(19) (a) B. M. Pyatin and R. G. Glushkov, *Khim.-Farm. Zh.* (*Engl. Trans.*), 488 (1968); (b) the pK_a determination was complicated by the facile decomposition of **5** at pH > 6; (c) determined spectrophotometrically (A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Butler and Tanner, London, 1962, p 69) using buffers with $\mu = 0.01$.
(20) T. K. Bradshaw and D. W. Hutchinson, *Chem. Soc. Rev.*, **6**, 43 (1977).
(21) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis," Wiley, New York, 1968, p 457.
(22) J. W. Clarke-Lewis, *Chem. Rev.*, **58**, 63 (1958).
(23) H. C. Van der Plas, "Ring Transformations of Heterocycles", Academic Press, New York, 1973, pp 125–134.
(24) K. H. Dudley and P. Hemmerich, *J. Org. Chem.*, **32**, 3049 (1967).
(25) R. W. Denney and A. Nickon, *Org. React.*, **20**, 133 (1973).
(26) R. M. Johnson and I. W. Siddiqi, "The Determination of Organic Peroxides", Pergamon Press, London, 1970, p 50.
(27) C. Kemal and T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 995 (1976).
(28) C. Kemal, T. W. Chan, and T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 405 (1977).
(29) The assay conditions are based on those described by J. Ayling, R. Pirson, W. Pirson, and G. Boehm, *Anal. Biochem.*, **51**, 80 (1973).
(30) C. Y. Huang and S. Kaufman, *J. Biol. Chem.*, **248**, 4242 (1973).
(31) The hydroxy adduct (**5**) was also tested at concentrations of up to 20 μM by using 4.67 μM 6,7-dimethyltetrahydropterin and the assay conditions described in ref 30. No inhibition was observed (S. Kaufman, personal communication).
(32) S. Udenfriend and J. R. Cooper, *J. Biol. Chem.*, **196**, 227 (1952).
(33) T. P. Waalkes and S. Udenfriend, *J. Lab. Clin. Med.*, **50**, 733 (1957).
(34) R. Gloor and E. L. Johnson, *J. Chromatogr. Sci.*, **15**, 413 (1977).
(35) The free base form of **2** was used in these experiments. Similar yields were obtained by using the mono- or ditrifluoroacetate salt of **2**.
(36) A. E. Mawdsley and K. D. Berlin, *Org. Prep. Proced. Int.*, **6**, 169 (1974).
(37) T. R. Sharp and R. D. Minard, private communication.
(38) Reference 21, p 471.
(39) A. Pohland, E. H. Flynn, R. G. Jones, and N. Shive, *J. Am. Chem. Soc.*, **73**, 3247 (1951).

Total Synthesis of Vernolepin. 2.[1] Stereocontrolled Synthesis of (±)-Vernolepin

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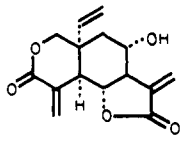
Abstract: The stereocontrolled total synthesis of the bis(α-methylene)lactonic sesquiterpene, vernolepin (**1**), has been achieved. This tumor inhibitor was synthesized in 11 steps starting from the cyclopropane ketone (**3**) as our key synthetic intermediate. This total synthesis has been constructed by a new synthetic strategy based on conformational analyses of the *exo*-olefinic system as IV. The key steps of the synthesis are (i) [2,3]-sigmatropic rearrangement of *p*-anisyl sulfoxide (**8** → **9**) and (ii) intramolecular conjugate reduction with sodium cyanoborohydride in HMPA followed by hydride exchange with diborane.

Vernolepin (**1**) and its congener vernomenin (**2**) have been isolated from *Vernonia hymenolepis* and shown to have sig-



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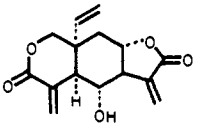
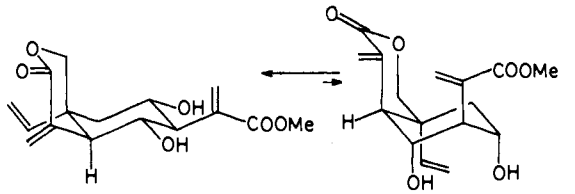


