Design and Synthesis of Potential DNA Cross-Linking Reagents Based on the Anthramycin Class of Minor Groove Binding Compounds†

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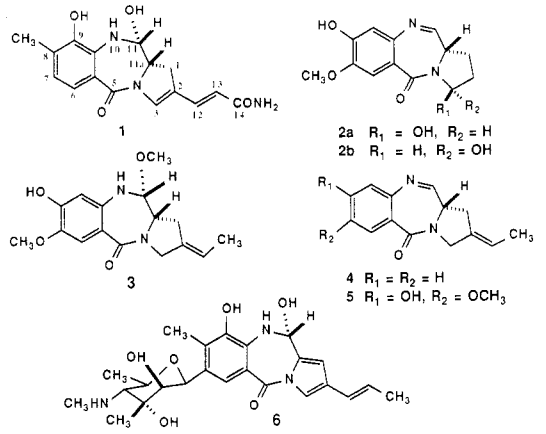
The use of computer molecular modeling to design a potential cross-linking reagent selective for the G-C rich regions of DNA is described. The target structure, the imino epoxide **7**, is synthesized in 11 steps from *N*-Boc-proline methyl ester (**8**). Redox chemistry of certain key intermediates affords several interesting intramolecular rearrangements such as the conversion of the thioimidate epoxide **22** to the tetracyclic product **26** via the transient species **23–25**. Reduction of the hydroxy tosylate **21** with aluminum amalgam also yields a tetracyclic compound, the tetrahydrofuran derivative **30**, a result of the cyclization of the unstable iminium alcohol **29** derived from the initial reduction intermediate, the dihydro derivative **28**. A fragmentation of the tetracycle **30** then yields directly the desired imino epoxide **7**, generating both reactive functionalities in a single step.

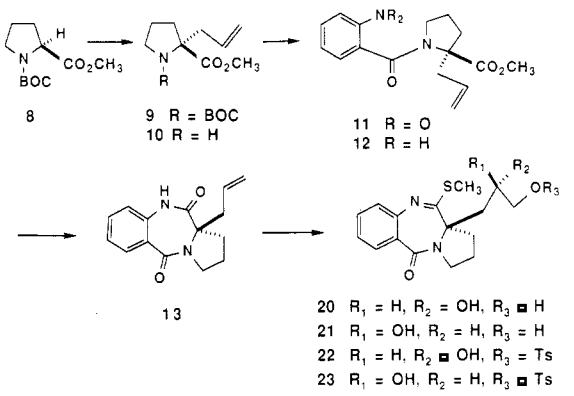
The antitumor antibiotics collectively known as the anthramycins have been postulated to exert their biological activity by covalently binding to the minor groove of DNA, thereby inhibiting nucleic acid synthesis.[1] A number of congeners of the prototypical compound anthramycin (**1**),[2] such as the neothramycins **2a** and **2b**,[3] tomaymycin (**3**),[4] prothracarcin (**4**),[5] pretomaymycin (**5**),[6] sibiromycin (**6**),[7] and several others[8] have been reported in recent years.



The initial step in the biochemical mechanism of action of the anthramycins involves diffusion into the minor groove of DNA. If a G-C base pair is present at the binding site, a reversible chemical reaction takes place, namely the addition of the N(2) nitrogen of the guanine residue to the N(10)–C(11) imino functionality of anthramycin (**1**) to form a labile aminal linkage as shown in Figure 1.[9] This mechanism is believed to account for the observed antitumor properties of these substances and has served as the basis for the design of more potent analogues.[10]

We have carried out extensive computer molecular modeling to determine the optimum site for attachment of a chemical linker (a tether with an electrophilic terminus) to the basic anthramycin system to allow the formation of a *second* covalent bond to a nucleophilic site on the complementary strand. The resulting construct is a DNA cross-link which would not only lend support to the biochemical mechanism but may also lead to enhanced antitumor activity in this series. Certain attachment sites could be readily excluded on the basis of these modeling studies. For example, securing the linker to various points on the benzo portion of the anthramycin nucleus led to the requirement of a long, floppy chain of at least five

Scheme I



atoms in order to reach a nucleophilic site across the double helix. On the other hand, examination of alternative attachment sites on the pyrrolidine substructure,

† Contribution no. 4458.

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(9) (a) Hurley, L. H.; Needham-VanDevander, D. R. *Acc. Chem. Res.* **1986**, *19*, 230. (b) Barkley, M. D.; Cheatham, S.; Thurston, D. E.; Hurley, L. H. *Biochemistry* **1986**, *25*, 3021–31.

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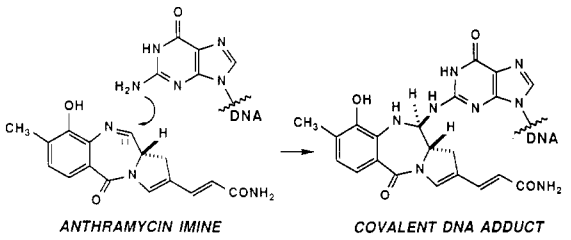


Figure 1.

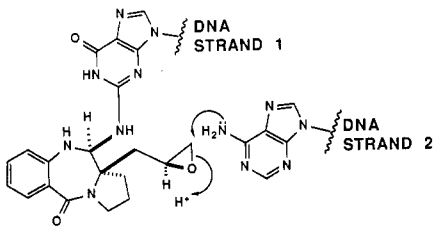
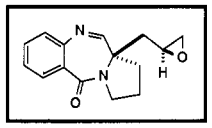


Figure 2.

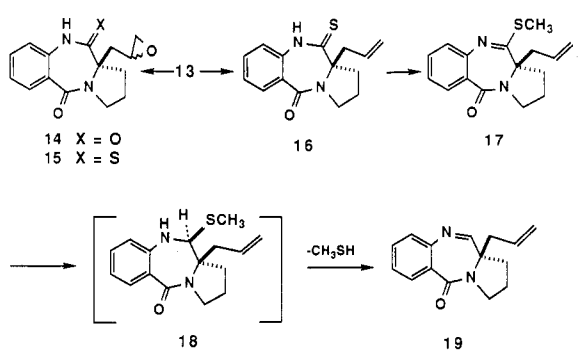
employing a variety of chemical linkers, led to the choice of the imino epoxide **7**, which was then targeted for synthesis.[11] As can be seen in Figure 2, this designed molecule offers the possibility of effecting a cross-link to the N(6) nitrogen of an adenine residue on the complementary strand by electrophilic attack of the epoxide tether at that site. Assuming some conformational mobility, this approach requires sufficient room in the minor groove to accommodate the additional nine atoms of the linker arm. In spite of the fact that no naturally occurring product in the series has anything larger than a hydrogen atom attached to the C(11a) position, our computer-modeling results indicate that the binding site does accommodate the linker at this attachment site.



