Chemical Co.), 100 mg (0.0004 mol) of $SbCl_3$, and CH_2Cl_2 (100 mL) was treated with 4.2 mL (0.032 mol) of diethylaminosulfur trifluoride at room temperature. The progress of the reaction was followed by GLC, and after 1 h, the light yellow solution was washed with aqueous $NaHCO_3$, dried (K_2CO_3), and filtered. The solution containing the α-fluoro sulfide[13] was treated with 8.6 g (0.04 mol) of 80% *m*-chloroperbenzoic acid and stirred at room temperature for 6 h. The reaction was filtered, and the filtrate was washed with aqueous $NaHSO_3$ and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo. Purification by flash chromatography (350 g of silica gel, 1/6 EtOAc/hexane) gave 2.3 g (64%) of **2** (Et_2O): mp 74–76 °C; ^1H NMR δ 3.78 (ddd, 1, *J* = 13.7, 12.9, 9.5 Hz), 4.16 (ddd, 1, *J* = 32.9, 12.9, 2.2 Hz), 5.27 (ddd, 1, *J* = 48.3, 9.5, 2.3 Hz), 7.61–7.98 (m, 5); ^{19}F NMR δ −180.68 (ddd, *J* = 47.9, 33.4, 14.1 Hz); MS (CI/CH_4) *m/z* 223 (MH^+). Anal. Calcd for $C_8H_8ClFO_2S$: C, 43.16; H, 3.62. Found: C, 43.03; H, 3.61.

1-Fluorovinyl Phenyl Sulfone (3). To a mixture of **2** (20.9 g, 0.0939 mol) and CH_2Cl_2 (200 mL) was slowly added 15.2 g (0.1 mol) of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). After 2 h at room temperature, GLC showed the disappearance of **2**. The reaction was washed with 1 N HCl, dried ($MgSO_4$), and concentrated. The resulting oil was dried under high vacuum for several hours and slowly crystallized, providing 15.1 g (86%) of **3** as light tan crystals: mp 35–38 °C (lit.[2] no melting point reported); ^1H NMR δ 5.43 (dd, 1, *J* = 12.5, 4.6 Hz, SO_2CH_aHF), 5.88 (dd, 1, *J* = 41.8, 4.6 Hz, SO_2CHH_bF), 7.58–7.99 (m, 5, Ph); ^{19}F NMR δ −115.52 (dd, *J* = 41.9, 12.6 Hz, CH_aH_bF); MS (CI/CH_4) *m/z* 187 (MH^+). Anal. Calcd for $C_8H_7FO_2S$: C, 51.60; H, 3.79. Found: C, 51.33; H, 3.89.

***erythro*- and *threo*-2-[2-Fluoro-2-(phenylsulfonyl)-ethyl]tetrahydrofuran (4a and 4b). Zinc Dust Procedure.** A mixture of 707 mg (3.8 mmol) of α-fluorovinyl phenyl sulfone, Zn dust (7.1 μm) (300 mg, 4.6 mmol), and THF (50 mL) was heated at 60 °C for 24 h under argon. The progress of the reaction was followed by GLC. After 24 h (see Table I) the reaction was cooled, filtered, and purified by flash chromatography (75 g of silica gel, 1/4 EtOAc/hexane) to provide 183 mg of **4a**, 470 mg of a mixture of **4a** and **4b**, and 75.5 mg of **4b** (overall yield: 728.5 mg, 74.3%).

4a: ^1H NMR δ 1.50–1.63 (m, 1), 1.86–1.99 (m, 2), 2.01–2.17 (m, 2), 2.17–2.35 (m, 1), 3.75 (dd, 1, *J* = 16.1, 7.4 Hz), 3.87 (ddd, 1, *J* = 15.1, 7.9 Hz), 4.06 (9 line m, 1), 5.39 (ddd, 1, *J* = 48.8, 11.1, 1.8 Hz), 7.53–8.00 (m, 5); MS (CI/CH_4) *m/z* 259 (MH^+). Anal. Calcd for $C_{12}H_{15}FO_3S$: C, 55.80; H, 5.85. Found: C, 55.44; H, 5.75.

4b: ^1H NMR δ 1.49–1.65 (m, 1), 1.84–1.96 (m, 2), 1.96–2.17 (m, 2), 2.43 (dddd, 1, *J* = 30.6, 15.2, 5.8, 4.6 Hz), 3.75 (dd, 1, *J* = 14.8, 7.2 Hz), 3.88 (dd, 1, *J* = 14.6, 7.5 Hz), 4.14 (dt, 1, *J* = 12.4, 7.0 Hz), 5.31 (ddd, 1, *J* = 48.3, 8.3, 4.2 Hz), 7.52–7.99 (m, 5); MS (CI/CH_4) *m/z* 259 (MH^+). Anal. Calcd for $C_{12}H_{15}FO_3S$: C, 55.80; H, 5.85. Found: C, 56.02; H, 5.87.