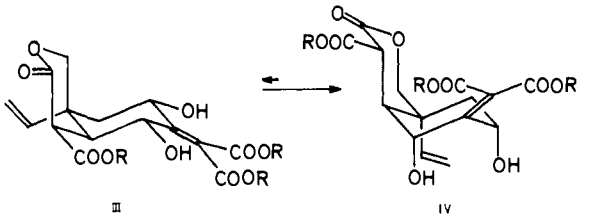
2

nificant in vitro cytotoxicity (KB) and in vivo tumor inhibitory activity against Walker intramuscular carcinosarcoma in rats.[2] Multifaced synthetic attack toward this complex natural product has led to the total syntheses by Grieco,[3] Danishefsky,[4] and ourselves,[5] and a formal total synthesis by Schlessinger[6] and many other valuable synthetic approaches.[7] This full paper, part 2, deals with accomplishment of our total synthesis of **1** from the key synthetic intermediate **3**,[8] the preparation of which has recently been reported in part 1[9] with experimental details.

Conformational analyses of vernolepin prototypes (I to IV) suggest the methodology of functionalization to our key synthetic intermediate (**3**) as shown in Scheme I. The following is our synthetic strategy principally based on the conformational analysis of the B ring of **1**. In the two possible conformers of the acidic methanolysate of **1**, it should exclusively exist in the form of the conformer I rather than II. However, the latter one, the thermodynamically unstable form, contains promising axial bondings to be introduced into the cyclohexanone ring of the precursor (**3**). The fixation of the conformation into the currently interesting type II could be ensured by taking another account for thermodynamic stability, i.e., steric interference in the *exo*-olefin system like III and IV. In this case, equilibration should occur largely into IV as the type II, since in III



I II



III IV

the spacial repulsion between the two hydroxyl groups and two carboalkoxy groups in the planarity would be much greater than the 1,3-diaxial interaction in the conformer IV.[10] Its two axial hydroxyl groups could further assist the stereospecific reduction of the exo-double bond by coordinating with hydride reagent for the hydride to attack from the α side. Introduction of their hydroxyl groups into axial orientation should thus be achieved after condensation of malonyl residue to the key compound (**3**). These conformational analysis and resulting strategy led us to succeed in the stereocontrolled total synthesis (Scheme I) which involves no separation process of stereoisomers at all.

Knoevenagel Condensation of Malonates with Key Compound. The first step for the total synthesis starting from **3** is Knoevenagel condensation, which was first achieved on **11**, as

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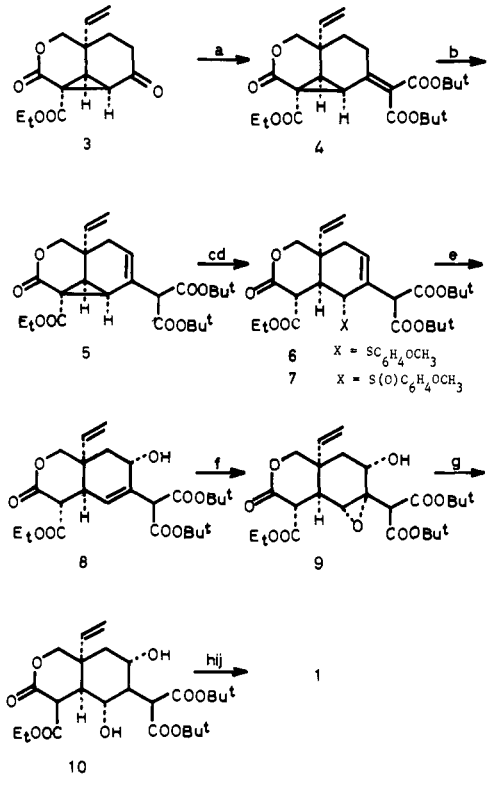
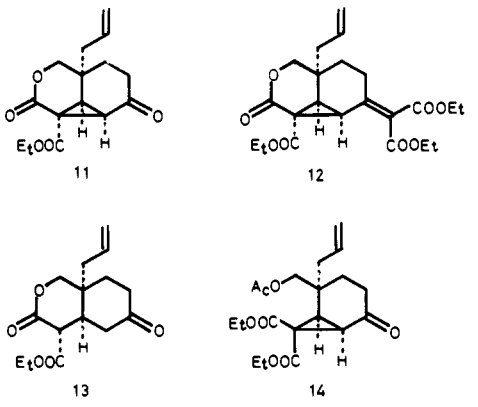
Scheme I