7

A number of methodologies have been reported for the construction of the basic anthramycin system.[12] Our particular application required an approach in which the problematic imino functionality of this class of compounds would be generated under conditions compatible with the pendant epoxide functionality. The successful preparation of the targeted imino epoxide **7** begins with *N*-Boc-proline methyl ester (**8**)[13] (Scheme I). This material is converted to the racemic[14] *N*-Boc-2-allylproline derivative (**9**) by first forming the anion at the α carbon with lithium hexamethyldisilazane at −78 °C in tetrahydrofuran and then quenching with allyl bromide.[15] The Boc protecting group is removed with hydrogen chloride in dioxane to afford the 2-allylproline methyl ester (**10**), in an overall yield of 96% based on **8**. Acylation of **10** with *o*-nitrobenzoyl chloride in the presence of triethylamine affords the amide **11** which

Scheme II



is selectively reduced with titanium trichloride in methanol to yield the corresponding aniline **12**. Treatment of **12** with 25% sodium methoxide/methanol at 25 °C closes the benzodiazepine ring to afford the tricyclic product **13**. The overall yield of the allylated benzodiazepine **13** is 74%, based on the starting material **8**.

At this time it was decided to determine the critical point in the synthesis for the introduction of the potentially reactive epoxide group, preferably toward the end of the preparation. After some effort, the C(11a)-allylbenzodiazepine **13** was found to be epoxidized with *m*-chloroperbenzoic acid in dichloroethane in the presence of Kishi's radical inhibitor[16] to yield the corresponding epoxy derivatives **14** (Scheme II). This study served to determine the optimum conditions for oxidation of the allyl group and to gauge the stability of the epoxide to the reaction conditions required for conversion of the aniline-derived amide group of the benzodiazepine nucleus to the imino function present in the target. In this connection, the epoxide **14** does not survive a number of conditions known to convert the benzodiazepine nucleus to its monothioamide derivative **15**, the appropriate precursor to the target **7**. Therefore, the epoxidation of the allyl substituent had to be postponed until later in the synthesis. Treatment of **13** with Lawesson's reagent[17] yields the desired monothioamide **16** which is alkylated with methyl iodide to afford the thioimidate ester **17**.[18] Various oxidations of the allyl group were again attempted on both intermediates **16** and **17**. However, no characterizable products could be isolated and an NMR examination of the reaction mixtures revealed that the allyl function was still intact, indicating that reactions at the more readily oxidized sulfur atom prevailed. However, reduction of the thioimidate ester **17** with aluminum amalgam is straightforward, affording the desired allyl imine **19** directly from the presumed intermediate dihydro thioimidate **18** which loses methyl mercaptan upon isolation and chromatography, possibly a result of the steric crowding at the adjacent carbon C(11a). A series of oxidation attempts were then directed at the allyl imine **19**. In every instance, the imino moiety was preferentially attacked, even under strongly acidic conditions, leaving the allyl substituent intact. Interestingly, **19** possesses antitumor activity,[19] providing the first indication that the

(11) For examples of epoxides as electrophiles, see: Ross, W. C. J.; *Biological Alkylating Agents*; Butterworth: London, 1962; pp 13–14. Prikrylova, V.; Lipavska, H.; Jizba, J. V.; Fuska, J.; Podojil, M.; Vanek, Z. *J. Antibiot.* **1985**, *38*, 1714–8.

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(13) Matoba, K.; Yonamoto, H.; Fukui, M.; Yamazaki, T. *Chem. Pharm. Bull.* **1984**, *32* (10), 3918–25.

(14) Seeback, D.; Boes, M.; Naef, R.; Schweizer, W. B. *J. Am. Chem. Soc.* **1983**, *105*, 5390–8.

(15) The dianion of Boc-proline, generated from 2 equiv of LDA at −78 °C over 30 min could also be allylated at the α carbon, albeit in somewhat lower yield.

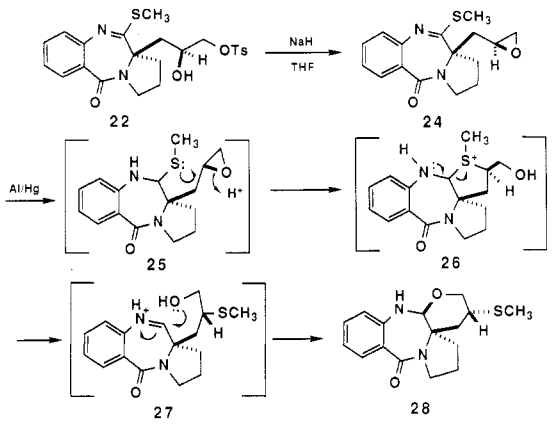
(16) Kishi, Y.; Aratani, M.; Tanino, H.; Fukuyama, T.; Goto, T.; Inoue, S.; Sugiura, S.; Kakoi, H. *J. Chem. Soc., Chem. Commun.* **1972**, 64.

(17) Freshly prepared Lawesson's reagent must be used for optimum yields. Thomsen, I.; Clausen, K.; Scheibye, S.; Lawesson, S. O. *Org. Synth.* **1984**, *62*, 158–64.

(18) Kaneko, T.; Wong, H.; Doyle, T. W. *Tetrahedron Lett.* **1983**, *24*, 5165. Kaneko, T.; Wong, H.; Doyle, T. W. *J. Antibiot.* **1984**, *36*, 1699. Kaneko, T.; Wong, H.; Doyle, T. W.; Rose, W. C.; Bradner, W. T. *J. Med. Chem.* **1985**, *28*, 388–92.

