(7) R. J. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, **93**, 2987 (1971).
(8) S. Penco, F. Angelucci, A. Vigevani, E. Arlandini, and F. Arcamone, *J. Antibiot.*, **30**, 764 (1977).
(9) S. T. Crooke, V. H. Duvernay, L. Galvan, and A. W. Prestayko, *Mol. Pharmacol*, **14**, 290 (1978).
(10) (a) S. Hori, M. Shirai, S. Hirano, T. Oki, T. Inui, S. Tsukagoshi, M. Ishizuka, T. Takeuchi, and H. Umezawa, *Gann*, **68**, 685 (1977); (b) T. Oki, *J. Antibiot.*, **30**, 570 (1977).
(11) R. K. Johnson, A. A. Ovejera, and A. Goldin, *Cancer Treat. Rep.*, **60**, 99 (1976).
(12) (a) G. Zbinden and E. Brändle, *Cancer Chemother. Rep., Part 1*, **59**, 707 (1975); (b) G. Zbinden, E. Bachman and C. Holderegger, *Antibiot. Chemother.*, **23**, 255 (1978).
(13) G. Zbinden, M. Pfister, and C. Holderegger, *Toxicol. Lett.*, **1**, 267 (1978).
(14) F. P. Mettler, D. E. Young, and J. M. Ward, *Cancer Res.*, **37**, 2705 (1977).
(15) W. F. Benedict, M. S. Baker, L. Haroun, E. Choi, and B. N. Ames, *Cancer Res.*, **37**, 2209 (1977).
(16) (a) H. Marquardt, F. S. Philips, and S. S. Sternberg, *Cancer Res.*, **36**, 2065 (1976); (b) H. Marquardt and H. Marquardt, *Cancer*, **40**, 1930 (1977).
(17) B. N. Ames, W. E. Durston, E. Yamasaki, and F. D. Lee, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2281 (1973).
(18) K. Umezawa, M. Sawamura, T. Matsushima, and T. Sugimura, *Cancer Res.*, **38**, 1782 (1978).
(19) N. R. Bachur, *Biochem. Pharmacol.*, **suppl. 2**, 207 (1974).

A Phenazine Analogue of Actinomycin D

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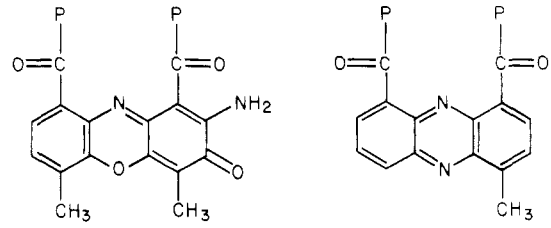
An analogue of actinomycin D (1), in which the phenoxazone chromophore has been replaced by a phenazine, has been synthesized and characterized. Although this compound (2) lacks the 2-amino group and does not possess the quinoid structure of 1, it does bind to DNA, but less tightly than either 1 or the 2-deamino derivative of 1. NMR and CD spectra indicate that the peptide conformations in 2 are approximately as in 1; there was no apparent asymmetry of the two peptide rings. Compound 2 inhibited nucleic acid synthesis in L1210 cell cultures more effectively than does 2-deaminoactinomycin D, but about one-tenth as well as does actinomycin D.

The potent activity of the antibiotic actinomycin D (1)



1 2

P = -Thr-D-Val-Pro-Sar-MeVal—

against several tumors[1-3] is well known; unfortunately the high toxicity of 1 limits its usefulness. Because 1 strongly inhibits DNA-dependent RNA synthesis[4] and also, to a lesser extent, DNA synthesis, its interaction with DNA has been extensively studied, and details of the mechanism of binding to DNA have been elaborated.[5-7] Sobell[6] has argued that hydrogen bonds linking groups on the peptide and sites on the guanine base are responsible for the G-C specificity of 1. Although it has been assumed that the antitumor activity of 1 is due to its inhibition of growth following the intercalative binding to DNA, it is quite possible that the distortions in helical DNA resulting from intercalactive binding are not completely responsible for observed biological effects. For example, Bachur[8] proposes that an intermediate free-radical metabolite may be the active form of 1 that causes DNA damage and cell death.

Although a planar chromophore is a prerequisite for intercalative binding, if the primary role of the chromophore is simply to align the peptide portion of the molecule relative to the DNA helix, chromophores other than that occurring naturally might function as well or better in this role. We have reasoned that an actinomycin analogue in which the pentapeptide lactone rings of 1 have been condensed with an approximately isosteric chromophore should be of value in investigating various possible mechanisms of action. No such analogues with variations of the chromophore nucleus have been reported in the literature, although a number of actinomycins with various substituents in the 2,[9,10] in the 4 and 6, and in the 7 positions of the phenoxazinone nucleus have been prepared.[10] Since phenazines exhibit antibiotic properties, presumably due to intercalative binding to DNA,[11] we selected 2, in which a phenazine residue replaces the phenoxazone system, for synthesis and study to determine the effect of this chromophore substitution on DNA binding and nucleic acid synthesis in cell culture. Some changes in the substitutions on the chromophore of 2 are dictated by synthetic accessibility. However, we have found[9] that loss of the 2-amino group in 1 does not prevent binding to DNA and does not destroy (but does decrease) the in vivo antitumor activity and ability to inhibit nucleic acid synthesis in tissue culture. Additionally, the chromophore 2 lacks the quinoid structure of 1. Unless it is metabolically activated to such a structure, 2 could not participate in the type of oxygen uptake catalysis proposed by Bachur[8] as the biologically important activity of 1. Thus, the more drastic changes in 2 are an important probe of the role of the chromophore in DNA binding and biological activity.