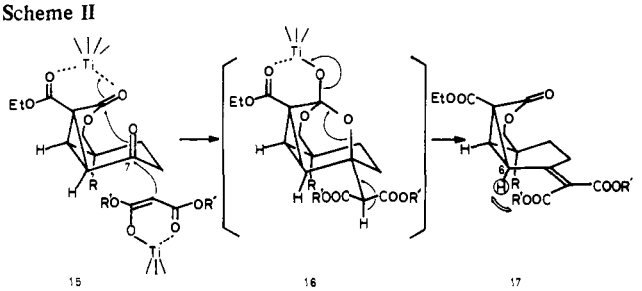


a model, in 88% yield to give **12** with diethylmalonate in the presence of titanium(IV) chloride and pyridine in THF. Lehnert[11] has described this condensation condition with simple cyclohexanone derivatives in high yields. Low yields

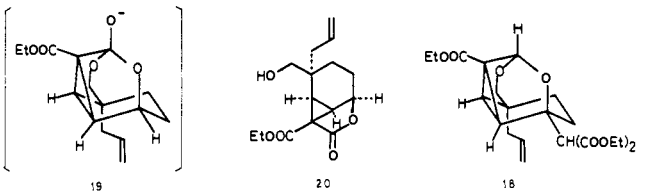


(almost no reaction occurred under the same condition) were, however, the case in compound **13**, precursor for **11**, which has active methine proton at the C-4 position, and the case in compound **14** with cyclopropane ring. The mechanism could be figured out like the sequence in Scheme II [**15** → orthoester **16** → **17**], which involves oxygen transfer from the 7 position to the 3 position. These two positions were found to be spatially

Scheme II



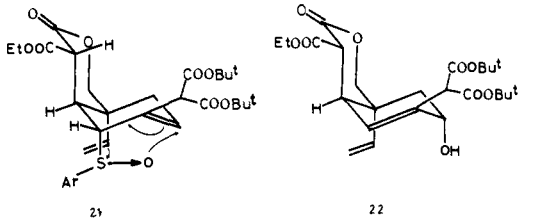
quite close to each other in a system like **18**, since the reduction of **12** (i.e., **17**, R = allyl, R′ = Et) with sodium borohydride afforded cyclic ketal **18** having similar caged ether bonds. Furthermore, sodium borohydride reduction of **11** (i.e., **15**, R = allyl, hydride attacking at the C-7 position), has been shown to give a transesterified product **20** via orthoester intermediate



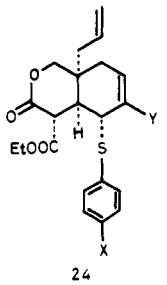
19 similar to **16**.[8] In the Knoevenagel condensation of **3** or **11**, such an intramolecular process is considered to render the initial step to proceed very smoothly. In the condensation of **3** with di-*tert*-butyl malonate, the initial step (**15** → **16**, R = vinyl, R′ = Bu′) proceeded very rapidly (in 10 min at 0 °C), but the dehydration step (**16** → **17**) completed in a fairly longer period while partial hydrolysis of the *tert*-butyl ester occurred to lower the yield (ca. 60%). Although addition of thionyl chloride into this reaction mixture (after 10 min) shortened the dehydration period, no improvement was observed in the yield of **17**. We found, later on, that only the *tert*-butyl compound **4** was the possible precursor for the total synthesis of **1**.

The condensed product **4** readily deconjugated in THF with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) at room temperature in 3 h. This deconjugation can be monitored by UV shift of the absorption maximum at λ_{238nm} (conjugated) into λ_{218nm} (unconjugated) in the course of reaction and was indeed confirmed after workup by appearance of NMR signal of a new olefinic proton at δ 5.97 ppm (broad doublet, J = 6 Hz). The driving force for this deconjugation is likely to be the relaxation of the spatial steric repulsion between the ester carbonyl group and the C-6-H in the same plane (see **17**). In the case of the triethyl ester **12** with smaller steric repulsion, this deconjugation largely completed in fairly drastic condition, potassium acetate with dicyclohexyl-18-crown-6 in acetonitrile at refluxing temperature, but incompleted with DBU in 3 h at room temperature.