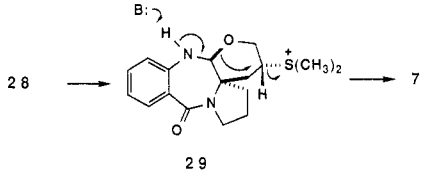
(19) In vitro antitumor activity was found at 0.12 μg/mL against the RPMI-7272 cell line; Regina Ruben, Du Pont Medical Products Department.

Scheme III



anthramycins can accommodate the chemical linker we have appended at C(11a) and still retain biological activity unless, of course, a different mechanism of action is now operative. The problem of the allyl group oxidation was solved when it was found that the thioimidate **17** can be cleanly oxidized at the allyl position by osmium tetraoxide without interference from the competing thioimidate, thereby affording the diasteromeric diols **20** and **21** (ratio 1:1) in 89% yield (Scheme I). These were converted to the corresponding tosylates **22** and **23**, which were readily separated and carried forward independently.[20]

The diastereomer **22** is converted to the desired (vide infra) epoxide **24** with sodium hydride in tetrahydrofuran (Scheme III). An unwanted rearrangement occurs, however, upon reduction of the thioimidate group of **24** with aluminum amalgam. The initial reaction product, the dihydro derivative **25**, presumably undergoes an intramolecular alkylation of the sulfide by the newly introduced epoxide before expulsion of methyl mercaptan can occur. The intermediate sulfonium ion **26** further rearranges to the tricyclic iminium sulfide **27**, which spontaneously recyclizes to the observed product, the tetracyclic species **28**. Although in principle **28** could be an effective precursor to our targeted compound via conversion to its sulfonium derivative **29** and base-catalyzed fragmentation to the imino epoxide **7**, further progress from **28** proved elusive.



Our successful preparation of **7** starts with the primary tosylate **22** which is converted to its dihydro derivative **30** without reduction of the tosylate group. In this case, the alkylation of the sulfide by the tosylate proved to be slower than with the epoxide **25**, perhaps reflecting the rate of closure of a six- vs five-membered ring. This retardation presumably facilitates the departure of methyl mercaptan and allows the iminium tosylate **31** to be formed. However, before any base could be added to convert the hydroxy tosylate of **31** to the desired epoxide, the *secondary* alcohol now adds to the imine, leading to the observed tetracyclic product **32**. The relative stereochemistry of **32** was elucidated by an X-ray structure determination and is presented in Figure 3. This result, of course, allows the assignment of the configuration of all previous intermediates in this synthesis. Fortunately, unlike the previous rearrangement product **28**, the tetracyclic tosylate **32** readily undergoes the fragmentation depicted in Scheme IV to yield directly the desired target structure, the imino epoxide **7**. Similarly, the diastereomeric tosylate **23** is converted into the tetracyclic isomer **33** and finally to the analogous imino epoxide **34**.

(20) For simplicity, only the chemistry of the diastereomeric tosylate **22** will be discussed in detail since the transformations of **23** are essentially identical. The Experimental Section includes the details of the key reactions in both diastereomeric series derived from **22** and **23**.

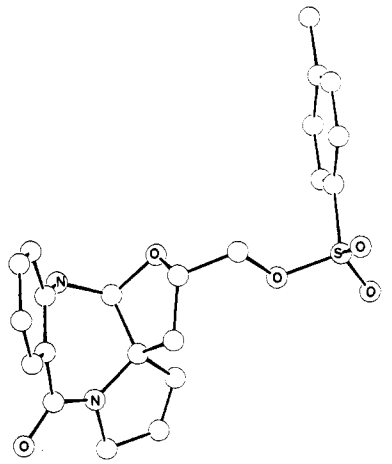
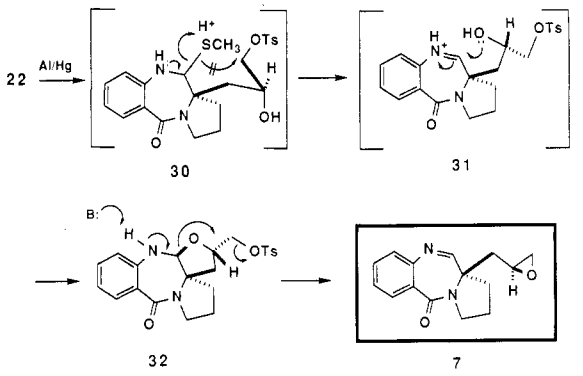
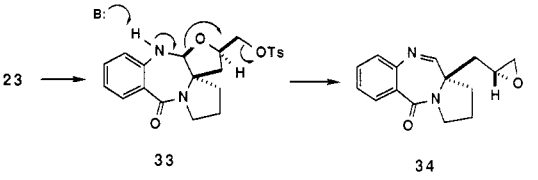


Figure 3. X-ray structure of the tetracyclic tosylate **32**.

Scheme IV





This final solution to the numerous synthetic problems outlined above is very satisfying since *both* sensitive groups present in the target molecule are generated in a single reaction and in the final step of the preparation. The biological and biochemical properties of **7** and **34** as well as related substances are currently under study.

Experimental Section

General Methods. Melting points were determined with a Fisher-Johns hotplate instrument or a Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet 7199 FT-IR spectrophotometer. Frequencies are reported in reciprocal centimeters (cm^{-1}) and were calibrated with use of polystyrene's 1601.8-cm^{-1} reference peak. ^1H NMR spectra were obtained with a General Electric Nicolet QE300 in the solvent indicated. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane ($\delta = 0.00$). Coupling constants (J) are given in cycles per second (Hz). Mass spectra were recorded at 70 eV on a VG Micromass 70-70H double-focusing high-resolution spectrometer. Column chromatography

was carried out with 230–400-mesh silica gel and with a Waters prep-500 instrument by using the solvent system indicated.