Benzoyl Peroxide Procedure. A mixture of **3** (380 mg, 2.0 mmol), benzoyl peroxide (20 mg, 0.08 mmol), and THF (30 mL) was refluxed for 9 h. Workup as described above gave 419 mg (80%) of a mixture of **4a** and **4b** as a colorless oil, which exhibited the same spectral properties as above.

2-[2-Fluoro-2-(phenylsulfonyl)ethyl]tetrahydro-2(and 5)-methylfuran (5a and 5b): purified by flash chromatography (1/5 EtOAc/hexane) to provide an inseparable mixture of **5a** and **5b** as a clear liquid; ^1H NMR δ 1.18–1.28 (m, 3), 1.40–2.52 (m, 6), 3.78–4.31 (m, 2), 5.19–5.52 (m, 2), 7.58–7.97 (m, 5); MS (CI/CH_4) *m/z* 273 (MH^+). Anal. Calcd for $C_{13}H_{17}FO_3S$: C, 57.33; H, 6.29. Found: C, 57.69; H, 6.42.

2-[2-Fluoro-2-(phenylsulfonyl)ethyl]-1,3-dioxolane (6): purified by flash chromatography (1/3 EtOAc/hexane) to provide **6** as a colorless oil; MS (CI/CH_4) *m/z* 261 (MH^+), 119 (MH^+ − HSO_2Ph, base peak); ^1H NMR δ 2.15–2.55 (m, 2), 3.80–4.07 (m, 4), 5.13 (dd, 1, *J* = 6.0 and 3.3 Hz), 5.38 (ddd, 1, *J* = 48.8, 10.0, and 2.7 Hz), 7.57–7.99 (m, 5); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.49 (d, *J* = 18.2 Hz), 65.78, 66.05, 100.55 (d, *J* = 218 Hz), 101.03, 130.15, 130.45, 135.51, 135.79. Anal. Calcd for $C_{11}H_{13}FO_4S$: C, 50.76; H, 5.03. Found: C, 50.87; H, 5.05.

2-[2-Fluoro-2-(phenylsulfonyl)ethyl]-1,4-dioxane (7): purified by flash chromatography (1/3 EtOAc/hexane) to provide **7** as a clear liquid: ^1H NMR δ 1.78–2.47 (m, 2), 3.34 (14 line m, 1), 3.53–3.66 (m, 1), 3.68–3.79 (m, 4.5), 3.94 (12 line m, 0.5), 5.26 (ddd, 0.5, *J* = 4,.0, 7.7, 4.3 Hz), 5.41 (ddd, 0.5, *J* = 48.8, 11.2, 1.8 Hz), 7.58–7.96 (m, 5); MS (CI/CH_4) *m/z* (MH^+); HRMS Calcd for $C_{12}H_{16}FO_4S$ 275.0753, found 275.0736.

1-Fluoro-1-(phenylsulfonyl)-3-pentanone (9). A mixture of **3** (340 mg, 2.0 mmol), benzoyl peroxide (10 mg, 0.04 mmol), AIBN (10 mg, 0.07 mmol), and propionaldehyde (40 mL) was refluxed for 20 h under argon. The reaction was concentrated under high vacuum to provide crude **9**. Attempted purification of **9** by flash chromatography provided a 3 to 1 mixture of **9** and (*E*)-2-(phenylsulfonyl)vinyl ethyl ketone (**11**).[10]

9: ^1H NMR δ 1.11 (t, 3, *J* = 7.4 Hz), 2.68 (g, 2, *J* = 7.4 Hz), 3.12 (m, 2), 5.71 (ddd, 1, *J* = 47.3, 8.9, 2.7 Hz), 7.56–7.97 (m, 5); MS/(CH/CH_4) *m/z* 245 (MH^+), 143 (base peak).

***erythro*- and *threo*-1-Fluoro-1-(phenylsulfonyl)-3-pentanol (10).** Fluoro ketone **9** from the above experiment was dissolved in EtOH (20 mL), and $NaBH_4$ (500 mg, 12 mmol) was added. After 6 h at room temperature the reaction was concentrated and partitioned between H_2O/EtOAc (20 mL/25 mL). The EtOAc extract was dried ($MgSO_4$), concentrated, and purified by flash chromatography on 80 g of silica gel (1/3 EtOAc/hexane and then 2/3) to give 253 mg (51%) of **10** as a mixture of diastereomers: ^1H NMR δ 0.94–1.00 (t, 3), 1.50–1.70 (m, 2), 1.94–2.13 (m, 0.5), 2.30–2.49 (m, 0.5), 3.86 (br d, 1), 5.43 (ddd, 0.5, *J* = 48.1, 7.2, 4.9 Hz), 5.50 (ddd, 0.5, *J* = 48.4, 10.6, 2.2 Hz), 7.58–7.97 (m, 5); ^{19}F NMR δ −182.03 (ddd, *J* = 48.9, 29.5, 15.7 Hz), −175.94 (ddd, *J* = 48.7, 39.3, 14.4 Hz); MS (CI/CH_4) *m/z* 247 (MH^+), 229 (MH^+ − H_2O); HRMS calcd for $C_{11}H_{15}FO_3S$ 274.0804 (MH^+), found 274.0806.