Synthesis. The synthesis of 2 was considered as three efforts: (1) the preparation of the 1,9-dicarboxyphenazine 5, (2) the synthesis of blocked pentapeptide lactone 13, and (3) the condensation of 5 and deblocked 13 to give the final product 2.

At the time this work was initiated, the literature contained no reference to the synthesis of 1,9-dicarboxyphenazine (4) or its derivatives. Breitmaier and Hollstein[12] reported the preparation of 4 and its methyl ester shortly thereafter.

The two 1,9-dicarboxyphenazines 4 and 5 were synthesized by the route shown in Scheme I. Condensation of the anthranilic acid with 2-bromo-3-nitrobenzoic acid[13] was carried out by the method of Flood,[14] using 1-propanol

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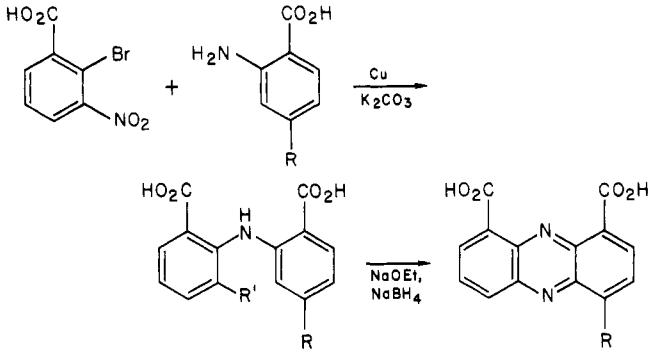
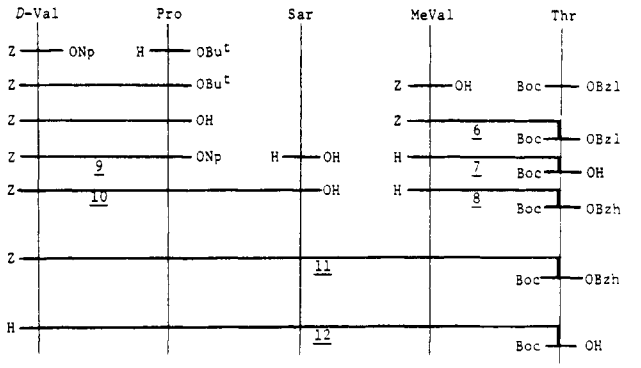
Scheme I



3a, R = CH_3; R′ = NO_2
3b, R = CH_3; R′ = NH_2
3c, R = H; R′ = NO_2

4, R = H
5, R = CH_3

Scheme II



12 → Boc-Thr-D-Val-Pro-Sar-MeVal

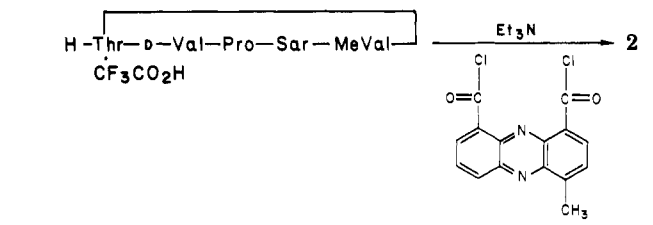
13

Boc = $-C(=O)OC(CH_3)_3$; Z = $-OC(=O)OCH_2C_6H_5$; Np = $-4-NO_2C_6H_4-$; Bzh = $-CH(C_6H_5)_2$; Bzl = $-CH_2C_6H_5$

instead of ethanol, to increase reaction temperature. Initially, we investigated the oxidative cyclization of amine **3b**, the product of catalytic reduction of **3a**. Heating in dry nitrobenzene (180 °C) for 1 h gave a product that was tentatively identified as a dibenzodiazepinone; analogous cyclization of 4,6-diaminodiphenyl-2,2′-dicarboxylate was reported by Bentley.[15] Similar heating of the dimethyl ester of **3b** in nitrobenzene caused decarboxylation, as also experienced by Bentley.[15] Reductive cyclization[12,14] of **3c** and **3a** yielded the phenazines **4** and **5**, respectively, which were characterized spectroscopically. Mass spectral patterns shown by **4** and **5** were nearly identical, including weak $M^+ + 2$ peaks; Flood[14] also reported $M^+ + 2$ peaks in the mass spectra of 1-carboxylic and 1,6-dicarboxylic phenazines. The patterns in the mass spectra of the trimethylsilylated derivatives of **4** and **5** were also almost identical; both showed M^+ peaks. The IR spectra of both **4** and **5** showed two carbonyl peaks—at 1700 and 1740 cm^{-1}—presumably because one of the two carboxy groups is involved in hydrogen bonding with the phenazine nitrogen.