L-(*tert*-Butyloxycarbonyl)proline Methyl Ester (8).[13] A solution of 50.0 g (435 mmol) of L-proline and 300 mL of methanol was cooled to 0 °C under N_2 and 35 mL (478 mmol) of thionyl chloride was added dropwise over 20 min. After refluxing for 1 h, the solvent was removed in vacuo to afford 77.9 g of a yellow oil which was then dissolved in 600 mL of *tert*-butyl alcohol (mechanical stirring) under N_2; then 150 mL (1.09 mol) of triethylamine was added and the solution was stirred 10 min. A solution of 95 g (0.44 mol) of di-*tert*-butyl dicarbonate in 100 mL of *tert*-butyl alcohol was added dropwise over 30 min. After stirring for 18 h, the white precipiate was filtered and the bulk of the solvent was evaporated. The oil was dissolved in 500 mL of ether and washed with 1 N HCl (2 × 500 mL), 500 mL of saturated sodium dicarbonate, and 500 mL of brine. The ether layer was dried ($MgSO_4$) and removed in vacuo. Further drying on a vacuum pump gave 82.1 g (82.4%) of **8** as a colorless oil (used without purification): NMR ($CDCl_3$, 300 MHz) δ 4.32 (dd, J = 8, 4 Hz, 0.4 H), 4.22 (dd, J = 8, 5 Hz, 0.6 H), 3.72 (s, 3 H), 3.3–3.6 (m, 2.6 H), 2.1–2.3 (m, 1.2 H), 1.8–2.0 (m, 3.5 H), 1.46 (s, 3.7 H), 1.41 (s, 6.6 H).

***N*-(*tert*-Butyloxycarbonyl)-2-(methoxycarbonyl)-2-(2-propenyl)pyrrolidine (9).** To a solution of 82.1 g (359 mmol) of **8** in 800 mL of dry THF cooled to −78 °C was added 394 mL of lithium hexamethyldisilazane (1 M in hexane) over 15 min. The solution was stirred 15 min. Allyl bromide was purified by passing an excess amount through 10 g of activity III basic alumina. A 47-mL (540 mmol) sample of this allyl bromide was added to the reaction over 5 min. The cold bath was removed and the solution was stirred for 1.5 h. After adding 50 mL of 1 N HCl and stirring 5 min, most of the solvent was evaporated. The product was dissolved in 500 mL of ether and was washed with 1 N HCl (2 × 300 mL), 300 mL of saturated $NaHCO_3$, and 300 mL of brine. Drying with $MgSO_4$ and evaporation gave 95.75 g (99%) of **9** as a tan oil. The crude product was one spot on TLC (25% ethyl acetate/hexanes). Further purification could be carried out by column chromatography (10% ethyl acetate/hexanes) to give pure **9** as a colorless oil: IR (neat) 2980, 2880, 1745, 1700, 1390, 1370, 1165 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 5.65–5.85 (m, 1 H), 5.50–5.2 (m, 2 H), 3.5–3.8 (m, 4 H), 3.3–3.45 (m, 1 H), 2.85–3.15 (m, 1 H), 2.60 (dd, 1 H, J = 14, 8 Hz), 1.7–2.2 (m, 4 H), 1.35–1.5 (m, 9 H); mass spectrum, m/e 228,154,149, measured 228.1224 (calcd for $C_{14}H_{23}NO_4 - C_3H_5$ ($M^+ - C_3H_5$), 228.1235).

2-(Methoxycarbonyl)-2-(2-propenyl)pyrrolidine Hydrochloride (10). To a solution of 95.7 g (356 mmol) of **9** in 250 mL of dry dioxane under N_2 was added hydrogen chloride gas in a steady stream of bubbles for 30 min. An ice bath was used to keep the mixture near room temperature. After spontaneous bubbling began, the addition was discontinued and the cold bath removed. After stirring for 20 h, the solvent was evaporated and the solid azeotroped with 300 mL of dry toluene. Drying on the vacuum pump to constant weight gave 73 g (quantitative) of **10** pure enough to use in the next reaction. A previous sample was crystallized 3 times from ether/ethanol to give pure **10** as tiny white needles: mp 122.5–123 °C; IR 2890, 2710, 2480, 1755, 1640 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 5.8–6.0 (m, 1 H), 5.2–5.4 (m, 2 H), 3.84 (s, 3 H), 3.5–3.7 (m, 2 H), 3.08 (dd, 1 H, J = 17, 7 Hz), 2.88 (dd, 1 H, J = 17, 7 Hz), 2.45 (m, 1 H), 2.15 (m, 2 H), 1.8–2.0 (m, 1 H). Anal. Calcd for $C_9H_{16}ClNO_2$: C, 52.56; H, 7.84; Cl, 17.24; N, 6.81. Found: C, 52.79; H, 7.84; Cl, 17.28; N, 6.81.

***N*-(2-Nitrobenzoyl)-2-(methoxycarbonyl)-2-(2-propenyl)pyrrolidine (11).** To a solution of 73 g (about 360 mmol) of amine **10** in 600 mL of dry CH_2Cl_2 under N_2 was added 220 mL (1.57 mol) of triethylamine. The thick white suspension was agitated with a good stirring device and cooled to 0 °C. *o*-Nitrobenzoyl chloride (69 mL, 0.51 mol) was added over 5 min. The cold bath was removed and the slurry was stirred for 16 h. Glycine (7.5 g) was added and the dark mixture was stirred 1 h. The CH_2Cl_2 was evaporated in vacuo, and the product was dissolved in 1 L of ether. The ether layer was washed with 1 N HCl (2 × 400 mL), saturated $NaHCO_3$ (2 × 400 mL), and 400 mL of brine. The ether layer was dried with $MgSO_4$ and evaporated, and the resulting oil was dried on a vacuum pump to constant weight. The yield was 140 g of crude **11** as a dark solid. This material was not pure but was used without purification. A previous sample was purified first by column chromatography (40% ethyl acetate/hexanes) and then by crystallization from hexanes/ethyl acetate to give pure **11** as pale needles: mp 79.5–81 °C; IR (neat) 3080, 2980, 2960, 2880, 1740, 1645, 1535, 1350 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 8.17 (d, 1 H, J = 8 Hz), 7.72 (t, 1 H, J = 8 Hz), 7.57 (t, 1 H, J = 8 Hz), 7.45 (d, 1 H, J = 8 Hz), 5.95–6.1 (m, 1 H), 5.2–5.3 (m, 2 H), 3.83 (s, 3 H), 3.15–3.45 (m, 3 H), 2.82 (dd, 1 H, J = 14, 8 Hz), 2.1–2.3 (m, 2 H), 1.8–2.05 (m, 2 H); mass spectrum, m/e 318.1201 (calcd for $C_{16}H_{18}N_2O_5$, 318.1215). Anal. Calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.51; H, 5.56; N, 8.52.