Tetrahydro-2-[2-(phenylsulfonyl)ethyl]furan (12). Phenyl vinyl sulfone (3.0 g, 17.8 mmol) and benzoyl peroxide (300 mg, 1.23 mmol) were dissolved in tetrahydrofuran (100 mL). The colorless solution was heated at a gentle reflux under argon, and the progress of the reaction was followed by GLC. After 6.5 h, the solvent was removed in vacuo (bath temperature 25 °C), and the product was purified by flash chromatography (ethyl acetate/hexane, 1/4, and then 1/3) to provide **12** as a colorless viscous oil (2.76 g, 64%); ^1H NMR δ 1.39–1.52 (m, 1), 1.75–2.05 (m, 5), 3.22 (14 line m, 2), 3.63–3.89 (m, 3), 7.53–7.95 (m, 5); MS (CI/CH_4) *m/z* 241 (MH^+). Anal. Calcd for $C_{12}H_{16}O_3S$: C, 59.97; H, 6.71. Found: C, 59.64; H, 6.74.

Acknowledgment. We thank Professor Earl Huyser for helpful discussions.

Registry No. 1, 27998-60-3; **2**, 125927-29-9; **3**, 114969-03-8; **4** (isomer 1), 125927-30-2; **4** (isomer 2), 125950-25-6; **5** (isomer 1), 125927-31-3; **5** (isomer 2), 125927-38-0; **6**, 125927-32-4; **7**, 125927-33-5; **9**, 125927-34-6; **10** (isomer 1), 125927-35-7; **10** (isomer 2), 125927-37-9; **11**, 108662-10-8; **12**, 125927-36-8; propionaldehyde, 123-38-6; phenyl vinyl sulfone, 5535-48-8; 2-methyltetrahydrofuran, 96-47-9; dioxolane, 646-06-0; 1,4-dioxane, 123-91-1; tetrahydrofuran, 109-99-9.

(13) A small sample of the α-fluoro sulfide precursor to **2** was prepared in $CDCl_3$: ^1H NMR δ 5.82 (ddd, 1, *J* = 52.3, 6.7, and 4.4 Hz); ^{19}F NMR δ −151.1 (ddd, *J* = 53.4, 18.0, and 13.5 Hz).

Development of a Drug-Release Strategy Based on the Reductive Fragmentation of Benzyl Carbamate Disulfides

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It has been shown that solid tumors frequently have inadequate vascularization and may exist in oxygen-deficient or hypoxic states.[1] Enhanced levels of reducing

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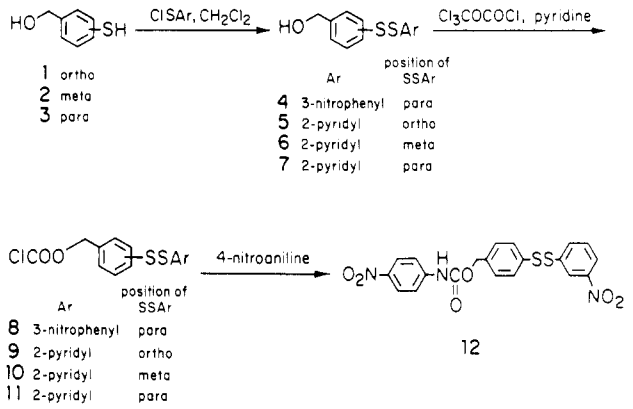
Scheme I



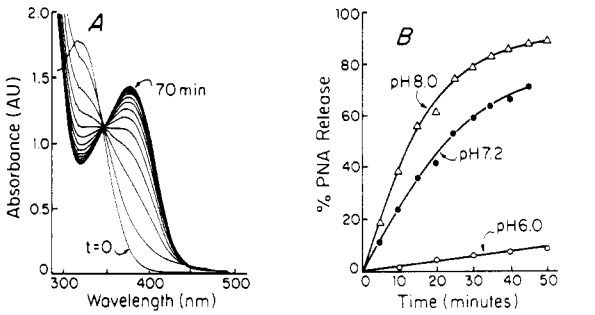


Figure 1. Reaction of **12** with dithiothreitol (DTT). (A) An aqueous solution of **12** at pH 8.0 was reduced with excess DTT, and the formation of *p*-nitroaniline (PNA) was monitored by UV/vis spectroscopy. The increased absorbance at 371 nm represents PNA release from the carbamate. (B) The effect of pH on the rate of PNA release.

agents such as NADH, NADPH, and glutathione have been associated with human tumor cell lines.[2] These observations have provided the impetus for much research toward the development and understanding of anticancer drugs that are bioreductively activated in the reducing environment of solid tumors.[1,3] Such agents may be of considerable therapeutic value.

We report the development of a prodrug strategy based on the reactivity of benzyl carbamate disulfide drug derivatives toward mild reducing agents. Upon disulfide bond reduction, appropriately substituted benzyl carbamates are shown to undergo fragmentation, and the amine-containing element of the carbamate is released. The use of this new fragmentation reaction for the development of mitomycin C (MMC) prodrugs is described.

