC) was performed using Merck 60-F-254 0.2 mm precoated silica gel plates. Compounds were visualized using ultraviolet light, iodine vapor, or ceric ammonium sulfate/sulfuric acid.

Solvents were freshly distilled prior to use. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were distilled from sodium/benzophenone ketyl. Dichloromethane (CH_2Cl_2), triethylamine (Et_3N) and acetonitrile were distilled from calcium hydride. Benzene and toluene were distilled from sodium metal.

n-Butyllithium was purchased from Aldrich Chemical Company and titrated using the method of Kofron (Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879-1880). All other reagents were used as received, or distilled or recrystallized as necessary. All air- or moisture-sensitive reactions were conducted in oven- or flame-dried glassware, and under an atmosphere of house nitrogen. Moisture-sensitive reagents were transferred through rubber septa using syringes or cannulas. Light-sensitive reactions were conducted in glassware wrapped with aluminum foil in a dark hood.

(Z,Z)-3,6-Bis[2'-(trimethylsilyl)ethanethio]-3,5-nonadiene-1,7-diyne (5).

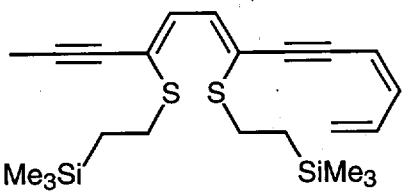


5

To a solution of tetrabromide **3** (Koreeda, M.; Yang, W. *J. Am. Chem. Soc.* **1994**, *116*, 10793-10794) (1.75 g, 2.55 mmol) in dry tetrahydrofuran (30 mL) at -78 °C was added dropwise *n*-butyllithium (1.5 M in hexanes, 6.80 mL, 10.20 mmol). The solution turned dark green after the addition was complete. After 10 min at -78 °C, the mixture was taken to room temperature and

kept stirring at that temperature for 1 h. It was then recooled to -78 °C, whereupon a stock solution of methyl iodide in tetrahydrofuran (0.636 M, 4.41 mL, 2.81 mmol) was added dropwise. The reaction mixture was gradually warmed up to room temperature over 12-h period and then the reaction was quenched with saturated aqueous ammonium chloride (20 mL). The resulting mixture was extracted with diethyl ether (2 x 30 mL) and the combined organic layers were washed first with water (50 mL), and then with brine (50 mL) and dried (Na_2SO_4). Removal of the solvent under reduced pressure provided a red oil, which was purified by silica gel flash column chromatography using hexanes as the eluent to provide the monomethylated alkyne **5** as an orange oil (388 mg, 40%): R_f(hexanes/diethyl ether, 10/1) 0.22; ^{1}H NMR (360 MHz, $CDCl_3$) δ 0.026 (s, 9H), 0.031 (s, 9H), 0.90 and 2.92 (AA'XX', 8H), 2.06 (s, 3H), 3.30 (s, 1H), 6.94 and 7.11 (ABq, 2H, J_{AB} = 11.6 Hz); ^{13}C NMR (90.6 MHz, $CDCl_3$) δ -1.77 (q) 4.65 (q), 18.32 (t), 18.55 (t), 28.94 (t), 28.99 (t), 78.01 (s), 81.37 (d), 81.77 (s), 92.08 (s), 118.57 (s), 122.90 (s), 130.49 (d), 133.64 (d), IR (neat) 3311, 2211, 2079 cm^{-1}; UV-Vis (EtOH) λmax 362.0 nm (ε 27488), 266.5 nm (ε 10293). HRMS (CI with ammonia): Calcd for $[C_{19}H_{32}S_2Si_2+H]^+$: m/z 381.1562. Found: m/z 381.1575.

(Z,Z,Z)-7,10-Bis[2'-(trimethylsilyl)ethanethio]-1,3,7,9-tridecatetraene-5,11-diyne (7).

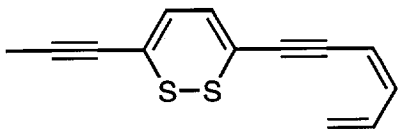


7

To a solution of **5** (455 mg, 1.20 mmol) in benzene (10 mL) were added in succession 1,2-*cis*-dichloroethene (180 mg, 140 mL, 1.80 mmol), *n*-butylamine (175 mg, 237 mL, 2.40 mmol), tetrakis(triphenylphosphine)palladium (71 mg, 0.06 mmol), and copper (I) iodide (24 mg, 0.12 mmol). The resulting solution was kept stirring at room temperature for 1 h. It was then diluted with hexanes/dichloromethane (5/1, 100 mL) and the solution was filtered through a short pad of silica gel using suction filtration. Removal of the solvent under reduced pressure gave a red oil, which was then dissolved in dry benzene (15 mL). To this solution at room temperature was added tetrakis(triphenylphosphine)palladium (71 mg, 0.06 mmol) followed by vinylmagnesium bromide