1,2,3,10,11,11a-Hexahydro-11a-(2-propenyl)-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (13). To a solution of 70 g (about 350 mmol) of crude **11** in 500 mL of methanol under N_2 was added 1.0 L of titanium trichloride (20% in H_2O) at a rate of 20 mL/min. After the addition, stirring was continued for 2 h. The purple solution was extracted with 1 L of CH_2Cl_2 followed by 10% methanol/CH_2Cl_2 (2 × 500 mL). The organic layers were dried with $MgSO_4$ and evaporated to give 52.9 g of crude material as a mixture of **12** and **13**.

The above product was dissolved in 100 mL of methanol and 200 mL of sodium methoxide (25% in methanol) was added. After stirring 18 h, the slurry was poured into 500 mL of 2 N HCl with external cooling in ice. Extraction with 500 mL of CH_2Cl_2 and then 10% methanol/CH_2Cl_2 (3 × 300 mL), drying ($MgSO_4$), and concentration in vacuo provided 49.8 g of crude **13**. Three batches of crystallizations (methanol/CH_2Cl_2 or ethyl acetate/hexanes) gave a total of 33.1 g (74%, 2 steps) of pure **13**. Two crystallizations from ethyl acetate/hexanes provided an analytical sample of **13** as colorless cubes: mp 198–199.5 °C; IR (KBr) 3200, 3080, 3000, 2980, 2940, 2880, 1675, 1665, 1630, 1615, 1580, 1480 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 8.45 (br, 1 H), 8.02 (d, 1 H, J = 8 Hz), 7.45 (t, 1 H, J = 8 Hz), 7.25 (t, 1 H, J = 8 Hz), 6.97 (d, 1 H, J = 8 Hz), 5.45–5.65 (m, 1 H), 5.08 (d, 1 H, J = 10 Hz), 4.86 (d, 1 H, J = 17 Hz), 4.03 (m, 1 H), 3.59 (m, 1 H), 3.07 (m, 1 H), 2.30 (dd, 1 H, J = 15, 7 Hz), 2.17 (dd, 1 H, J = 15, 7 Hz), 1.7–2.1 (m, 3 H); mass spectrum, m/e 215, 187, 146. Anal. Calcd for $C_{15}H_{16}N_2O_2$: C, 70.29; H, 6.29; N, 10.93. Found: C, 70.45; H, 6.27; N, 10.98.

11a-(2,3-Epoxypropyl)-1,2,3,10,11,11a-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11-dione (14). A mixture of 100 mg (0.34 mmol) of **13** and 4.3 mL (0.43 mmol) of *m*-chloroperoxybenzoic acid (0.1 M MCPBA in dichloroethane) with 5 mg of 3-*tert*-butyl-4-hydroxy-5-methylphenyl sulfide was refluxed under N_2 for 5 h. The reaction was not complete as judged by TLC (75% ethyl acetate/hexanes) and 2.0 mL of the MCPBA solution was added and refluxing was continued another 3 h. The reaction was partitioned between saturated $NaHCO_3$/10% methanol–CH_2Cl_2, dried, and evaporated. Column chromatography provided 86 mg (81%) of pure **14** as a white solid: NMR ($CDCl_3$, 300 MHz) δ 8.04 (d, 1 H, J = 8 Hz), 7.72 (br, 1 H), 7.50 (t, 1 H, J = 8 Hz), 7.26 (m, 1 H), 6.94 (t, 1 H, J = 8 Hz), 4.12 (m, 1 H), 3.70 (m, 1 H), 3.26 (m, 1 H), 2.84 (m, 1 H), 2.70 (m, 1 H), 1.6–2.3 (m, 6 H).

1,2,3,10,11,11a-Hexahydro-11a-(2-propenyl)-11-thioxo-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (16). A mixture of 10.00 g (39.4 mmol) of **13** and 8.1 g (20 mmol) of Lawesson's reagent[16] in 200 mL of dry glyme was refluxed under N_2 for 3 h. All but 50 mL of the solvent was evaporated and 200 mL of saturated K_2CO_3 was added. After stirring 0.5 h, the slurry was extracted twice with enough 30% methanol/CH_2Cl_2 to bring the yellow solid into solution. Drying and evaporation gave 15.2 g of crude **16**. An analytical sample was prepared by first column chromatography on silica gel followed by repeated crystallization from ethyl acetate/methanol to give pure **16** as yellow cubes: mp 244.5–246 °C; IR (KBr) 3160, 2980, 2920, 2880, 1600, 1580, 1535, 1480 cm^{-1}; NMR (DMSO-d_6, 300 MHz) δ 12.50 (br, 1 H), 7.82 (d, 1 H, J = 8 Hz), 7.58 (t, 1 H, J = 8 Hz), 7.34 (m, 2 H), 5.4–5.6 (m, 1 H), 5.04 (d, 1 H, J = 11 Hze, 4.74 (d, 1 H, J = 18 Hz), 3.80 (m, 1 H), 3.3–3.5 (m, 2 H), 2.1–2.3 (m, 3 H), 1.76 (m, 2 H); mass spectrum, m/e 272.0975 (calcd for $C_{15}H_{16}N_2OS$, 272.0983). Anal. Calcd for $C_{15}H_{16}N_3OS$: C, 66.15; H, 5.92; N, 10.29; S, 11.77. Found: C, 65.94; H, 5.98; N, 10.26; S, 11.95.

11-(Methylthio)-11a-(2-propenyl)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (17). A mixture of 15.2 g (about 40 mmol) of crude **16**, 6.1 mL (100 mmol) of methyl

iodide, 16.6 g (120 mmol) powdered K_2CO_3, and 200 mL of THF was refluxed for 4 h. The reaction was monitored by using TLC (10% acetone/toluene, multiple developments). Most of the solvent was evaporated and the slurry was partitioned between water/CH_2Cl_2 (2 × 200 mL). After drying ($MgSO_4$) and evaporation, 16.2 g of a red oil remained. The sample was purified by column chromatography on silica gel (10:1, ethyl acetate/hexanes) and 9.42 g (84%) of reasonably pure **16** were obtained. MPLC provided a pure sample of **16** (clear oil): IR (neat) 3070, 2980, 2920, 2880, 1735, 1625, 1605, 1595, 1560 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 8.01 (d, 1 H, J = 8 Hz), 7.50 (t, 1 H, J = 8 Hz), 7.24 (m, 2 H), 5.4–5.6 (m, 1 H), 5.06 (d, 1 H, J = 10 Hz), 4.84 (d, 1 H, J = 17 Hz), 4.08 (m, 1 H), 3.54 (m, 1 H), 2.90 (m, 1 H), 2.44 (s, 3 H), 1.9–2.4 (m, 5 H); mass spectrum, m/e 286.1136 (calcd for $C_{16}H_{18}N_2OS$, 286.1139).