Results and Discussion

Model Studies. The general method used for the preparation of benzyl carbamate disulfides is shown in Scheme I. Condensation of the mercaptobenzyl alcohols **1–3**[4] with 3-nitrobenzenesulfenyl chloride[5] or with 2-pyridinesulfenyl chloride resulted in the formation of the disulfides **4–7**. The corresponding chloroformates **8–11** were prepared by reacting **4–7** with trichloromethyl chloroformate (diphosgene).[6] Treatment of the chloroformate **8** with 4-nitroaniline gave the benzyl carbamate disulfide **12**.

Scheme II

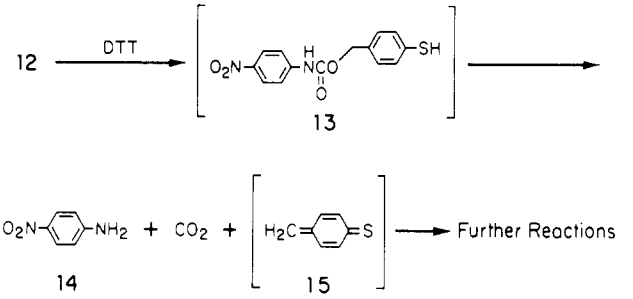
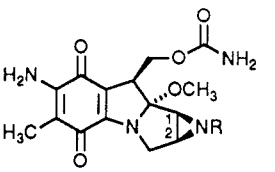
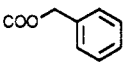
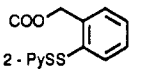
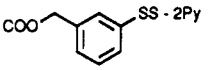
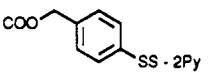
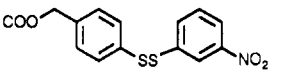


Table I. Structures and Cytotoxic Activities of the MMC Derivatives



mitomycin derivatives	R	IC_{50},[a] μM Namalwa	IC_{50},[a] μM HSB-2
16	H (MMC)	5	4
17		10	8
18		2	2
19		>10[b]	>10[c]
20		0.2	0.2
21		0.07	0.1

[a] Cells exposed to drug for 1 h at 37 °C in phosphate-buffered saline (pH 7.2). Cytotoxic effect (IC_{50}) expressed as concentration required to kill 50% of the cells. [b] 32% cell kill at 10 μM. [c] 26% cell kill at 10 μM.

In aqueous methanol buffered between pH 6 and 8, **12** was stable for several days. However, upon treatment with dithiothreitol,[7] the disulfide bond was reduced and *p*-nitroaniline (**14**) was released. The course of reaction was monitored by UV/vis spectroscopy, in which **14** (λ_{max} 371 nm) was easily distinguished from the starting material **12** (λ_{max} 313 nm) (Figure 1A). It was demonstrated that the rate of formation of **14** was pH-dependent (Figure 1B), in that the reaction was relatively slow at pH 6.0, but significantly faster under neutral or basic conditions.

The presumed pathway for the elimination of **14** from **12** is shown in Scheme II. The proposed mechanism is based on the chemically related fragmentations of benzisoxazolyl carbamates under basic conditions,[8] amidobenzyl carbamates after amide bond hydrolysis,[9] and nitrobenzyl

(1) (a) Sartorelli, A. C. *Biochem. Pharm.* **1986**, *35*, 67–69. (b) Sartorelli, A. C. *Cancer Res.* **1988**, *48*, 775–778. (c) Lin, T. S.; Wang, L.; Antonini, I.; Cosby, L. A.; Shiba, D. A.; Kirkpatrick, L.; Sartorelli, A. C. *J. Med. Chem.* **1986**, *29*, 84–89. (d) Kennedy, K. A. *Anti-Cancer Drug Design* **1987**, *2*, 181–194. (e) Fracasso, P. M.; Sartorelli, A. C. *Cancer Res.* **1986**, *46*, 3939–3944.

(2) (a) Russo, A.; DeGraff, W.; Friedman, N.; Mitchell, J. B. *Cancer Res.* **1986**, *46*, 2845–2848. (b) DeGraff, W. G.; Russo, A.; Mitchell, J. B. *J. Biol. Chem.* **1985**, *260*, 8312–8315. (c) Lemasters, J. J.; Ji, S.; Thurman, R. G. *Science* **1981**, *213*, 661–663.

(3) Moore, H. W.; Czerniak, R.; Hamdan, A. *Drugs Exptl. Clin. Res.* **1986**, *12*, 475–494.

(4) Grice, R.; Owens, L. N. *J. Chem. Soc.* **1963**, 1947–1954.

(5) Matsueda, R.; Aiba, K. *Chem. Lett.* **1978**, 951–952.

(6) Kurita, K.; Iwakura, Y. *Org. Synth.* **1979**, *59*, 195–201.

(7) Cleland, W. W. *Biochemistry* **1964**, *3*, 480–482.

(8) Kemp, D. S.; Hoyng, C. F. *Tetrahedron Lett.* **1975**, 4625–4628.

(9) Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. *J. Med. Chem.* **1981**, *24*, 479–480.