(1.0 M in tetrahydrofuran, 2.4 mL, 2.4 mmol). The mixture was stirred for 12 h at that temperature and then the reaction was quenched with saturated aqueous ammonium chloride (30 mL). The aqueous layer was back-extracted with hexanes (30 mL). The combined organic layers were washed first with water (30 mL), and then with brine (30 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a red oil, which was purified by silica gel flash column chromatography using hexanes/dichloromethane (15/1) as the eluent to give *cis*-diene-yne **7** as a yellow oil (305 mg, 59% for two steps): R_f(hexanes ^{1}H NMR (360 MHz, $CDCl_3$) d 0.027 (s, 9H), 0.033 (s, 9H), 0.92 and 2.92 (AA'XX', 8H), 2.07 (s, 3H), 5.32 (d, 1H, J = 9.3 Hz) 5.43 (d, 1H, J = 16.2 Hz), 5.72 (d, 1H, J = 10.7 Hz), 6.45 (t, 1H, J = 10.7 Hz), 6.84-6.99 (m, 3H); ^{13}C NMR (90.6 MHz, $CDCl_3$) δ -1.77, 4.69, 17.51, 18.48, 28.70, 28.97, 78.31, 91.57, 96.48, 109.43, 120.22, 120.46, 121.10, 131.15, 132.79, 133.40, 134.14, 140.72. HRMS (CI with ammonia): Calcd for $[C_{23}H_{36}S_2Si_2+H]^+$: m/z 433.1875. Found: m/z 433.1860.

Thiarubrine C (1).

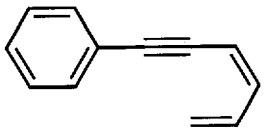


1

A 25-mL round-bottomed flask was charged with tetrabutylammonium fluoride trihydrate (439 mg, 1.39 mmol) and the flask was then connected to a vacuum pump and, in order to remove water, was heated until the solid melted. The pumping continued for 0.5 h without the heating. The anhydrous tetrabutylammonium fluoride thus obtained was then dissolved in dry tetrahydrofuran (4 mL) and *cis*-diene-yne **7** (50 mg, 0.11 mmol) in tetrahydrofuran (1 mL) was added dropwise at room temperature. The solution turned dark red. It was then cooled to -40 °C and trifluoroacetic anhydride (148 mg, 99 mL, 0.695 mmol) was added dropwise. After 10 min at -40 °C, the reaction mixture was taken to room temperature and stirred for 4 h and then saturated aqueous sodium bicarbonate (10 mL) was added. The resulting suspension was stirred at room temperature for 15 min and then poured into a 100-mL Erlenmeyer flask containing diethyl ether (10 mL). Iodine (295 mg, 1.10 mmol) in aqueous potassium iodide (10%, 8 mL) was added with stirring. The resulting

mixture was stirred at room temperature for 15 min and aqueous sodium thiosulfate (0.1 M, 15 mL) was added. The resulting mixture was then extracted with diethyl ether (20 mL) and the organic layer was washed first with saturated aqueous sodium bicarbonate (20 mL), and then with brine (20 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a red oil, which was purified by silica gel flash column chromatography using hexanes as the eluent to give thiarubrine C (**1**) (Constabel, C. P.; Balza, F.; Towers, G. H. N. *Phytochemistry* **1988**, *27*, 3533-3535) as a red oil (6 - 8.5 mg, 24-34%): ^{1}H NMR (360 MHz, CD_3CN) δ 2.06 (s, 3H), 5.40 (d, 1H, J = 10.1 Hz), 5.52 (d, 1H, J = 17.0 Hz), 5.72 (d, 1H, J = 11.1 Hz), 6.59 (dd, 1H, J = 11.1 Hz), 6.61 and 6.68 (ABq, 2H, J_{AB} = 6.8 Hz), 6.79-6.87 (m, 1H); UV-Vis (EtOH) λmax 486.0 nm (ε 1645), 345.0 nm (ε 10660), 276.0 nm (ε 1534).

(*Z*)-3,5-Hexadien-1-ynylbenzene (8)

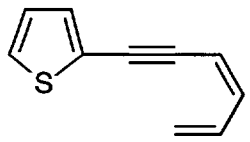


8

A 50-mL round-bottomed flask was charged with phenylacetylene (408 mg, 0.45 mL, 4.0 mmol), *cis*-1,2-dichloroethene (485 mg, 0.38 mL, 5.0 mmol), *n*-butylamine (740 mg, 1.0 mL, 10.0 mmol), and benzene (10 mL) at room temperature. Tetrakis(triphenylphosphine)palladium (231 mg, 0.2 mmol) and copper (I) iodide (76 mg, 0.4 mmol) were then added. The resulting mixture was stirred at room temperature for 1.5 h and then diluted with hexanes/dichloromethane (5/1, 100 mL). The resulting solution was filtered through a short pad of silica gel using suction filtration. Removal of the solvent under reduced pressure gave a yellow oil, which was then dissolved in dry benzene (15 mL). To this solution at room temperature was added tetrakis(triphenylphosphine)palladium (231 mg, 0.2 mmol) followed by vinylmagnesium bromide (1.0 M in tetrahydrofuran, 8.0 mL, 8.0 mmol). The mixture was stirred at that temperature for 12 h and then quenched with saturated aqueous ammonium chloride (30 mL). It was then extracted with hexanes (30 mL). The organic layer was