11a-(2-Propenyl)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (19). A mixture of 1.29 g (4.48 mmol) of **17** and aluminum amalgam[21] (prepared from 1.22 g Al foil) in 50 mL of 20% H_2O/THF was stirred at 0 °C under N_2 for 20 h. The gray slurry was filtered through Celite washing with THF. The THF was removed in vacuo, and the residue was partitioned between brine/10% methanol–CH_2Cl_2 (3×). After drying ($MgSO_4$) and evaporation, 1.21 g of a foam was obtained. Column chromatography on silica gel (50–100% ethyl acetate/hexanes) produced 339 mg (31%) of pure **19** as a white solid: mp 134–136 °C; IR (KBr) 3070, 2970, 2910, 2870, 1615, 1595, 1560, 1490 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 8.05 (d, 1 H, J = 7 Hz), 7.72 (s, 1 H), 7.54 (t, 1 H, J = 7 Hz), 7.34 (m, 2 H), 5.4–5.6 (m, 1 H), 5.06 (d, 1 H, J = 11 Hz), 4.84 (d, 1 H, J = 17 Hz), 4.06 (m, 1 H), 3.56 (m, 1 H), 2.3–2.5 (m, 2 H), 2.18 (dd, 1 H, J = 13, 7 Hz), 1.8–2.1 (m, 3 H); mass spectrum, m/e 199.0870 (calcd for $C_{15}H_{16}N_2O - C_3H_5$, 199.0871); UV ($CH_3CN$) λ_{max} 221 nm (ϵ 18 100), 249 nm (ϵ 8460), 324 nm (ϵ 2390).

11-(Methylthio)-11a-(2,3-dihydroxypropyl)-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepin-5-one (20 and 21). To a solution of 4.35 g (15.1 mmol) of thioimidate **17**[22] and 4.67 g (34.6 mmol) of *N*-methylmorpholine *N*-oxide in 160 mL of 25% H_2O/acetone were added 70 drops of a 5% solution of osmium tetraoxide in methylene chloride. After stirring 20 h under N_2, 18 g of $NaHSO_3$/Florisil (1/12) was added and stirring continued for 30 min. The slurry was filtered and the acetone was evaporated. The remaining material was partitioned between brine/10% methanol–CH_2Cl_2 (3×), dried, and evaporated. Column chromatography on silical gel (0–5% methanol/ethyl acetate) gave 4.30 g (89%) of a mixture of pure **20** and **21** (ratio = 1.06:1) as a white solid which was inseparable by TLC in all solvent systems investigated: IR ($CHCl_3$) 3400, 2930, 2880, 1605, 1595, 1560, 1360, 1190, 1170 cm^{-1}; NMR ($CDCl_3$, 300 MHz) shows no olefin peaks in the range of δ 4.2–6.7; mass spectrum, m/e 320.1202 (calcd for $C_{16}H_{20}N_2O_3S$, 320.1194).

11-(Methylthio)-11a-[2-hydroxy-3-[(*p*-tolylsulfonyl)-oxy]propyl]-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]-benzodiazepin-5-one (22 and 23). To a solution of 1.188 g (3.69 mmol) of diols **20** and **21** in 30 mL of dry pyridine was added 1.06 g (5.53 mmol) of *p*-toluenesulfonyl chloride under N_2. After being stirred for 18 h at room temperature, the tan solution was added to 150 mL of ethyl acetate which was then washed with 1 N HCl (2×), saturated $NaHCO_3$, and brine. After drying with $MgSO_4$ and evaporation, 2.08 g of crude material was obtained. Column chromatography on silica gel followed by MPLC (both using 50% ethyl acetate/hexanes) afforded 647 mg of **23** (less polar component) and 672 mg of **22** (more polar component). The total yield was 75%. Their R_f values were 0.15 and 0.10, respectively (50% ethyl acetate/hexanes).

The less polar component was crystallized twice from CH_2Cl_2/ether to give pure **22** as white cubes: mp 190–193 °C; IR (KBr) 3430, 3060, 2980, 2960, 2920, 2880, 1600, 1555, 1495 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 7.89 (d, 1 H, J = 8 Hz), 7.67 (d, 2 H, J = 8 Hz), 7.46 (t, 1 H, J = 8 Hz), 7.33 (d, 2 H, J = 8 Hz), 4.13 (m, 1 H), 3.78 (m, 1 H), 3.4–3.6 (m, 3 H), 2.96 (dd, 1 H, J = 14, 4 Hz), 2.46 (s, 3 H), 2.42 (s, 3 H), 1.2–2.2 (m, 6 H). Anal. Calcd for $C_{23}H_{26}N_2O_5S_2$: C, 58.21; H, 5.52; N, 5.90; S, 13.51. Found: C, 57.92; H, 5.36; N, 5.79; S, 13.45.

The more polar component was crystallized from CH_2Cl_2/ether to give pure **23** as colorless cubes: mp 150–152 °C; NMR ($CDCl_3$, 300 MHz) δ 7.95 (d, 1 H, J = 8 Hz), 7.66 (d, 2 H, J = 8 Hz), 7.44 (t, 1 H, J = 8 Hz), 7.32 (d, 2 H, J = 8 Hz), 7.21 (t, 1 H, J = 8 Hz), 7.14 (d, 1 H, J = 8 Hz), 3.98 (m, 1 H), 3.77 (m, 1 H), 3.45–3.6 (m, 3 H), 3.04 (dt, 1 H, J = 14, 6 Hz), 2.45 (s, 3 H), 2.42 (s, 3 H), 2.25–2.4 (m, 1 H), 2.19 (m, 1 H), 1.85–2.05 (m, 2 H), 1.74 (m, 1 H), 1.32 (dd, 1 *H*, J = 15, 8 Hz). Anal. Calcd for $C_{23}H_{26}N_2O_5S_2$: C, 58.21; H, 5.52; N, 5.90; S, 13.51. Found: C, 58.05; H, 5.75; N, 5.93; S, 13.67.