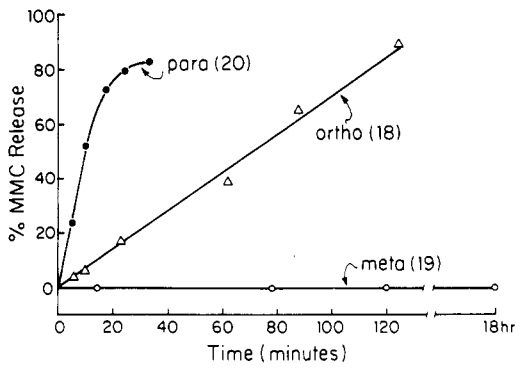


Figure 2. Release of MMC from the ortho, meta, and para benzyl carbamate disulfides, **18–20**. The disulfides were reduced with excess DTT at pH 7.2, and MMC release was quantified by HPLC.

halides and carbamates after reduction of the nitro group.[1c,10] These reactions are initiated by the release of electron density from the phenyl heteroatom into the π-system, followed by liberation of CO_2 (or halide ions from nitrobenzyl halide reduction) and the expulsion of the amine component of the carbamate.

Synthesis and Reactivity of MMC Prodrugs. The MMC derivatives **17–21** were prepared by condensation of MMC with benzyl chloroformate or with the chloroformates **8–11** (Table I). The stabilities and reactivities of the drug derivatives thus obtained were monitored by HPLC. At pH 7.2, the three MMC benzyl carbamate disulfides, **18–20**, underwent rapid reduction with dithiothreitol. In all three cases the half-lives for reduction ranged from 3 to 5 min, but subsequent elimination of MMC occurred at differing rates (Figure 2). Under the reducing conditions, the para disulfide (**20**) released MMC most quickly ($t_{1/2}$ 10 min), and the ortho disulfide (**18**) was significantly slower ($t_{1/2}$ 72 min). As expected, the meta isomer (**19**) and the MMC benzyl carbamate (**17**) did not release any MMC even after 18 h. These results established that mild reducing conditions are capable of effecting the fragmentation of appropriately substituted benzyl carbamate disulfides and that the amine component is released in high yield.

In Vitro Experiments. The drug derivatives were tested for in vitro cytotoxic activity on two human lymphoid cell lines, Namalwa and HSB-2, using a [^{3}H]thymidine incorporation assay. The results show that the most active MMC derivatives, **18**, **20**, and **21**, were those that were capable of undergoing thiol-mediated benzyl carbamate fragmentation (Table I). In fact, these compounds were more active than MMC itself. The most potent of these derivatives was the disulfide **21**, which was 40–70-fold more cytotoxic than MMC. The noncleavable MMC derivatives (**17** and **19**) were significantly less cytotoxic. While the generality of these findings would require related studies with several more cleavable and noncleavable MMC benzyl carbamate disulfides, the fact that the noncleavable carbamates, **17** and **19**, were found to be less cytotoxic than MMC is consistent with previous reports in the literature concerning the activities of MMC aziridinyl carbamates and amides.[11,12]

The enhanced activities of the cleavable MMC benzyl carbamate disulfides might be due to the lipophilic character of the prodrugs and the ease with which they can penetrate into cells and undergo subsequent fragmentation. Additionally, the thioquinone methides presumed to be formed in the reaction (**15** and the corresponding ortho isomer), may react with biological nucleophiles and enhance the activity of the released drug. It has been proposed that iminoquinone methides, which are generated from the reduction of nitrobenzyl derivatives, have cytotoxic activity.[10]

In summary, we have shown that ortho and para benzyl carbamate disulfides undergo reductive fragmentation and that the amine component of the carbamate is released in high yield. The in vitro results indicate that prodrugs based upon this fragmentation reaction warrant further investigation for their in vivo activities.

Experimental Section

Melting points are uncorrected. NMR spectra were obtained at 360 or 80 MHz. High-resolution mass spectrometry (HRMS) were obtained in the electron-impact mode. Elemental analyses of **18–20** were corrected for ethyl acetate and H_2O present in the samples as indicated by NMR spectroscopy. Attempts to remove these solvents under high vacuum or by reprecipitation of the drugs were not successful. *o*-, *m*-, and *p*-mercaptobenzyl alcohols, **1–3**, were prepared according to the procedures of Grice and Owens.[4]

General Procedure for the Preparation of Mixed Disulfides 4–7: Preparation of 2-(2-Pyridinyldithio)benzenemethanol (5). A solution of 0.75 g (3.4 mmol) of 2,2′-dithiodipyridine (Sigma Chemical Co.) in 25 mL of CH_2Cl_2 was cooled to 0 °C, and Cl_2 gas was bubbled in for 20 min. The resulting suspension was allowed to warm up to 23 °C and then stirred for 1.5 h. All volatile material was removed under high vacuum, leaving 2-pyridinesulfenyl chloride as a fine yellow powder. An analogous procedure was used for the preparation of 3-nitrobenzenesulfenyl chloride.[5]