washed first with water (30 mL) and then with brine (30 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a yellow oil, which was purified by silica gel flash column chromatography using hexanes as the eluent to provide diene-yne **8** as a colorless oil (550 mg, 85% for two steps): R_f(hexanes) 0.27; ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.32 (ddd, 1H, J = 10.2, 1.6, 0.9 Hz), 5.42 (dddd, 1H, J = 17.0, 1.6, 0.9, 0.9 Hz), 5.69 (d, 1H, J = 10.6 Hz), 6.45 (ddd, 1H, J =10.8, 10.6, 0.9 Hz), 6.93- 7.04 (m, 1H, J = 17.0, 10.8 Hz), 7.25-7.33 (m, 3H), 7.44-7.48 (m, 2H); ^{13}C NMR (90.6 MHz, $CDCl_3$) δ 86.42 (s), 95.71 (s), 109.85 (d), 120.46 (t), 123.35 (s), 128.27 (d), 128.33 (d), 131.46 (d), 134.11 (d), 140.26 (d); IR (neat) 1789, 1723, 1634, 1599, 1489, 1457, 1320, 1267 cm^{-1}. HRMS (EI 70 ev): Calcd for $C_{12}H_{10}$: m/z 154.0783. Found: m/z 154.0784.

2-[(*Z*)-3,5-Hexadien-1-ynyl]thiophene (9).



9

A 25-mL round-bottomed flask was charged with 2-iodothiophene (1.05 g, 0.55 mL, 5.0 mmol), (trimethylsilyl)acetylene (600 mg, 0.87 mL, 6.0 mmol), *n*-butylamine (1.46 g, 1.97 mL, 20.0 mmol), and benzene (10 mL) at room temperature. Bis(triphenylphosphine)palladium chloride (140 mg, 0.2 mmol), and copper (I) iodide (76 mg, 0.4 mmol) were then added. The dark blue solution was stirred at room temperature for 12 h and then diluted with hexanes/dichloromethane (5/1, 100 mL). The solution was filtered through a short pad of silica gel using suction filtration. Removal of the solvent under reduced pressure gave a yellow oil, which was then dissolved in methanol (20 mL). To this solution at room temperature was added potassium hydroxide (1.0 M in water, 5 mL, 5.0 mmol). It was then stirred at that temperature for 2 h and then diluted with diethyl ether (30 mL). The mixture was washed with water (20 mL). The aqueous layer was back-extracted with diethyl ether (30 mL) and the combined organic layers were washed first with water (50 mL), and then with brine (50 mL), and dried (Na_2SO_4). Removal of the solvent by careful rotary evaporation gave a yellow oil, which was then mixed with tetrakis(triphenylphosphine)palladium (289 mg, 0.25 mmol), *cis*-1,2-dichloroethene (1.00 g, 0.78 mL, 10.0 mmol), *n*-butylamine (740 mg, 1.0 mL, 10.0 mmol),

copper (I) iodide (95 mg, 0.50 mmol), and dry benzene (10 mL). The resulting mixture was stirred at room temperature for 1.5 h and then diluted with hexanes/dichloromethane (5/1, 100 mL). The resulting solution was filtered through a short pad of silica gel using suction filtration. Removal of the solvent under reduced pressure gave a yellow oil, which was then dissolved in dry benzene (15 mL), to which tetrakis(triphenylphosphine)palladium (289 mg, 0.25 mmol) was added followed by vinylmagnesium bromide (1.0 M in tetrahydrofuran, 10.0 mL, 10.0 mmol) at room temperature. The mixture was stirred for 12 h at that temperature and then the reaction was quenched with saturated aqueous ammonium chloride (30 mL). The resulting mixture was extracted with hexanes (30 mL). The organic layer was washed first with water (30 mL), and then with brine (30 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a colorless oil, which was purified by silica gel flash column chromatography using hexanes as the eluent to give diene-yne **9** as a colorless oil (280 mg, 35% for four steps): R_f(hexanes) 0.35; ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.33 (dd, 1H, J = 10.2, 0.8), 5.42 (ddd, 1H, J = 16.9, 0.7, 0.7 Hz), 5.68 (d, 1H, J = 10.8 Hz), 6.44 (dd, 1H, J = 11.0, 10.8 Hz), 6.88-7.00 (m, 2H), 7.21-7.28 (m, 2H); ^{13}C NMR (90.6 MHz, $CDCl_3$) δ 88.73 (s), 90.32 (s), 109.41 (d), 120.69 (t), 123.31 (s), 127.09 (d), 127.32 (d), 131.71 (d), 134.04 (d), 140.17 (d); IR (neat) 2186, 1511, 1437, 1416, 1192 cm^{-1}. HRMS (EI 70 ev): Calcd for $C_{10}H_8S$: m/z 160.0347. Found: m/z 160.0354.

Materials and Methods for DNA Experiments.

Reagents were of the highest purity available and were used without further purification. Materials were purchased from the following suppliers: glycerol, Sigma Chemical Company; tris(hydroxymethyl)aminomethane (Tris), Acros Chemical Company; diethylenetriaminepentaacetic acid (DETAPAC), Fluka Chemical Company; xylene cyanol bromophenol and sodium dodecyl sulfate (SDS), United States Biochemical; HPLC-grade methanol, acetonitrile, Fischer; ethidium bromide pellets, Gibco BRL; ethanol, McCormick Distilling Company; supercoiled pBR322 DNA, superoxide dismutase, catalase, Boehringer Mannheim; Seakem ME Agarose, FMC. All other reagents were purchased from Aldrich Chemical Company. Water was distilled, deionized, and

glass redistilled. Densitometry of ethidium bromide stained agarose gels was performed using an Alpha Innotech IS-1000 digital imaging system.