Reduction of Epoxide 24. The reduction was performed in the same manner as with **33** using 0.18 g (6.8 mmol) of aluminum foil, 171 mg (0.57 mmol) of **24**, and 10 mL of 20% H_2O/THF under N_2. After workup, 172 mg of a solid was obtained. An NMR ($CDCl_3$, 300 MHz) spectrum indicated a 1/1 mixture of **25** and **28** along with other impurities. The resonances indicative of **25** were δ 5.44 (br d, N_{10}-H, J = 6 Hz), 4.68 (d, C_{11}-H, J = 6 Hz), 3.00 (m, C_{13}-H), 2.77 (t, C_{14}-H, J = 4 Hz). Column chromatography on silica gel (50–100% ethyl acetate/hexanes) gave 66 mg of pure **28** as a white amorphous solid: IR (KBr) 3060, 2920, 2880, 1610, 1590, 1550 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 8.51 (dd, 1 H, J = 8, 1 Hz), 7.43 (td, 1 H, J = 8, 1 Hz), 7.31 (td, 1 H, J = 8, 1 Hz), 7.17 (dd, 1 H, J = 8, 1 Hz), 4.45 (s, 1 H), 3.45–4.0 (m, 44), 2.75 (br, 1 H), 2.52 (dd, 1 H, J = 14, 9 Hz), 2.39 (dt, 1 H, J = 12, 7 Hz), 2.07 (s, 3 H), 1.65–2.15 (m, 5 H); mass spectrum, m/e 304.1203 (calcd for $C_{16}H_{20}N_2O_2S$, 304.1246).

Preparation of Tetracyclic Tosylate 32. Fresh mercury–aluminum amalgam[21] (from 0.86 g of aluminum foil) was stirred with 1.50 g (3.16 mmol) of **22** in 32 mL of 25% H_2O/THF in the same manner as for the preparation of **33**. Column chromatography on silica gel (75% ethyl acetate/hexanes) gave 0.94 g (70%) of **32** as a light yellow foam. Several recrystallizations from ethyl acetate/methanol gave pure **32** as small white flakes: mp 166–168 °C; IR (KBr) 3430, 3060, 2960, 2880, 1625, 1580, 1485, 1360, 1190, 1180 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 7.69 (d, 3 H, J = 8 Hz), 7.34 (d, 2 H, J = 8 Hz), 7.21 (t, 1 H, J = 8 Hz), 7.07 (t, 1 H, J = 8 Hz), 6.58 (d, 1 H, J = 8 Hz), 5.01 (d, 1 H, J = 3 Hz), 4.15 (br, 1 H), 3.95–4.1 (m, 1 H), 3.7–3.9 (m, 2 H), 3.57 (dd, 1 H, J = 10, 4 Hz), 3.45 (dd, 1 H, J = 10, 7 Hz), 2.48 (s, 3 H), 2.25–2.35 (m, 1 H), 1.9–2.1 (m, 3 H), 1.70 (dd, 1 H, J = 13, 6 Hz), 1.57 (m, 1 H); mass spectrum, m/e 308, 280, 256, 243, 428.1393 (calcd for $C_{22}H_{24}N_2O_5S$, 428.1406).

Preparation of Tetracyclic Tosylate 33. A solution of 1.50 g (3.16 mmol) of **23** in 32 mL of 25% H_2O/THF was cooled to 0 °C under N_2. Freshly prepared aluminum amalgam[21] from 0.86 g (32 mmol) of aluminum foil was immediately added to the above solution. After stirring at 0 °C for 18 h, the gray slurry was filtered through Celite and the THF was evaporated. After partitioning the residue between brine/10% MeOH–CH_2Cl_2, the organic layer was dried (Na_2SO_4) and evaporated to yield 1.21 g of crude product as a foam. Column chromatography on silica gel gave 1.11 g of pure material which was recrystallized from CH_2Cl_2/methanol-/ethyl acetate to give 0.85 g (63%) of **33** as tiny white flakes: mp 198.5–199.5 °C; IR (KBr) 3380, 2960, 2930, 2880, 1620, 1605, 1580, 1480, 1365, 1175 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, 3 H, J = 8 Hz), 7.25–7.35 (m, 3 H), 7.08 (t, 1 H, J = 8 Hz), 6.83 (d, 1 H, J = 8 Hz), 4.95 (s, 1 H), 4.17 (br, 1 H), 3.65–3.95 (m, 5 H), 2.45 (s, 3 H), 2.32 (m, 1 H), 1.8–2.2 (m, 4 H), 1.68 (dd, 1 H, J = 12, 5 Hz); mass spectrum, m/e 428, 308, 280, 148. Anal. Calcd for $C_{22}H_{24}N_2O_5S$: C, 61.67; H, 5.65; N, 6.54; S, 7.48. Found: C, 61.95; H, 5.83; N, 6.47; S, 7.54.

Preparation of the Imino Epoxide 7. To a stirred solution of the tosylate **32** (300 mg, 0.70 mmol) in 20 mL of dry THF at 0 °C was added 1 mL of 1 M $LiN(TMS)_2$ in THF (1 mmol) dropwise, and the mixture was stirred for 1.5 h at the same temperature. At the end of the stirring it was partitioned between EtOAc (50 mL) and cold water (10 mL) and the EtOAc layer was washed with brine. After drying over Na_2SO_4 the solvents were evaporated to give a white solid residue (220 mg). It was recrystallized from EtOAc–MeOH–hexane to afford 86 mg of pure epoxide **7**: mp 165–168 °C (48% yield); NMR ($CDCl_3$) hydrate

(21) The aluminum amalgam should be prepared immediately prior to use according to: Myers, A. I.; Durandetta, J. L. *J. Org. Chem.* **1975**, *40*, 2021–2025.