Introduction of Hydroxyl Group at C-8 Position. Introduction of hydroxyl group to compound **5** eventuated via indirect method involving [2,3]-sigmatropic rearrangement of **21**, and the first step for the purpose was to obtain a thioaryl adduct as **6**. Phenyl sulfinyl anion cleaved the cyclopropane ring[12] of **5** by selective attacking from the α side at the allylic C-6 position. Similar opening was observed with sodium phenyl selenide[9] instead of sulfide in THF but failed with alkoxides such as potassium superoxide in the presence of crown ether or any other oxygen anion as nucleophile. This may be rationalized by the soft-soft interaction of HSAB principle, and only the phenyl and anisyl sulfinyl anions were found to produce the useful adduct **6** (vide infra). Conformation of the thiophenyl group was analyzed to be axial by NMR which showed the following coupling constants, $J_{5,6}$ = 3 Hz and $J_{4,5}$ = 10 Hz; and, thus, the corresponding sulfoxide should also have the same axial orientation like **21**, which is favorable for [2,3]-sigmatropic rearrangement into **22** having axial C-8 hydroxyl group in the α configuration.



Oxidation of the sulfide **6** with *m*-chloroperbenzoic acid (mCPBA) at −78 °C in methylene chloride gave diastereomeric mixture (practically 1:1) of the corresponding sulfoxides **7** indicating the same *m/e* M + 1$^+$ peak in the field desorption mass spectra. This mixture (**7**), without separation, was heated in ethanol in the presence of trimethyl phosphite as thiophile,[13] and both of them were observed to proceed [2,3]-sigmatropy in slightly different velocity. The rearrangement was best accomplished in 4 h with the *p*-methoxyphenyl sulfoxides (**7**) by heating at 70 °C to give 87% yield of the allylic alcohol **8.** The reason why we chose the anisyl group instead of the phenyl one follows the reasoning that the major side reaction which occurred during this rearrangement was 1,2-syn elimination of sulfenic acid to afford the diene **23** with concomitant double bond isomerization. In the general form **24,** we have studied[14]



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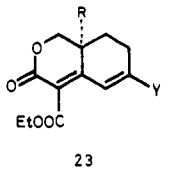

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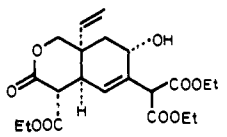

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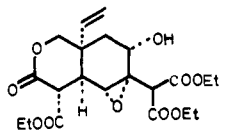

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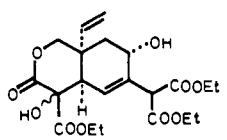

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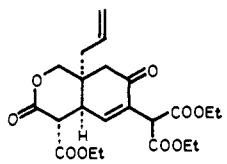
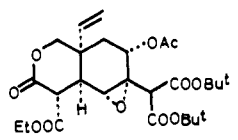


28

the substituent effects of X (OMe, H, Cl, and NO_2) and Y (OEt and 2-malonyl) on the ratio of the rearrangement vs. elimination and have disclosed that sigmatropy predominated in the more electron donative X and in the less electron donative Y substituents.

Preparation of All the Asymmetric Centers. The allyl alcohol **8** was converted into the desired epoxide **9** in 81% yield from **8** with mCPBA at room temperature for 30 h in methylene chloride in the presence of water which was indispensable to diminish the acidity of *m*-chlorobenzoic acid produced in the medium during the reaction. Oxidation of **25,** for instance, aiming for **26,** with mCPBA in aqueous THF or in methylene chloride with sodium carbonate afforded undesired **27** [*m/e* 440 (M$^+$); δ 5.55 (H-6, d, *J* = 4.5 Hz), 3.40 ppm (H-5, d, *J* = 4.5 Hz)], while vanadyloxy-acac catalyzed oxidation[15] of the allyl analogue of **25** gave in very low yield the enone **28** [*m/e* 436 (M$^+$); δ 6.75 (H-6, d, *J* = 5 Hz), 3.31 (H-5, dd, *J* = 5 and 8 Hz), 2.56 ppm (H-9, s)]. The epoxide **26** was detectable only in an NMR tube with triethyl ester **25** and mCPBA in deuteriochloroform and deuterium oxide, but it was so unstable to be isolated in pure state without partial decomposition by using silica gel for chromatographic separation. These troubles obliged us to prepare the di-*tert*-butyl malonyl compounds. Treatment of the di-*tert*-butyl malonyl epoxide **9** with acetic anhydride in pyridine afforded the crystalline acetate **29.** Both **9** and **29** were found to be stable; this stability may be because the bulkiness of *tert*-butyl groups may prevent the oxirane from coming in contact with silica gel. However, epoxide **9** was very labile to zinc chloride, triethylamine, or sodium borohydride etc. to give complex decomposed mixture.