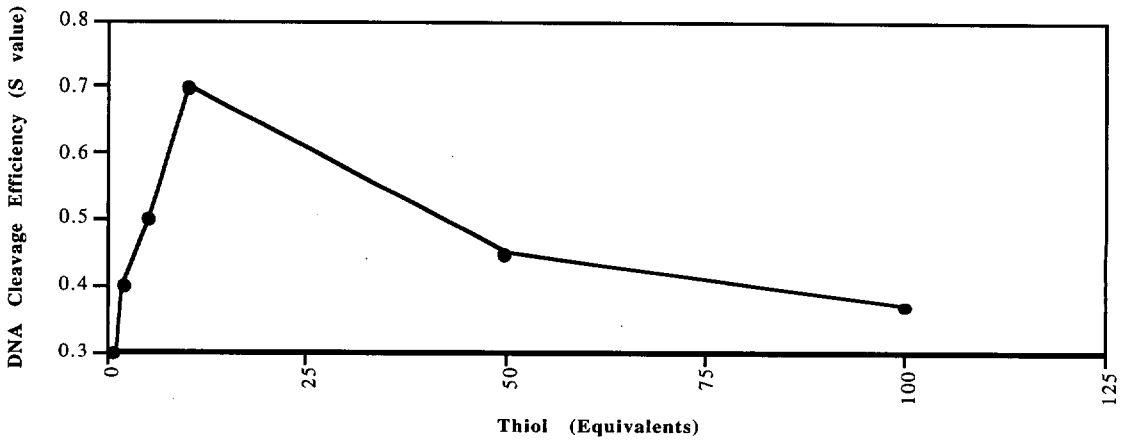
General Experimental Methods for DNA-Cleavage Assays.

In a typical assay (final concentrations: 50 mM sodium phosphate buffer, pH 7.0, 2 mM 2-mercaptoethanol, and 37.8 μM bp of pBR322 DNA) a solution containing the compound of interest (2 μL of a stock solution in acetonitrile) was added to a mixture of buffer (2 μL of 500 mM sodium phosphate solution, pH 7.0), water (12 μL), and pBR322 DNA (2 μL of 0.25 μg/μL solution in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) followed by 2-mercaptoethanol (hereafter referred to as thiol) (2 μL of a freshly prepared aqueous stock solution). The resulting mixture was agitated on a vortex mixer for 4-5 sec, spun for 20-30 sec in a tabletop microcentrifuge, and incubated at 37 °C for 14 h in the dark. The final reactions contained 10% acetonitrile by volume. Stock solutions were prepared and shipped in acetonitrile, then stored at 4 °C for four days or less. Acetonitrile solutions of **1** contain a trace impurity that comigrates (TLC) with the decomposition product that results from deliberate prolonged exposure of a small amount of the stock solution to ambient lighting. This impurity is therefore presumed to be the corresponding thiophene derivative (for photodecomposition of 1,2-dithiins, see: Block, E.; Page, J.; Toscano, J. P.; Wang, C.-X.; Zhang, X.; DeOrazio, R.; Guo, C.; Sheridan, R. S.; Towers, C. H. N. *J. Am. Chem. Soc.* **1996**, *118*, 4719-4720). It is, thus, important to reiterate that the thiophene derivative **9** is inactive as a thiol-dependent DNA-cleaving agent under the conditions used in our experiments. Additives used in mechanistic experiments were placed in the reaction mixture prior to the addition of the compound of interest. Preparation of reactions was carried out under red light (darkroom light) and all reactions were incubated in the dark.

Gel Electrophoresis and Quantitation of DNA Cleavage.

Following incubation, 4 μL of 50% glycerol loading buffer, containing 0.1% bromophenol blue, 150 mM EDTA, 1% SDS in 2M Tris, 1M acetate, pH 8.0, was added and the reaction mixture agitated on a vortex mixer for 3-4 sec, centrifuged for 20-30 sec in a tabletop microcentrifuge,

loaded immediately onto a 0.9% agarose gel which was cast containing ethidium bromide (1 μg/mL), and electrophoresed at 90 V for approximately 2 h in TAE buffer (40 mM Tris Base, 20 mM acetate, 1 mM EDTA, pH 8.0). The amount of DNA in each band was quantitated using an Alpha Innotech IS-1000 Digital Imaging System. The values reported are uncorrected for differential ethidium staining of form I, II, and III DNA (see: Vinograd and Bauer *J. Mol. Biol.* **1968**, *33*, 141-171).



Supporting Information Figure 1. DNA Cleavage by Thiarubrine C (**1**, 100 μM) in the Presence of Varying Thiol Concentrations.[a,b,c]

[a] Reactions were performed as described above.

[b] Values reflect the average of multiple experiments and the standard error is less than 4%.

[c] The S-value is the mean number of strand breaks per plasmid and is calculated using the equation: S = - ln (% form I).

Supporting Information Table 1. Cleavage of Plasmid DNA by Thiarubrine C (**1**) and the Effect of Various Additives.[a]

Reaction[a]	% Form I Remaining	S[b]
DNA Alone	88	0.1
Thiol alone (2 mM)	85	0.2
1 alone (100 μM)	85	0.2
1 alone[c] (1 mM)	79	0.2
Std. Rxn: **1** (100 μM) + thiol (2 mM)	50	0.7
Std. Rxn + Additive		
Methanol (1M)	86	0.2
Methanol (200 mM)	80	0.2
Ethanol (1M)	87	0.1
Ethanol (200 mM)	77	0.3
Mannitol (100 mM)	78	0.3
Mannitol (20 mM)	71	0.3
Desferal (5 mM)	67	0.4
DETAPAC (10 mM)	76	0.3
DETAPAC (1 mM)	70	0.4
SOD (100 μg/mL)	60	0.5
Catalase (100 μg/mL)	88	0.1
Degassed Std. Rxn.[d]	69	0.4

[a] Assays were performed as described above.