(22) This compound should either be used immediately or repurified by MPLC prior to use. This ensures that the subsequent reaction is complete in less than 1 day and the amount of osmium tetraoxide used is sufficient for complete reaction.

of **7** δ 8.24 (d, J = 8 Hz, 1 H), 7.26 (t, J = 8 Hz, 1 H), 6.86 (t, J = 8 Hz, 1 H), 6.68 (d, J = 8 Hz, 1 H), 5.47 (d, J = 6 Hz, 1 H), 4.81 (d, J = 6 Hz, 1 H), 3.86 (dd, J = 9 Hz, 10 Hz, 1 H), 3.74 (m, 1 H), 3.03 (m, 1 H), 2.72 (t, J = 4 Hz, 1 H), 2.66–1.40 (m, 7 H); IR (KBr) 3392, 3263, 3045, 2972, 2929, 1612, 1594, 1575, 1553, 1489, 1439, 1405, 1337, 1129, 1117 cm^{-1}; HRMS, calcd for $C_{15}H_{16}N_2O_2$ 256.1212, found 256.1205.

Preparation of the Imino Epoxide 34. To a stirred solution of the tosylate **33** (100 mg, 0.257 mmol) in 10 mL of dry THF at 0 °C was added 0.26 mL of 1 M $LiN(TMS)_2$ in THF (0.26 mmol) dropwise, and the mixture was stirred for 0.5 h at 0 °C. At the end of the stirring 20 mL of EtOAc was added and the solution was washed with 5 mL of cold water and 5 mL of brine. After drying over Na_2SO_4 the solvents were evaporated off to give a white solid residue of the epoxide **34**. It was recrystallized from EtOAc–MeOH–hexane to afford 42 mg of the pure epoxide **34**: mp 151.5–153 °C (70.1% yield); NMR ($CDCl_3$) imino epoxide **34** δ 8.04 (d, J = 8 Hz, 1 H), 7.77 (s, 1 H), 7.53 (t, J = 8 Hz, 1 H), 7.34 (d, J = 8 Hz, 1 H), 7.33 (t, J = 8 Hz, 1 H), 4.13 (m, 1 H), 3.60 (m, 1 H), 2.97 (m, 1 H), 2.8–1.4 (m, 7 H), hydrate of **34** δ 8.10 (d, J = 8 Hz, 1 H), 7.23 (t, J = 8 Hz, 1 H), 6.80 (t, J = 8 Hz, 1 H), 6.75 (d, J = 8 Hz, 1 H), 5.38 (d, J = 6 Hz, 1 H), 4.40 (d, J = 6 Hz, 1 H), 3.88 (m, 2 H), 3.03 (m, 1 H), 2.8–1.4 (m, 8 H); IR (KBr) 3394, 3289, 2980, 2914, 2822, 1601, 1558, 1553, 1491, 1472, 1403, 1346, 1121 cm^{-1}; HRMS, CH_3OH adduct of **34**, calcd for $C_{16}H_{20}N_2O_3$ 288.1474, found 288.1464; hydrate of **34**, calcd for $C_{15}H_{18}N_2O_3$ 274.1318, found 274.1270; imino epoxide **34**, calcd for $C_{15}H_{16}N_2O_2$ 256,1212, found 256.1243.

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Supplementary Material Available: Detailed X-ray crystal data for compound **32** (atomic coordinates, bond lengths, bond angles, etc.) (5 pages). Ordering information is given on any current masthead page.

Studies on the Total Synthesis of Bouvardin and Deoxybouvardin: Cyclic Hexapeptide Cyclization Studies and Preparation of Key Partial Structures

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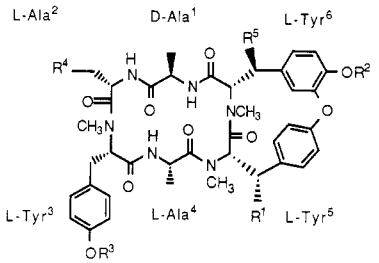
The total synthesis of *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Tyr-*N*-Me-Tyr) (**9**), *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-Me-Gly-*N*-Me-Gly) (**10**), and *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala-*N*-$(CH_3)CH_2CH_2$-(*p*-C_6H_4)-O-(*m*-C_6H_4)$CH_2CH_2C(O)$) (**11**) are detailed and constitute the parent 18-membered (**9**, **10**) and 26-membered (**11**) monocyclic peptide skeletons of the exceptionally potent, naturally occurring, bicyclic hexapeptide antitumor antibiotics bouvardin (**1**), deoxybouvardin (**2**, RA-V), RA-I–RA-IV, RA-VI, and RA-VII. The preparation of *cyclo*-(D-Ala-Ala-*N*-Me-Tyr(OCH_3)-Ala) (**12**), a conformationally constrained 12-membered cyclic tetrapeptide constituting a monocyclic, skeletal substructure of the naturally occurring materials, is detailed. Macrocyclization studies revealed no apparent preference for 12-membered vs 18-membered vs 26-membered ring closure and each represent a macrocyclization reaction which is facilitated with closure conducted at a N-terminus D-amino acid site (D-Ala).

Bouvardin (**1**, NSC 259968) and deoxybouvardin (**2**), bicyclic hexapeptides isolated initially from *Bouvardia ternifolia* (Rubiacea) and unambiguously identified by single-crystal X-ray structure analysis (bouvardin) and chemical correlation (deoxybouvardin),[2] are the initial members of a class of selective, exceptionally potent antitumor antibiotics[2-4] now including the additional, provisionally named, bicyclic hexapeptides RA-I–RA-VII.[3-5]

Bouvardin (**1**) and related agents inhibit protein synthesis[6]



	R^1	R^2	R^3	R^4	R^5	
1	OH	H	CH_3	H	H	bouvardin[2]
2	H	H	CH_3	H	H	deoxybouvardin,[2] (RA-V)[3,4]
3	H	H	CH_3	OH	H	RA-I[3]
4	H	CH_3	H	H	H	RA-II[3]
5	H	CH_3	CH_3	OH	H	RA-III[3,4]
6	H	CH_3	CH_3	H	OH	RA-IV[3,4]
7	H	CH_3	CH_3	H	H	*O*-methyl deoxybouvardin, (RA-VII)[3,4]
8	OH	CH_3	CH_3	H	H	*O*-methyl bouvardin[5]

by binding to the eukaryotic 80S ribosome and subsequently inhibit EF1-dependent binding of aminoacyl-tRNA *and* EF2-dependent translocation of peptidyl-

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