Originally, we proposed the synthesis of linear pentapeptide **12** by successive condensation of amino acids on the N terminal of the growing peptide chain beginning with **7**, as was accomplished in the synthesis of the analogous didemethyl analogue.[16] However, tripeptide H-Sar-MeVal-Thr(Boc)-OH appeared to be unstable and yields were low; therefore, we investigated other approaches. Scheme II shows the sequence of steps used in the successful

Scheme III



preparation of **12** and the cyclic pentapeptide **13**. Z-D-Val-Pro-OH was prepared according to the procedure of Meienhofer[17] but was immediately converted to the *p*-nitrophenyl ester **9**, which was condensed with sarcosine to give **10** in 55–75% yields. Although **10** was also obtained by reaction of **9** with sarcosine *tert*-butyl ester followed by hydrolysis, the production of H-Sar-O-*t*-Bu by hydrogenolysis of Z-Sar-*O*-*t*-Bu was not always reproducible; in some cases an odor of ammonia was detected after hydrogenolysis, and little H-Sar-*O*-*t*-Bu was obtained. Meienhofer[17] reported the preparation of **7** by reaction of Boc-L-Thr-OH with the mixed anhydride of Z-MeVal-OH; in the present work, synthesis of **7** was carried out according to our previously reported method,[16] followed by conversion of **7** to **8** by the method of Aboderin.[18] Condensation of **8** with **10**, using dicyclohexylcarbodiimide (DCC) in acetone, afforded **11** in 55–80% purified yields. Hydrogenolysis to **12** was followed by conversion to the *p*-nitrophenyl ester and cyclization[16] to give **13**. Yields in the final step were low, either via the –Np ester or by use of hydroxysuccinimide/DCC. No cyclic decapeptide was detected.

Gel filtration on Sephadex LH-20 was most effective in purifying a number of these peptides. The cyclic peptide **13** was characterized by its mass spectrum (*m/e* M^+ 581) and its TLC behavior which was very similar to that of the homologous didemethyl cyclic lactone.[16] Synthetic actinomycin D prepared from **13** [deblocking, condensing with 2-nitro-3-(benzyloxy)-4-methylbenzoyl chloride, reducing, and oxidizing under controlled conditions] was found to be essentially identical with natural **1** by comparing TLC, NMR spectra, and abilities to inhibit nucleic acid syntheses in L1210 cell cultures.

The coupling of **5** with deblocked **13** was effected via the acid chloride of **5**, indicated in Scheme III. Preliminary experiments indicated that when DCC condensation was attempted, the product was largely the acylurea; attempts to prepare the hydroxysuccinimide ester of **5** yielded only a monoester. The diacid chloride was generated in situ by the use of $SOCl_2$–DMF[19] and immediately was condensed with deblocked **13** in the presence of Et_3N. The formation of product was best followed by reversed phase TLC. The product was purified by gel filtration, thick-layer chromatography, and crystallization.

Results and Conclusions

Because of the role of peptide–DNA interaction in the Sobell model[6] for actinomycin binding, it is important to assess the effect of chromophore substitution on the peptide conformation. The CD spectrum of actinomycin at wavelengths below about 230 nm is principally due to absorption by the peptide rings[20] and is sensitive to their conformation.[21] The CD spectra of **1** and **2**, with their absorption spectra, are shown in Figure 1. In the range 200–230 nm, the CD spectra are nearly superimposable, indicating that the peptide rings are in similar conformations in the two compounds. The longer wavelength

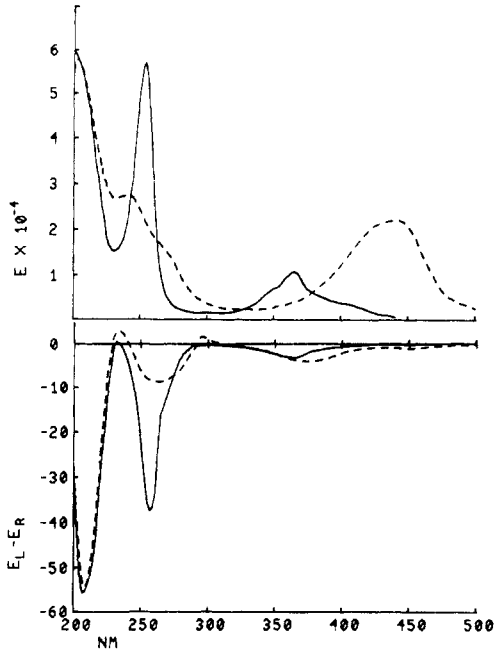


Figure 1. Absorption (top) and circular dichroism (bottom) spectra in 0.01 M phosphate buffer (pH 7) containing EDTA, 10^{-5} M; actinomycin D (---), 3×10^{-5} M; and **2** (—), 3×10^{-6} M.