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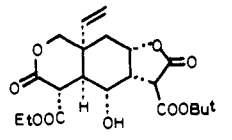

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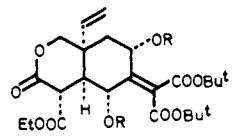
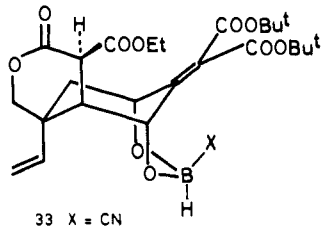
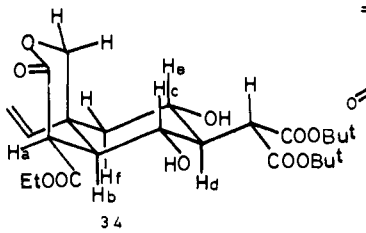


31 R = H
32 R = Ac



33 X = CN
= H



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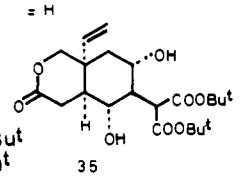

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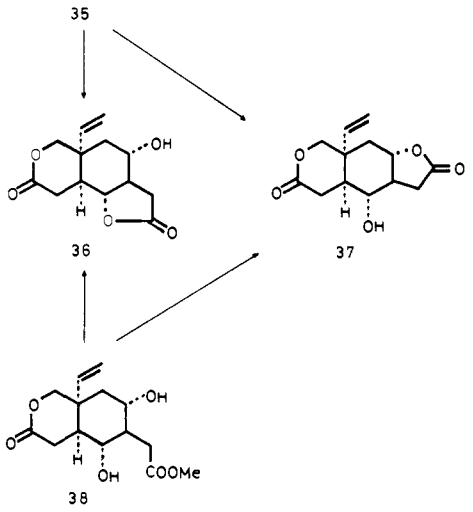
Treatment of **9** with sodium cyanoborohydride dissolved in wet THF or anhydrous DME afforded γ-lactone **30** (as the major product) derived from a 7-epi product which might form by conjugate reduction of an intermediate **31** *inter*molecularly attacked by a hydride in the medium from the β side. Various attempts were then made for the *intra*molecular hydride reduction from the α side with the assistance of the 8α-hydroxyl group of **9.** We found that suspended sodium cyanoborohydride in HMPA[16] containing **9** cleaved its C-7–O bond in the oxirane ring which was accompanied by electron donation from the malonyl side chain to give **31,** interestingly without any further double-bond reduction. NMR analysis of the diol **31** ($J_{5,6}$ = 4.5, $J_{8,9'}$ = 6 and 8 Hz) and its crystalline diacetate **32** ($J_{5,6}$ = 2, $J_{8,9'}$ = 8.5 and 8.5 Hz) showed that both of the two hydroxyl groups located in axial orientation,[17] which is the same conformer as IV in the beginning conformational discussion.

Based on the above preliminary examination, in attempt to convert **9** into compound **10,** we succeeded in the conversion (in 73.4% overall yield) in a one-flask reaction without isolating the intermediate product (**31**) under such two-step conditions, treatment of **9** with sodium cyanoborohydride in HMPA at 30 °C for 3 h, and subsequent addition of diborane–THF into the reaction mixture at −45 °C. These conditions were constructed as follows: (1) to avoid intermolecular hydride attack, sodium cyanoborohydride was suspended in anhydrous HMPA solution of **9** so that only its hydroxyl group would pick up the reagent; (2) the resultant boron moiety coordinated with the oxygen on the oxirane ring to cleave it with concomitant formation of the exo-double bond to form a (hypothetical) boron intermediate (**33;** X = CN, having very low reducing activity); (3) diborane mixed at low temperature exchanged the cyano group of the boron intermediate hydride (**33;** X = H); and (4) this hydride could now attack intramolecularly the C-7 carbon from the α side to form conjugate reduction product **10** [mp 129 °C, *m/e* (FD) 499 (M + 1$^+$)] with inversion of the conformation.