A solution of 0.5 g (3.6 mmol) of 2-mercaptobenzenemethanol (**1**)[4] in 10 mL of CH_2Cl_2 was added over a 3-min period to a stirred suspension of 0.63 g (4.32 mmol) of 2-pyridinesulfenyl chloride in 50 mL of CH_2Cl_2. After 5 min, the mixture was extracted with saturated $NaHCO_3$ and saturated NaCl and dried ($MgSO_4$). The product was purified by flash chromatography on a 2 × 20 cm SiO_2 column using 30% ethyl acetate in petroleum ether as eluant. A fine white solid was obtained (570 mg, 64%): mp 50–52 °C; ^{1}H NMR ($CDCl_3$) δ 4.60 (br d, J = 4.6 Hz, 1 H, OH), 4.90 (d, J = 4.6 Hz, 2 H, $ArCH_2$), 7.0–7.8 (m, 7 H, ArH), 8.4–8.5 (m, 1 H, ArH); HRMS m/e 249.0285 (calcd 249.0282). Anal. Calcd for $C_{12}H_{11}NO_2S_2$: C, 57.80; H, 4.45; N, 5.62. Found: C, 57.57; H, 4.40; N, 5.61.

4-[(3-Nitrophenyl)dithio]benzenemethanol (4): yield 41%; mp 83–84 °C; ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 1 H, OH), 4.68 (s, 2 H, $ArCH_2$), 7.2–7.6 (q, J = 6.2, 7.7 Hz, 4 H, ArH), 7.7–8.5 (m, 4 H, ArH); HRMS m/e 293.0183 (calcd 293.0180).

3-(2-Pyridinyldithio)benzenemethanol (6): yield 86%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.8 (s, 1 H, OH), 4.65 (s, 2 H, $ArCH_2$), 6.9–7.8 (m, 7 H, ArH) 8.35–8.60 (m, 1 H, ArH). HRMS m/e 249.0283 (calcd 249.0282). Anal. Calcd for $C_{12}H_{11}NO_2S_2$: C, 57.80; H, 4.45; N, 5.62. Found: C, 57.00; H, 4.52; N, 5.46.

4-(2-Pyridinyldithio)benzenemethanol (7): yield 55%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.4–1.9 (br s, 1 H, OH), 4.65 (s, 2 H, $ArCH_2$), 6.9–7.7 (m, 7 H, ArH), 8.3–8.6 (m, 1 H, ArH); HRMS m/e 249.0286 (calcd 249.0282). Anal. Calcd for $C_{12}H_{11}NO_2S_2$: C, 57.80; H, 4.45; N, 5.62. Found: C, 57.45; H, 4.76; N, 5.85.

General Procedure for the Preparation of Carbamates 12 and 17–21. Preparation of 2-(2-Pyridinyldithio)benzyl MMC-1a-carboxylate (18). A solution of 105 mg (0.42 mmol) of benzyl alcohol **5** and 0.034 mL of pyridine (0.42 mmol) in 1 mL of dry dioxane was added over a 3-min period to a stirred solution of 0.025 mL (0.211 mmol) of trichloromethyl chloroformate in 0.5 mL of dioxane. After the mixture was stirred for 15 min, a solution of MMC (70 mg, 0.211 mmol) and triethylamine (0.17 mL, 0.84 mmol) in 4 mL of dioxane was rapidly added. After 5 min, the solvents were evaporated, and a solution of the residue in CH_2Cl_2 was extracted with saturated $NaHCO_3$ and saturated

(10) Teicher, B. A.; Sartorelli, A. C. *J. Med. Chem.* **1980**, *23*, 955–960.
(11) Sasaki, H.; Mukai, E.; Hashida, M.; Kimura, T.; Sezaki, H. *Int. J. Pharmaceutics* **1983**, *15*, 49–59.
(12) (a) Kinoshita, S.; Uzu, K.; Nakana, K.; Shimizu, M.; Takahashi, T.; Matsui, M. *J. Med. Chem.* **1971**, *14*, 103–109. (b) Matsui, M.; Yamada, Y.; Uzu, K.; Hirata, T. *J. Antibiot.* **1968**, *21*, 189–198.

NaCl and dried ($MgSO_4$). The product was purified by flash chromatography on a 2 × 20 cm SiO_2 column by first separating nonpolar material with 30% ethyl acetate in petroleum ether (300 mL) and then eluting the carbamate with 5% methanol in chloroform. The product, **18**, was obtained as an amorphous blue solid which was dissolved in 3 mL of CH_2Cl_2 and added dropwise to 30 mL of petroleum ether. A fine blue solid was obtained (80 mg, 63%): mp 96–98 °C; ^1H NMR (py-d_5) δ 1.90 (s, 3 H, CH_3), 3.07 (s, 3 H, OCH_3), 3.4–3.55 (m, 2 H), 3.8–4.05 (m, 3 H), 4.6–4.9 (m, 4 H), 5.35–5.70 (m, 4 H), 6.8–7.7 (m, 7 H, ArH), 8.35 (d, J = 6.3 Hz, 1 H, ArH); IR (KBr) ν 3400, 1692, 1600, 1552 cm^{-1}; UV/vis (CH_3OH) λ_{max} 365 nm (log ϵ 4.32); HRMS m/e 610.1467 (calcd 610.1431). Anal. Calcd for $C_{28}H_{27}N_5O_7S_2 \cdot H_2O \cdot {}^1/_2EtOAc$: C, 53.60; H, 4.96; N, 10.43. Found: C, 53.76; H, 4.76; N, 10.00.