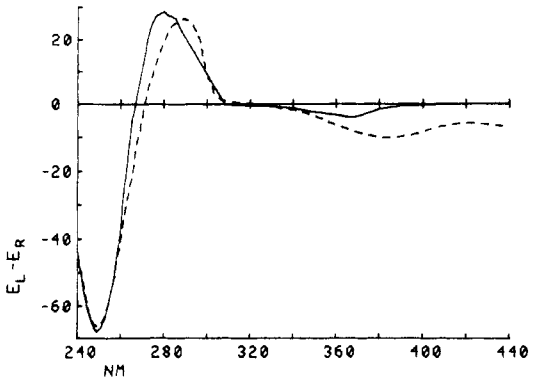


Figure 2. CD spectra of DNA–drug complexes; DNA(P)/drug ratio, 10:1. Concentrations and line types as in Figure 1.

CD bands are not comparable because of the difference in absorption spectra.

Another interesting correspondence is seen in the CD spectra (Figure 2) of mixtures (10:1) of DNA and **1** or **2**. The peaks in the range 240–300 nm are principally due to DNA, but those at about 250 nm are considerably stronger than the sum of the DNA and drug peaks. These are very similar in the two spectra despite the large difference in the spectra of the uncomplexed drugs in this region (Figure 1).

The NMR spectra of actinomycins are also sensitive to the conformation of the peptide rings. Assignments were based on similarities with the spectrum of **1** and on D_2O-exchange experiments. The spectrum in the aromatic region has been fully assigned and is consistent with the structure. The resonance assigned to valyl NH protons had not exchanged with D_2O after 50 h. No asymmetry between the two peptide rings in **2** is detectable. The resonances of the two valyl –NH protons in the NMR spectrum of **2** ($CDCl_3$ solution) appear at the same position (8.4 ppm), which is much closer to the two valyl –NH resonances in 2-deaminoactinomycin D (8.58 and 8.50 ppm) than those in **1** (8.10 and 7.94 ppm).[9] However, the resonances of the two threonyl –NH protons (again at the same position, 7.28 ppm) in **2** are very close to that of the

Table I. Effect of Actinomycin and Derivatives on the T_m of DNA and on Nucleic Acid Syntheses

compd	ΔT_m, °C[a]	inhibn of nucleic acid synth:[b] ED_{50}, μM DNA	RNA
1	7.1	0.6	0.015
2-deamino-AMD[c]	9.9	63	0.7
2	3.4	9.2	0.23

[a] ΔT_m = T_m of DNA-drug complex minus T_m of DNA. Concentration of drug, 5.2×10^{-6} M; of DNA (based on P), 5.2×10^{-5} M in 0.01 M phosphate buffer (pH 7), 10^{-5} M EDTA, and 5% Me_2SO. [b] ED_{50} is the concentration of drug necessary to reduce, by 50%, incorporation of tritiated thymidine in the DNA or tritiated uridine in the RNA of actively growing L1210 cells in growth medium containing 1% Me_2SO. [c] AMD = actinomycin D.

α-threonyl –NH in **1** (7.21 ppm); the β-threonyl –NH resonance in **1** is at 7.84 ppm. The upfield region (0–7 ppm) of the spectra of **1** and **2** are strikingly similar. These NMR spectra support the conclusion that the main features of the peptide ring conformation within each ring and between the two rings is as in **1**. The main differences are that the asymmetry observed in **1** is missing in **2** and the hydrogen bond between the valyl NH on one ring and the valyl carbonyl of the other ring is stronger in **2** than in **1**.

An increase in the thermal denaturation temperature (ΔT_m) of DNA upon complex formation with a drug indicates stabilization of the helix. We assume that the ΔT_m value (3.4 °C) of the DNA complex with **2** (Table I) reflects intercalative binding, which is generally accepted as the mode of binding of **1** (ΔT_m = 7.1 °C). These data show that **2** binds to DNA more weakly than actinomycin D or 2-deaminoactinomycin D. Compound **2** was only about one-tenth as effective as **1** in inhibiting nucleic acid syntheses in L1210 cells (Table I), but it was several times more active than its congener 2-deaminoactinomycin D. The ratios of the ED_{50} values for DNA/RNA synthesis for all three compounds are roughly comparable, those of **1** and **2** being almost identical.

The relative ΔT_m values for **1**, **2**, and 2-deaminoactinomycin D indicate that the chromophore definitely functions as more than a simple "bookmark" in the binding to DNA. Thus, although the ΔT_m values indicate weaker binding of **2** to DNA, compared to 2-deaminoactinomycin D, the more potent inhibition of DNA and RNA synthesis of **2** suggests that the phenazine analogue is possibly superior and is at least of interest for further study, including additional synthetic modification.