The conformation of the product **10** was proven to be **34** by its NMR spectra that showed all axial protons on the B ring to couple in J_{bc} = 11, J_{cd} = 11, J_{de} = 10, and J_{ef} = 9 Hz, respectively. The fact that two hydroxyl groups and a malonyl group locate all in equatorial orientation was further confirmed

by conversion of **10** into so-called bisnorvernolepin (**36**) in three steps. Thus, decarboethoxylation of **10** with basic alumina in heating aqueous dioxane[18] gave **35** [δ 3.01 (H-4$_\beta$, dd, J = 1 and 19 Hz) and 2.60 ppm (H-4$_\alpha$, dd, J = 7 and 19 Hz)] which was subsequently treated with hot trifluoroacetic acid and then heated at 160 °C for 0.5 h to afford a 2:1 mixture of **36** and **37** (bisnorvernomenin). Compound **36** was identical in NMR and TLC with authentic bisnorvernolepin prepared from **38** which was kindly furnished by Professor S. Danishefsky.



Total Synthesis of Vernolepin. The total synthesis of vernolepin from **10** was accomplished in 22% overall yield according to the following four steps without any isolation process of the intermediates. The series of the reaction conditions were intended as follows: first, hydrolysis of ethyl ester of **10** was carried out with Amberlite IRA400 in methanol; secondly, the hydrolysate was eluted from the resin with aqueous trifluoroacetic acid which was evaporated after 30 min at room temperature (during this period presumable formation of γ-lactone dicarboxylic acid); thirdly, the residue was subjected under Mannich reaction condition,[19] diethylamine and formalin followed by sodium acetate and acetic acid. The reaction mixture was extracted with methylene chloride, and the neutral fraction of the extract was chromatographed on silica gel to afford, in 22% overall yield, (±)-vernolepin (**1**), mp 206 °C, whose spectral data were all identical with those already reported.[3,4] A very small amount of vernomenin (**2**) obtained from the same reaction mixture showed an identical NMR spectrum with the reported one.[3,4] The yield of this isomer was below 5%.

In summary, (±)-vernolepin (**1**) was obtained in 4.6% yield in 11 steps from the key compound, which has been prepared in 26% overall yield from dihydroanisyl alcohol.[8,9]

Experimental Section[20]