[b] S is the mean number of strand breaks per plasmid molecule and is calculated using the equation: S = - ln (% Form I DNA). Values reflect the average of multiple experiments. Standard errors in these measurements are less than 4%.

[c] Result of a single experiment. This reaction contained 25% acetonitrile by volume.

[d] Degassing achieved by three freeze-pump-thaw cycles.

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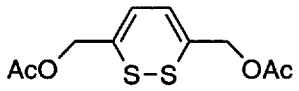

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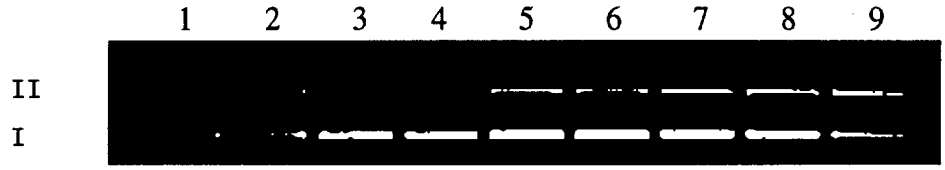
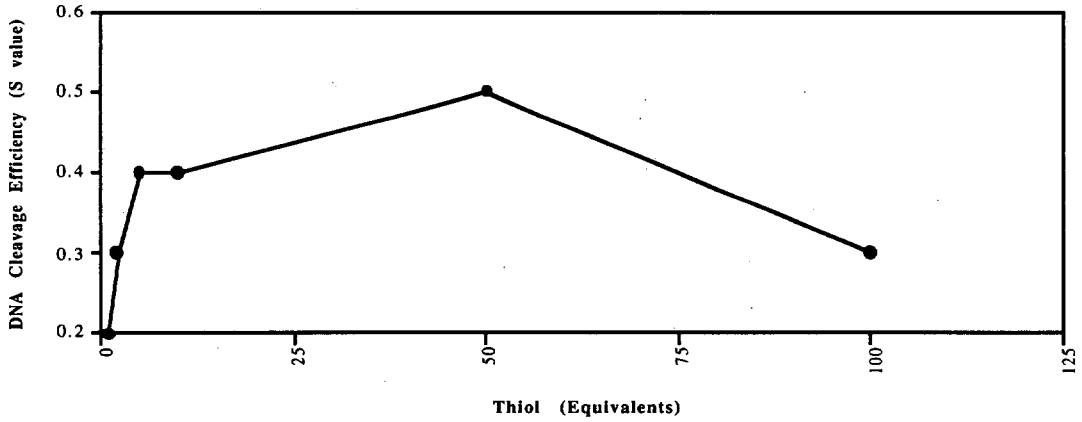
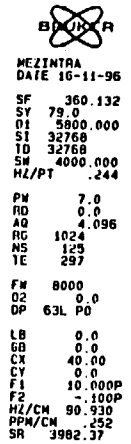


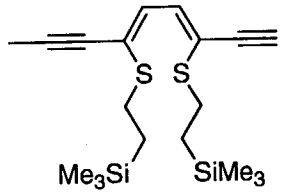
Supporting Information Figure 2. DNA Cleavage by Varying Concentrations of the Dithiin Analog **10** in the Presence of 20 Equivalents of Thiol. Assays were performed as described above. The number in parenthesis following the description of each lane indicates the S-value (mean number of strand breaks per plasmid molecule) for each lane and is calculated utilizing the equation $S = -\ln f_I$, where f_I is the fraction of plasmid in a given lane, present as form I. Values reported here are the average of three experiments and the standard error in these measurements is less than 2%. Lane 1, DNA alone (0.2); lane 2, 100 μM **10** (0.2); lane 3, 2 mM 2-mercaptoethanol (0.2); lane 4, 1 μM **10** + thiol (0.3); lane 5, 5 μM **10** + thiol (0.3); lane 6, 10 μM **10** + thiol (0.3); lane 7, 25 μM **10** + thiol (0.4); lane 8, 50 μM **10** + thiol (0.4); lane 9, 100 μM **10** + thiol (0.5).



Supporting Information Figure 3. DNA Cleavage by **10** (100 μM) in the Presence of Varying Thiol Concentrations.[a,b]

[a] Reactions were performed as described above.
[b] Values reflect the average of multiple experiments and the standard error is less than 4%.