Experimental Section

Melting points, uncorrected, were determined on a Fisher-Johns apparatus. Magnesium sulfate was used as drying agent; evaporations were done under reduced pressure on a rotary evaporator. Gel filtrations were accomplished on two Sephadex LH-20 columns (86 × 2.5 cm; 30 × 2.5 cm) using MeOH, flow rate 100 mL/h. Thick-layer chromatography was done on 2-mm silica gel F-254 (E. Merck) plates. Thin-layer chromatography was accomplished as follows: (A) on Whatman reversed-phase KC_{18} plates with solvent system CH_3CN–0.2 N NH_4Cl (3:2); on silica gel (0.25 mm GF, Analtech) plates with solvent systems (B) H_2O; (C) $CHCl_3$–MeOH (50:1); (D) $CHCl_3$–MeOH (10:1); (E) acetone–$CHCl_3$ (2:1); (F) EtOAc–acetone (2:1); (G) $CHCl_3$–MeOH (1:1); (H) $CHCl_3$–MeOH (100:1); detection was accomplished by UV light, by iodine vapors, or ninhydrin solution.

Calf thymus DNA (Miles Laboratories) solutions were prepared and stored as described previously.[9]

Spectra were determined on the following instruments: NMR, on a Varian EM 390 or XL-100-15 spectrometer equipped with Fourier transform; UV-visible and thermal denaturation curves,

on a Perkin-Elmer Model 575 spectrophotometer equipped with digital temperature controller, temperature programmer, auto 5-cell programmer and thermoelectric cell holders; CD, on a Durrum-Jasco ORD/UV spectrophotometer equipped with a Sproul Scientific SS-20 CD modification; MS, on a LKB 9000 GC–MS interfaced with a PDP12 computer.

The organization and analysis of the data base associated with this investigation were carried out using the PROPHET system, a unique national computer resource sponsored by the NIH. Information about PROPHET, including how to apply for access, can be obtained from the Director, Chemical/Biological Information-Handling Program, Division of Research Resources, National Institutes of Health, Bethesda, Md. 20014.

Thermal denaturation and L1210 nucleic acid synthesis inhibition assay were performed as described previously.[9]

2,2'-Dicarboxy-5'-methyl-6-nitrodiphenylamine (3a). Copper (electrolytic dust, Fisher Scientific), 5 mg, and K_2CO_3, 90 mg (0.65 mmol), were added to a solution of 100 mg (0.41 mmol) of 2-bromo-3-nitrobenzoic acid[13] and 62 mg (0.41 mmol) of 2-amino-4-methylbenzoic acid (Aldrich) in 1 mL of propanol, and the mixture was heated under reflux for 2 h. The bright-orange solid in the hot reaction mixture was collected on a filter and dissolved in water. After removal of the solid copper, the aqueous solution was acidified with concentrated HCl, yielding a voluminous bright-yellow solid, which was collected and washed with water: yield 108 mg (84%); mp 276–278 °C dec; UV λ_{max} (MeOH) 218 mm (ϵ 27 700), 269 (12 000), 332 (8950); TLC R_f (solvent system A) 0.92; NMR (Me_2SO-d_6) δ 2.17 (s, 3, CH_3), 6.53 (s, 1), 6.73 (d, 1), 7.28 (t, 1), 7.8 (d, 1), 8.13 (d, 2). Anal. ($C_{15}H_{12}N_2O_6$) C, H; N: calcd, 8.86; found, 8.40.

2,2'-Dicarboxy-6-nitrodiphenylamine (3c). Using the method described for the synthesis of **3a**, anthranilic acid was condensed with 2-bromo-3-nitrobenzoic acid to produce **3c**, a yellow solid, in 80% yield: mp 268–269 °C dec; NMR (Me_2SO-d_6) δ 6.7 (d, 1), 6.9 (t, 1), 7.2 (m, 2), 7.88 (m, 1), 8.12 (d, 2).

4-Methyl-1,9-dicarboxyphenazine (5). Finely powdered **3a** (440 mg, 1.39 mmol) was added with stirring to a solution of 1.12 g (49 mmol) of sodium in 15 mL of ethanol heated to 80 °C under nitrogen, producing a very dark solution. Sodium borohydride, 263 mg (6.9 mmol), was added and heating under reflux continued for 18 h. The cooled reaction mixture was dissolved in H_2O, ethanol was removed by distillation iv, and the aqueous solution was acidified with concentrated HCl, precipitating 380 mg of brown-green solid. Several recrystallizations from DMF and from methyl cellosolve yielded 79 mg (20%) of green-gold solid **5**, with a decomposition point above 250 °C: TLC R_f (solvent system A) 0.87; IR 1700, 1740 cm^{-1}; UV λ_{max} (95% EtOH) 207 nm (ϵ 37 800), 254 (67 500), 353 (sh)(10 900), 369 (19 100); NMR (CF_3CO_2D) δ 3.38 (s, 3, CH_3), 8.43 (m, 2), 9.1 (m, 1), 9.4 (m, 2); MS m/e 284 ($M^+ + 2$), 266 ($M^+ + 2 - H_2O$), 238 ($M^+ - CO_2$), 220 ($M^+ - CO_2 - H_2O$); MS [(Me_3Si)$_2$ derivative] m/e 426 (M^+), 411, 321, 263. Anal. ($C_{15}H_{10}N_2O_4 \cdot {}^1/_8H_2O$) C, H, N.