4-Nitrophenylcarbamic acid [4-[(3-nitrophenyl)dithio]-phenyl]methyl ester (12): yield 41%; yellow solid; mp 158–160 °C; ^1H NMR ($CDCl_3$) δ 1.60 (s, 1 H, NH), 5.25 (s, 2 H, CH_2OH), 7.2–8.4 (m, 12 H, ArH); UV/vis λ_{max} 313 nm (log ϵ 4.18); HRMS m/e 457.0408 (calcd 457.0402).

Benzyl MMC-1a-carboxylate (17): yield 55% blue powder; mp 100–101 °C (lit.[11] mp 102–103 °C).

3-(2-Pyridinyldithio)benzyl MMC-1a-carboxylate (19): yield 98%; blue powder; mp 90–92 °C; ^1H NMR (py-d_5) δ 1.90 (s, 3 H, CH_3), 3.05 (s, 3 H, OCH_3), 3.3–3.5 (m, 2 H), 3.7 (m, 2 H), 3.9–4.0 (m, 2 H), 4.5–4.8 (m, 4 H), 5.0–5.1 (m, 2 H), 5.50 (dd, J = 5.0, 6.5 Hz, 1 H), 6.8–7.7 (m, 7 H, ArH), 8.3–8.4 (m, 1 H, ArH); IR (KBr) ν 3400, 2920, 1690, 1550 cm^{-1}; UV/vis (CH_3OH) λ_{max} 357 nm (log ϵ 4.31); HRMS m/e 610.1414 (calcd 610.1431). Anal. Calcd for $C_{28}H_{27}N_5O_7S_2 \cdot H_2O \cdot {}^1/_2EtOAc$: C, 53.60; H, 4.96; N, 10.43. Found: C, 53.88; H, 4.66; N, 10.31.

4-(2-Pyridinyldithio)benzyl MMC-1a-carboxylate (20): yield 92%; blue powder; mp 99 °C dec; ^1H NMR (py-d_5) δ 1.95 (s, 3 H, CH_3), 3.15 (s, 3 H, OCH_3), 3.4–4.2 (m, 6 H), 4.6–5.0 (m, 4 H), 5.20 (s, 2 H, $ArCH_2$), 5.6 (dd, J = 4.6, 6.3 Hz, 1 H), 6.9–7.8 (m, 7 H, ArH), 8.35–8.5 (m, 1 H, ArH); IR (KBr) ν 3400, 2929, 1690, 1552 cm^{-1}; UV/vis (CH_3OH) λ_{max} 356 nm (log ϵ 4.31); HRMS m/e 610.1382 (calcd 610.1431). Anal. Calcd for $C_{28}H_{27}N_5O_7S_2 \cdot {}^1/_2H_2O \cdot {}^1/_2EtOAc$: C, 54.36; H, 4.88; N, 10.57. Found: C, 54.20; H, 4.55; N, 10.68.

4-[(3-Nitrophenyl)dithio]benzyl MMC-1a-carboxylate (21): prepared from MMC and chloroformate **8** according to the previously described methods; yield 70%; blue powder; mp 97–98 °C; ^1H NMR (py-d_5) δ 1.85 (s, 3 H, CH_3), 3.03 (s, 3 H, OCH_3), 3.35–3.42 (m, 2 H), 3.65 (d, J = 4.5 Hz, 1 H), 3.85–3.95 (m, 2 H), 4.55 (d, J = 13.2 Hz, 1 H), 4.70 (t, J = 8.0 Hz, 1 H), 4.75–4.85 (m, 3 H), 5.0–5.1 (m, 2 H, $ArCH_2$), 5.5 (dd, J = 4.5, 6.1 Hz, 1 H), 7.2–7.9 (m, 7 H), 8.30 (m, 1 H, ArH); IR (KBr) ν 3400, 2920, 1690, 1600, 1560, 1350 cm^{-1}; UV/vis (CH_3OH) λ_{max} (log ϵ) 356 (4.32), 242 (4.50); HRMS m/e 654.1322 (calcd 654.1328).

Reaction of 12 with Dithiothreitol. To a 4:1 CH_3OH/H_2O solution at room temperature containing **12** (0.08 mM) in tris(hydroxymethyl)aminomethane buffer (17 mM) and ethylenediamine tetraacetic acid (0.08 mM) at a final pH of 6.0, 7.2, or 8.0, was added excess dithiothreitol. p-Nitroaniline release (λ_{max} 371 nm, log ϵ 4.34) was measured by UV/vis spectroscopy and confirmed by HPLC analysis (Waters-μ-Bondapak column, 20% CH_3OH in 10 mM CH_3COOH (pH 4), monitored at 254 nm).