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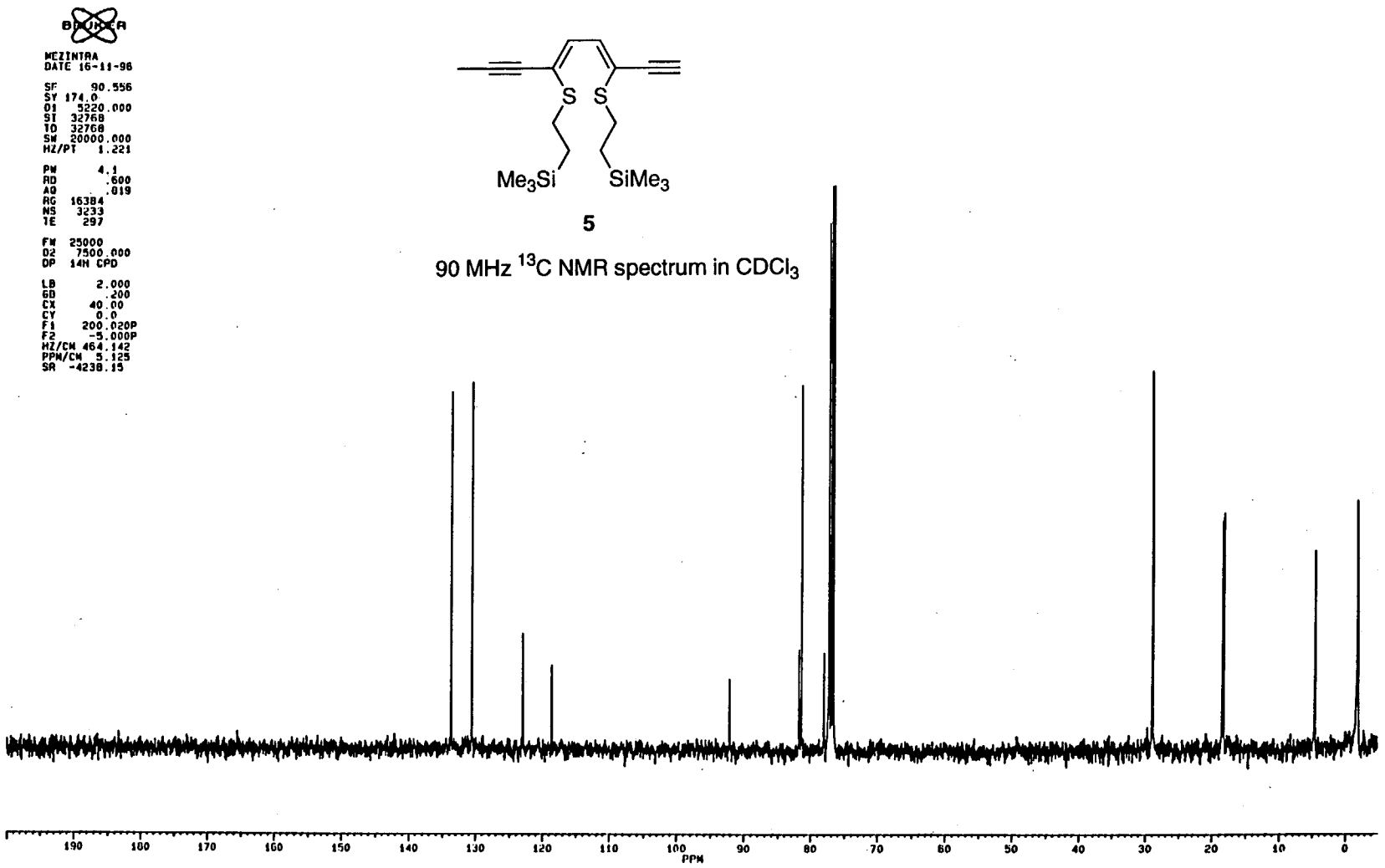
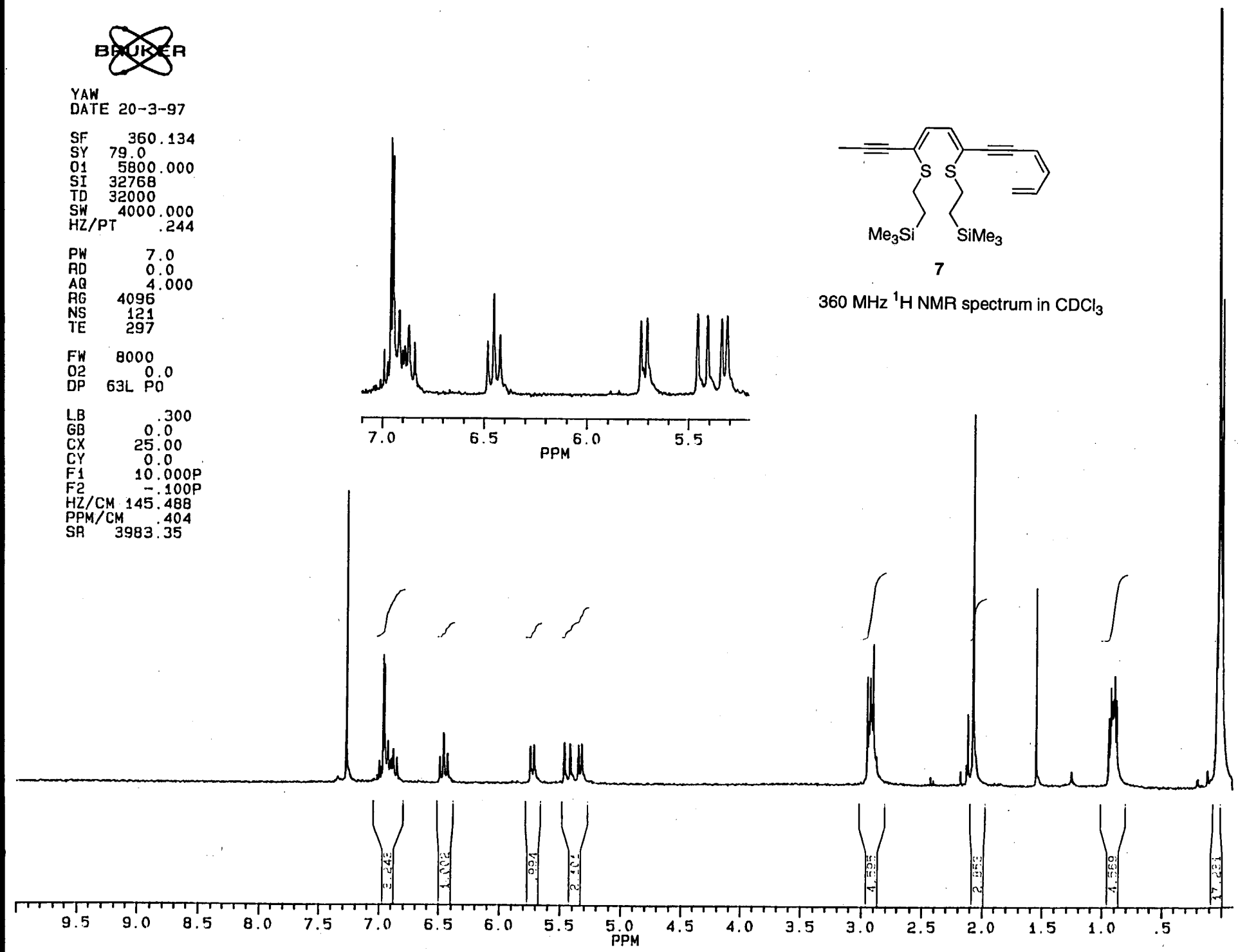
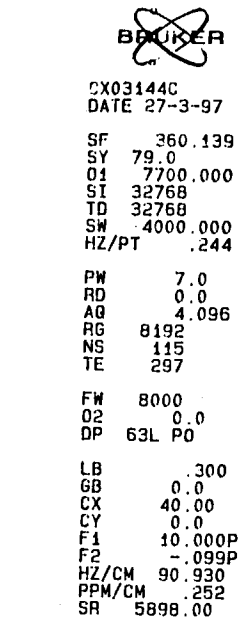
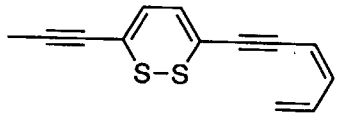
360 MHz ^{1}H NMR spectrum in $CDCl_3$