1,9-Dicarboxyphenazine (4). By a procedure similar to that used in preparing **5**, **3c** was converted to **4** in 50% crude yield; purification involved repeated precipitation from DMF and from methyl cellosolve solutions by addition of H_2O. Purified yield was 19%: mp >350 °C; TLC R_f (solvent system A) 0.92; IR 1700, 1740 cm^{-1}; UV λ_{max} (95% EtOH) 205 (ϵ 30 600), 250 (59 100), 352 (sh)(10 500), 370 (17 200); NMR (CF_3CO_2H) δ 8.9 (t, 2), 9.4 (d, 2), 9.8 (d, 2); MS m/e 270 ($M^+ + 2$), 252, 224, 206; MS [(Me_3Si)$_2$ derivative] m/e 412 (M^+), 397, 312, 249. Anal. ($C_{14}H_8N_2O_4 \cdot 0.5H_2O$) C, H, N.

***O*-(L-*N*-Methylvalyl)-*N*-(*tert*-butyloxycarbonyl)threonine (7).** The procedure used was that described for the preparation of the analogous *O*-(L-valyl)-*N*-Boc-L-Thr-OH.[16] DCC-mediated condensation of Z-*N*-MeVal-OH and Boc-Thr-OBzl afforded **6** as a syrup, which was subjected to hydrogenolysis. The ester **7** was isolated as a white crystalline solid after recrystallization from H_2O in 35–50% yields (based on Boc-L-Thr-OBzl): mp 199–204 °C; TLC R_f (solvent system B) 0.5.

***O*-(L-*N*-Methylvalyl)-*N*-(*tert*-butyloxycarbonyl)-L-threonine Benzhydryl Ester (8).** Using the method of Aboderin,[18] the tosylate salt of **7** was treated with diphenyldiazomethane.[22] Purification of crude syrupy **8** on a dry column (Woelm silica gel, activity III, 25 × 3 cm) using EtOAc–hexane (1:10) solvent yielded a light yellow syrup that slowly crystallized. Recrystallization from EtOAc–hexane (1:10) gave a white solid: mp 81–83 °C; total yield, including second and third crops, 83%; TLC R_f (solvent system C) 0.3. Anal. ($C_{28}H_{38}N_2O_6$) C, H, N.

(Benzyloxycarbonyl)-D-valyl-L-proline *p*-Nitrophenyl Ester (9). Z-D-Val-Pro-*O*-*t*-Bu,[17] 1.5 g (3.72 mmol), was mixed with 3 mL of CF_3CO_2H; after standing for 4 h, the solution was concentrated iv, and the residual syrup was dissolved in $CHCl_3$, washed successively with an aqueous solution of Et_3N, H_2O, 1 M citric acid solution and saturated NaCl solution. The organic layer was dried and concentrated to give a quantitative yield of Z-D-Val-Pro-OH, as a "foamy" solid: TLC (solvent system C) R_f 0.2. This blocked dipeptide (1.04 g, 3 mmol) was converted to the *p*-nitrophenyl ester.[23,24] The crude syrupy product, 1.87 g, was purified on a Sephadex column (30 cm) to give 1.23 g (87%) of **9**, a colorless syrup: TLC R_f (solvent system C) 0.5, R_f (solvent system D) 0.9.

(Benzyloxycarbonyl)-D-valyl-L-prolylsarcosine (10). A mixture of 998 mg (2.14 mmol) of **9**, 196 mg (2.2 mmol) of sarcosine, and 0.32 mL (2.3 mmol) of Et_3N in 3 mL of DMF and 0.3 mL of H_2O was stirred at room temperature for 48 h. It was concentrated iv to a yellow syrup, which was dissolved in EtOAc; the solution was washed twice with 1 M citric acid solution and twice with saturated NaCl solution, dried, and concentrated to give 1.17 g of yellow syrup, which was purified on a Sephadex column (86 cm). After 225 mL had been collected, 2-mL fractions were collected; fractions 41–53 contained 454 mg of colorless syrup (**10**), showing only a trace of impurity on TLC. Fractions 31–40 (44 mg) and 53–65 (232 mg) were combined and repurified on the Sephadex column, yielding 32 mg of **10**: total yield 486 mg (54%); TLC R_f (solvent system D) 0.3.

***O*-[(Benzyloxycarbonyl)-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl]-*N*-(*tert*-butyloxycarbonyl)-L-threonine Benzhydryl Ester (11).** To a solution of 463 mg (1.1 mmol) of **10** and 407 mg (0.82 mmol) of **8** in 1 mL of dry acetone cooled in an ice–H_2O bath was added 206 mg (1.0 mmol) of DCC. The mixture was stirred in the ice bath for 30 min and then at room temperature for 72 h. The mixture was worked up[16] as was **6**, yielding 905 mg of syrup, which was purified on a Sephadex column (86 cm). After collection of 220 mL, 2-mL fractions were collected; fractions 11–19 contained 307 mg of **11**; fractions 20–35 containing 389 mg of slightly impure material were repurified on the same column to give 106 mg of **11**: total yield 57%; TLC R_f (solvent system H, developed twice) 0.2.