Reaction of MMC Benzyl Carbamate Disulfides 18–20 with Dithiothreitol. To a 4:1 CH_3OH/H_2O solution at 30 °C containing **18**, **19**, or **20** (0.81 mM) in tris(hydroxymethyl)-aminomethane buffer (17 mM) and ethylenediamine tetraacetic acid (0.08 mM) at pH 7.2 was added dithiothreitol (final concentration 2 mM). The release of MMC was measured by HPLC using a 10-cm Whatman Partasil 5 ODS-3 reverse phase (C-18) column and the following gradient system: 30% CH_3OH in 0.1% acetate (pH 6) to 95% CH_3OH in 6 min; continued for 8 min; flow rate 2 mL/min; monitored at 340 nm.

Cytotoxicity Studies. In vitro experiments were done using HSB2 (human T cell leukemia) and Namalwa (Burkitts lymphoma) cells obtained from American Type Culture Collection (Rockville, MD). The cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37 °C in 5% CO_2 humid atmosphere. Serial dilutions (in triplicate) of drugs were made in phosphate buffered saline (pH 7.2) and 100 μL of each dilution was added to 96-well microtiter plates. To each well was added a suspension of 10^5 cells in 100 μL of phosphate buffered saline (pH 7.2). The cells were incubated for 1 h at 37 °C, washed twice, and resuspended in 200 μL of culture medium. After incubation at 37 °C for 19 h, 50 μL of 1 μCi [6-^3H]thymidine (New England Nuclear, 15 Ci/mmol) was added to each well, and incubation was continued for 4 h at 37 °C. The cells were transferred to Millititer sv plates (Millipore) and precipitated with 25% cold trichloroacetic acid (TCA). The precipitates were washed 10 times with 5% cold TCA. Filters were dried, punched, and counted in Econofluor liquid scintillation fluid (New England Nuclear). All counts were corrected by subtraction of background counts. Cytotoxicity was expressed as the percent of [^3H]thymidine incorporated into DNA relative to untreated controls.

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Supplementary Material Available: ^1H NMR spectra for **4**, **12**, and **18–21** (3 pages). Ordering information is given on any current masthead page.

Nickel(0)-Catalyzed Cycloaddition of Silyl Diynes with Carbon Dioxide to Silyl Bicyclic α-Pyrones

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Mono- and bicyclic α-pyrones are useful intermediates in organic synthesis.[1] Furthermore monocyclic and annulated α-pyrone ring systems are found in several biologically active natural products.[1a-c] Thus it is interesting to develop a convenient synthetic method for functionalized α-pyrones. A silyl-substituted α-pyrone is attractive because the silyl substituent attached to an sp^2-carbon atom is known to be easily converted into a variety of functional groups,[2] i.e., halogen, acyl, and hydroxy groups[3] along with a hydrogen atom. Examples of the synthesis of silyl α-pyrones, however, are few. Formation of 3- and 5-(trimethylsilyl)-substituted 6-ethoxy-4-methyl-α-pyrones by the rhodium-catalyzed carbonylation of 1-carbethoxy-3-methyl-2-(trimethylsilyl)cyclopropene has been described.[4]

Recently we have reported the Ni(0)–trialkylphosphine complex-catalyzed one-step bicyclic α-pyrone synthesis from diynes and CO_2. A remarkable effect of the phosphine ligand on this reaction was observed: monodentate trialkylphosphine ligands such as $P(n\text{-}C_8H_{17})_3$ and tricyclohexylphosphine (PCy_3) are effective for the reaction of terminally dialkyl-substituted diynes[5] while the reaction of unsubstituted diynes requires the use of a functionalized

(1) (a) Staunton, J. In *Comprehensive Organic Chemistry*; Sammes, P. G., Ed.; Pergamon: Oxford, England, 1979; Vol. 4, p 629. (b) Ellis, G. P. In *Comprehensive Heterocyclic Chemistry*; Boulton, A. J., McKillop, A., Ed.; Pergamon: Oxford, England, 1984; Vol. 3, p 675. (c) Dieter, R. K.; Fishpaugh, J. R. *J. Org. Chem.* **1983**, *48*, 4439 and references cited therein. (d) Jung, M. E.; Lowe, J. A.; Lyster, M. A.; Node, M.; Pfluger, R. W.; Brown, R. W. *Tetrahedron* **1984**, *40*, 4751.

(2) Colvin, E. W. *Silicon in Organic Synthesis*; Butterworths: London, 1981; Chapter 7.

(3) Tamao, K.; Kumada, M.; Maeda, K. *Tetrahedron Lett.* **1984**, *25*, 321.

(4) Cho, S. H.; Liebeskind, L. S. *J. Org. Chem.* **1987**, *52*, 2631.

(5) Tsuda, T.; Morikawa, S.; Saegusa, T. *J. Org. Chem.* **1988**, *53*, 3140. See also: Tsuda, T.; Sumiya, R.; Saegusa, T. *Synth. Commun.* **1987**, *17*, 147.

0022-3263/90/1955-2978$02.50/0