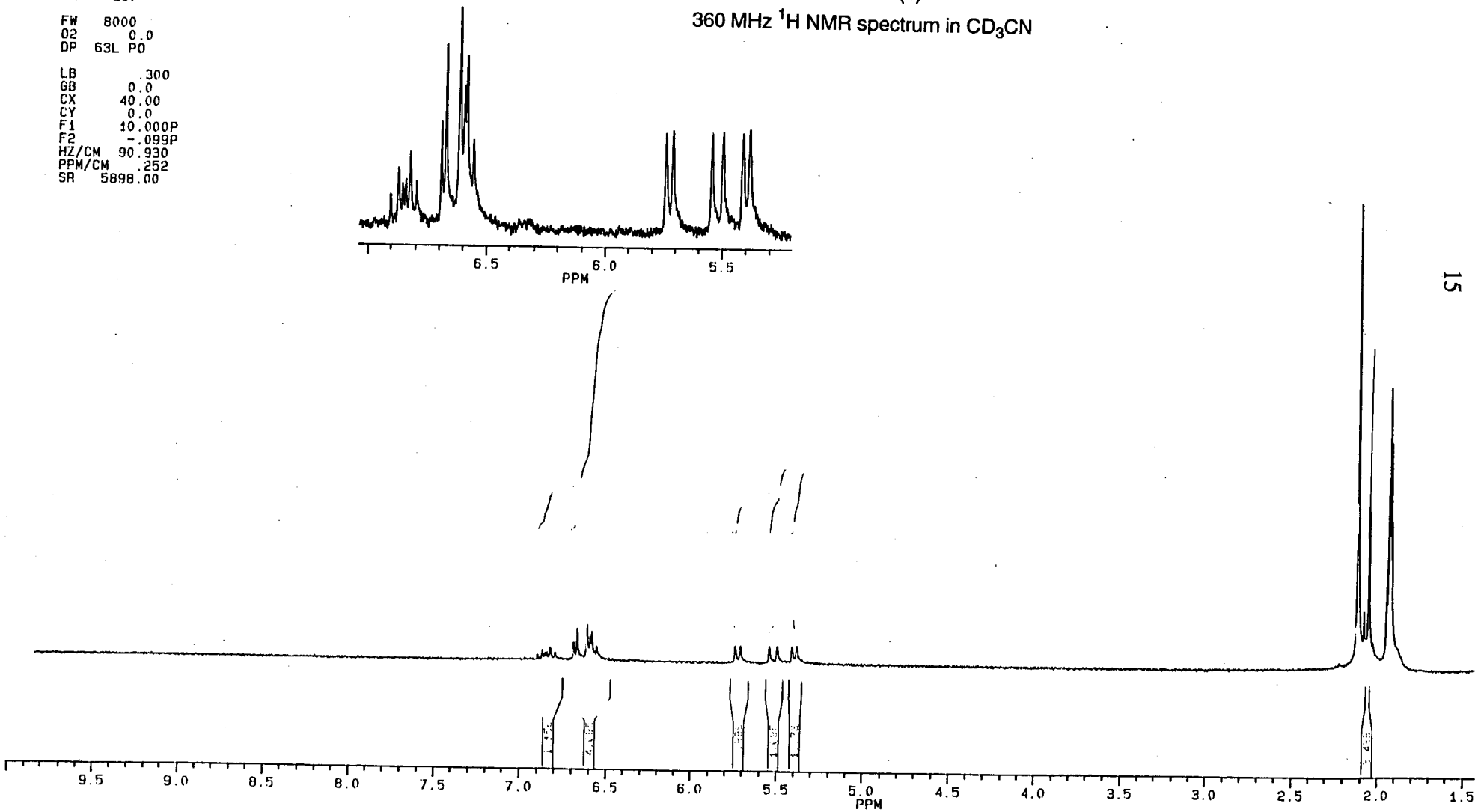


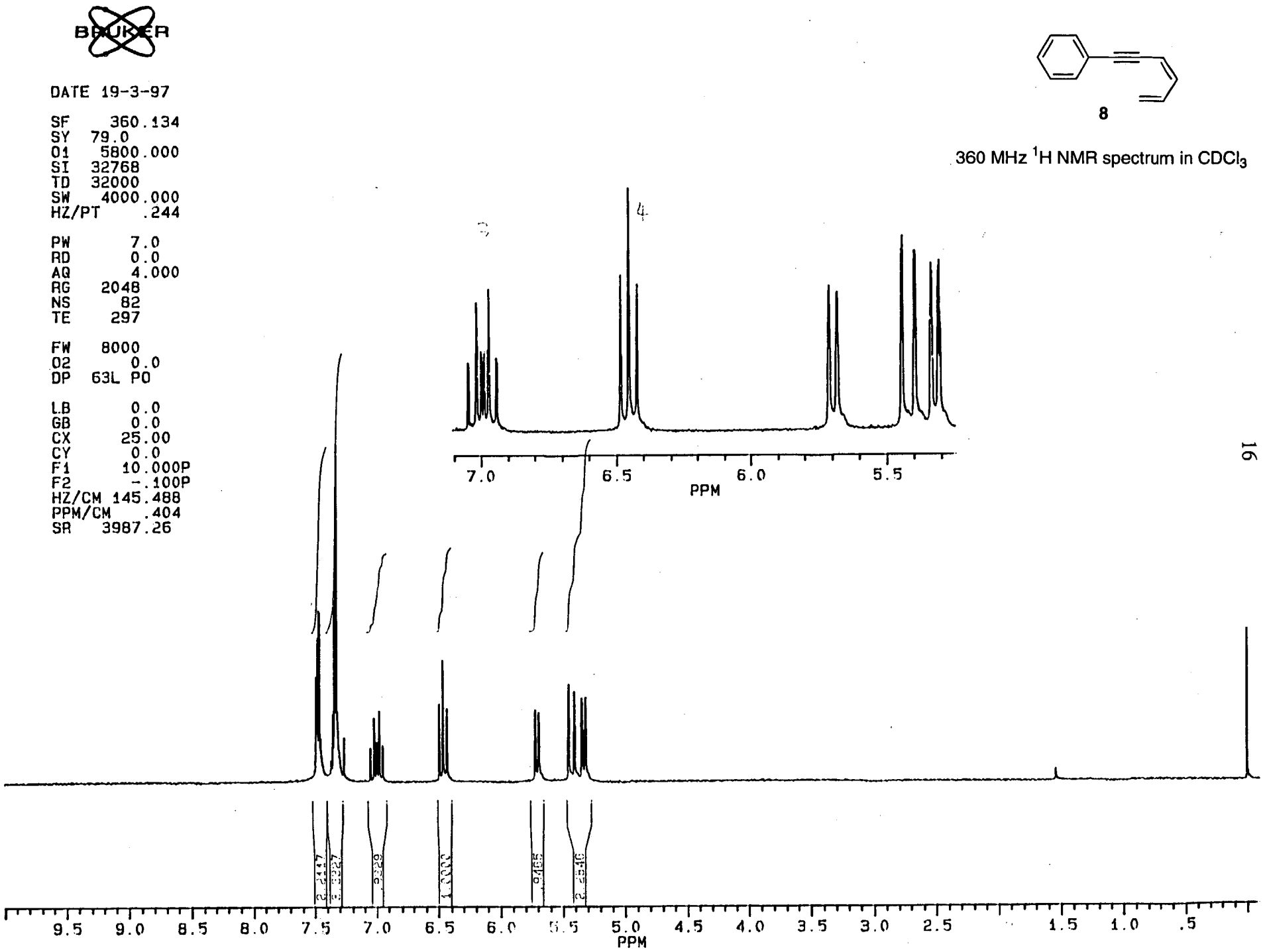


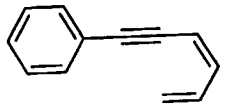


thiarubrine C (**1**)

360 MHz ^{1}H NMR spectrum in CD_3CN







8

90 MHz ^{13}C NMR spectrum in $CDCl_3$

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