***O*-(D-Valyl-L-prolylsarcosyl-L-*N*-methylvalyl)-*N*-(*tert*-butyloxycarbonyl)-L-threonine (12).** Hydrogenolysis of 220 mg (0.24 mmol) of **11** in 50 mL of ethanol over 200 mg of Pd black yielded, after removal of catalyst and solvent and trituration of the residue with hexane to remove diphenylmethane, 127 mg (86%) of white solid: mp 107–109 °C; TLC R_f (solvent system B, developed twice) 0.1.

***N*-(*tert*-Butyloxycarbonyl)-L-threonyl-D-valyl-L-prolylsarcosyl-L-methylvaline-(*threonine hydroxyl*) Lactone (13). Method A.** The turbid solution of 155 mg (0.26 mmol) of **12** and 49 mg (0.26 mmol) of *p*-toluenesulfonic acid monohydrate in 8.2 mL of H_2O was lyophilized, giving a white solid that was then mixed with 109 mg (0.34 mmol) of bis(*p*-nitrophenyl) sulfite[23] and dissolved in a solution of 0.15 mL (1.85 mmol) of distilled pyridine and 10 mL of EtOAc. The solution was heated at 50–55 °C for 2 h, concentrated to a syrup, and triturated with dry ether. The residual syrup, after removal of ether, was dissolved in 5 mL of DMF, a drop of glacial HOAc was added, and the solution was added dropwise with stirring, over a 1-h period, to 400 mL of dry pyridine at 50–55 °C. The solution was stirred at this temperature for 2.5 h, the pyridine was removed by distillation, and the residual syrup was dissolved in EtOAc. The solution was washed successively with 1 M citric acid solution, dilute $NaHCO_3$ solution, and saturated NaCl solution, dried, and concentrated to give 145 mg of a yellow syrup which was purified twice on a Sephadex column (86 cm); after 235 mL of eluate had been collected, 2-mL fractions were collected; from fractions 10–14 was obtained a white residue which, after recrystallization from EtOAc–hexane, yielded 22 mg (14%) of **13**: mp 210–214 °C; TLC (solvent system E) R_f 0.7; MS 581 (M^+).

Method B.[25] To a solution of **12**, 634 mg (1.06 mmol), and 377 mg (3.28 mmol) of *N*-hydroxysuccinimide in 1.07 L of dry THF, cooled in an ice–H_2O bath, was added with stirring a solution of

DCC, 684 mg (3.28 mmol), in 10 mL of THF. After the solution was stirred for 3 h and ice-bath cooled, it was allowed to stand for 3 days at room temperature, concentrated, and worked up as in method A to secure 165 mg (27%) of **13**, mp 214–216 °C.

4-Methyl-1,9-bis[carbonyl-L-threonyl-D-valyl-L-propylsarcosyl-L-*N*-methylvaline-(*threonine hydroxyl*) Lactone] Phenazine (2). All glassware was carefully dried and reactions were protected against moisture. A solution of 90 mg (0.16 mmol) of blocked pentapeptide **13** in 0.5 mL of trifluoroacetic acid was allowed to stand for 30 min and then concentrated, leaving a pale amber residue which was dissolved in 0.2 mL of dry DMF and 75 μL (0.55 mmol) of triethylamine.

The acid chloride of **5** was prepared by mixing 14.6 mg (0.052 mmol) of **5** (dried iv) with 0.3 mL of dry DMF and 12 μL (0.17 mmol) of $SOCl_2$, freshly distilled. After the mixture had been stirred for 5 min, solution occurred, followed immediately by separation of a yellow solid. The mixture was heated at 40 °C for 30 min and concentrated, leaving a yellow-green solid residue, which was slurried in 0.2 mL of dry DMF and added to the DMF solution of peptide and triethylamine.

The mixture was stirred at room temperature and the course of reaction followed by TLC [on Whatman reversed phase KC_{18} plates (solvent system A)]. After 5 h, the mixture showed two yellow spots, one at R_f 0.9, corresponding to **5**, and one at R_f 0.4, which was desired product **2**. After 6 days, some **5** was still seen on TLC. Precipitated Et_3N·HCl was removed by filtration and washed with a small volume of DMF; addition of H_2O to the filtrate caused separation of a small amount of **5**, which was removed by filtration. Concentration of the filtrate left 182 mg of yellow oil: TLC R_f (solvent system F) 0.5, 0.1, R_f (solvent system G) 0.9, 0.6; white fluorescence at origin in both systems. This was purified on the Sephadex column (86 cm). After 216 mL of colorless eluate had been collected, 2-mL fractions were collected and TLC of yellow fractions examined. Fractions 6–23 showed a major yellow spot, R_f 0.5 (solvent system F), with a minor brown spot at the origin. From fractions 40–46 was obtained 33 mg (yellow oil mixed with whitish solid), showing two spots on TLC (solvent system G), R_f 0.9, 0.6, with streaking; fractions 104–128 yielded 2 mg of yellow solid, identical on reversed phase TLC with **5**.

Concentration of fractions 6–23 yielded 34 mg (54% crude yield) of yellow solid, which was partially purified by several recrystallizations from EtOAc and from acetone, and finally by thick-layer chromatography using EtOAc–acetone (2:1); the yellow band at R_f 0.5 was cut out and extracted with acetone, yielding 19 mg (30%) of **2**: bright yellow crystalline solid; mp 243.5–245 °C; TLC R_f (solvent system F) 0.5, R_f (solvent system A) 0.38; UV λ_{max} (0.01 M phosphate buffer, pH 7) 197 nm (ε 64 300), 254 (57 000), 350 (sh)(7330), 365 (11 300). Anal. ($C_{61}H_{84}N_{12}O_{14}$·H_2O) C, H, N.

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References and Notes

(1) S. Farber, *J. Am. Med. Assoc.*, **198**, 826 (1966).
(2) J. L. Lewis, Jr., *Cancer*, **30**, 1517 (1972).
(3) E. Frei III, *Cancer Chemother. Rep.*, **58**, 49 (1974).
(4) E. Reich, *Cancer Res.*, **23**, 1428 (1963).
(5) W. Müller and D. M. Crothers, *J. Mol. Biol*, **35**, 251 (1968).
(6) H. M. Sobell and S. C. Jain, *J. Mol. Biol.*, **68**, 21 (1972).
(7) M. Waring, *J. Mol. Biol*, **54**, 247 (1970).
(8) N. R. Bachur, M. V. Gee, and S. L. Gordon, *Proc. Am. Assoc. Cancer Res.*, **19**, 75 (1978).
(9) C. W. Mosher, K. F. Kuhlmann, D. G. Kleid, and D. W. Henry, *J. Med. Chem.*, **20**, 1055 (1977).
(10) J. Meienhofer and E. Atherton in "Structure–Activity Relationships among the Semisynthetic Antibiotics", Academic Press, New York and San Francisco, 1977, pp 427–529.
(11) U. Hollstein and R. J. Van Gemert, Jr., *Biochemistry*, **10**, 497 (1971); U. Hollstein and P. L. Butler, *ibid.*, **11**, 1345 (1972).
(12) E. Breitmaier and U. Hollstein, *J Org. Chem.*, **41**, 2104 (1976).
(13) (a) F. C. Whitmore, P. J. Culhane and H. T. Neher, "Organic Syntheses", Collect. Vol. I, Wiley, New York and London, 1964, p 56. (b) P. J. Culhane, *ibid.*, p 125;
(14) M. E. Flood, R. B. Herbert, and F. G. Holliman, *J. Chem. Soc., Perkin Trans. 1*, 622 (1972).
(15) R. K. Bentley and F. G. Holliman, *J. Chem. Soc. C*, 2447 (1970).
(16) C. W. Mosher and L. Goodman, *J. Org. Chem.*, **37**, 2928 (1972).
(17) J. Meienhofer, *J. Am. Chem. Soc.*, **92**, 3771 (1970).
(18) A. A. Aboderin, G. R. Delpierre, and J. S. Fruton, *J. Am. Chem. Soc.*, **87**, 5469 (1965).
(19) H. H. Bosshard, R. Mory, M. Schmid, and Hch. Zollinger, *Helv. Chim. Acta*, **42**, 1653 (1959).
(20) S. N. Timasheff, H. Susi, R. Townend, L. Stevens, M. J. Gorbunoff, and T. F. Kumosinski, in "Conformation of Biopolymers", G. N. Ramachandran, Ed., Academic Press, New York, 1967.
(21) F. Ascoli, P. DeSantis, M. Lener, and M. Savino, *Biopolymers*, **11**, 1173 (1972).
(22) J. B. Miller, *J. Org. Chem.*, **24**, 560 (1959).
(23) B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, **43**, 1763 (1960).
(24) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 624 (1957).
(25) H. Kinoshita and H. Kotake, *Bull. Chem. Soc. Jpn.*, **50**, 280 (1977).

7-(Aminoethyl) Ether and Thioether of Daunomycinone[1]

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One-step treatment of daunomycinone with excess 2-aminoethanethiol and 2-aminoethanol in trifluoroacetic acid afforded at C-7 the thioether (77% yield) and ether (30% after recycling), respectively. Stereoselectivity for the natural 7*S* over the 7*R* configuration was greater for the ether (97:3) than for the thioether (2.5:1). Esterification of daunomycin at C-7 with β-alanine was accomplished through the mixed anhydride of Z(OMe)-β-alanine. Preliminary biological tests suggest that the antitumor and DNA interactive properties of the anthracyclines can be retained in such structures.

The chemically labile glycosidic bond at the 7 position of daunorubicin (**1**) and adriamycin (**2**) is cleaved rapidly in the metabolism of these useful anticancer drugs. Both hydrolytic and reductive glycosidases deactivate **1** and **2**

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