Knoevenagel Condensation of 3 and Deconjugation of 4. A solution of titanium tetrachloride (3.45 g, 18.2 mmol) in carbon tetrachloride (2 mL) was added dropwise to ice-cooled dry tetrahydrofuran (20 mL) over 15 min under nitrogen atmosphere. Yellow precipitate occurred in the reaction mixture, into which was added a solution of the vinyl ketone **3** (1.45 g, 5.49 mmol), di-*tert*-butyl malonate (2.0 g, 9.26 mmol), and pyridine (6 mL, 74.2 mmol) in tetrahydrofuran (10 mL) at 0 °C. After the reaction mixture was stirred for 16 h at room temperature, additional titanium tetrachloride (0.86 g, 4.55 mmol) in CCl_4 (1 mL) was added and stirred for an additional 6 h at room temperature. Ether (50 mL) was added to the reaction mixture and the resulting precipitate was removed by filtration. The filtrate was washed with water, sodium bicarbonate, and water, dried over anhydrous sodium sulfate, and then evaporated to give 1.77 g (69.8%) of the crude conjugated ester **4**: ^{1}H NMR ($CDCl_3$), δ 5.90 (1 H, dd, J = 11, 17.5 Hz), 5.28 (1 H, d, J = 11 Hz), 5.22 (1 H, d, J = 17.5 Hz), 4.27 (2 H, q, J = 7 Hz), 4.20 (2 H, ABq, J = 11 Hz), 3.57 (1 H, d, J = 9 Hz), 3.10 (1 H, dt, J = 4, 18 Hz), 2.46 (1 H, d, J = 9 Hz), 2.44 (1 H, dt, J = 6, 18 Hz), 1.75 (2 H, m), 1.51 (9 H, s), 1.47 (9 H, s), 1.31 (3 H, t, J = 7 Hz), which was used for the next reaction without further purification. The crude **4** (1.77 g, 3.83 mmol) was dissolved in THF (25 mL) and then stirred with DBU (1.0 g, 6.77 mmol) for 3 h at room temperature. Removal of the solvent under reduced pressure gave an oil which was chromatographed on a silica gel column (10 g). Elution with ether-hexane (2:1) gave 1.42 g (55.9% from **3**) of the deconjugated ester **5**, and elution with ether gave 60 mg of recovered **3**. Crystallizations from ether-hexane-petroleum ether afforded analytically pure **5**, mp 86-87 °C: ^{1}H NMR ($CDCl_3$) δ 5.97 (1 H, brd, J = 6 Hz), 5.94 (1 H, dd, J = 11, 17 Hz), 5.26 (1 H, d, J = 11 Hz), 5.18 (1 H, d, J = 17 Hz), 4.24 (2H, q, J = 7 Hz), 4.05 (2 H, ABq, J = 11 Hz), 3.94 (1 H, s), 2.84 (1 H, d, J = 9.5 Hz), 2.55 (1 H, brd, J = 18), 2.40 (1 H, d, J = 9.5 Hz), 2.20 (1 H, dd, J = 6, 18 Hz), 1.44 (18 H, s), 1.29 (3 H, t, J = 7 Hz); Analysis ($C_{25}H_{34}O_8$): C, H.

Opening the Cyclopropane Ring of 5 and [2,3]-Sigmatropic Rearrangement of 7. Sodium *p*-methoxythiophenolate, prepared from *p*-methoxythiophenol (1.4 g, 10 mmol) and sodium hydride (0.4 g, 10 mmol, 60% mineral oil, washed with dry petroleum ether) in THF (60 mL) at room temperature under argon atmosphere, was mixed with a solution of the deconjugated ester **5** (4.6 g, 10 mmol) in THF (20 mL). After stirring for 30 min, the reaction mixture was poured into cold 0.1 N HCl (100 mL) and extracted with ether. The extract was washed with sodium bicarbonate, water, and brine, dried over anhydrous sodium sulfate, and then evaporated. The residue was chromatographed on silica gel column (150 g, eluted with ether-hexane, 1:1, then ether-hexane, 3:1). Elution with ether-hexane (3:1) gave 5.17 g (86.2%) of the sulfide **6**: ^{1}H NMR ($CDCl_3$) δ 7.20 (4 H, m), 5.96 (1 H, t, J = 4 Hz), 5.94 (1 H, dd, J = 12, 18 Hz), 5.28 (1 H, d, J = 12 Hz), 5.24 (1 H, d, J = 18 Hz), 4.35 (1 H, s), 4.19 (2 H, ABq, J = 9 Hz), 4.00 (2 H, dq, J = 7, 7 Hz), 3.80 (3 H, s), 3.58 (1 H, d, J = 10 Hz), 3.25 (1 H, brs), 3.04 (1 H, dd, J = 3, 10 Hz), 2.32 (2 H, dABq, J = 4, 18 Hz), 1.50 (9 H, s), 1.44 (9 H, s), 1.16 (3 H, t, J = 7 Hz); ν ($CHCl_3$) 2930, 1740, 1730, 1597, 1498, 1375 cm^{-1}; *m/e* (FD) 603 (M + 1$^+$).

To a cold (−78 °C) solution of the sulfide **6** (5.17 g, 8.6 mmol) in methylene chloride (100 mL) was added a solution of 80% mCPBA (1.85 g, 8.6 mmol) in methylene chloride (10 mL). After stirring for 30 min, the reaction mixture was treated with saturated sodium sulfite solution, and then the cooling bath was removed. When the organic layer became negative to KI-starch test paper, it was washed with sodium bicarbonate solution and water, and dried over anhydrous sodium sulfate. Removal of the solvent gave 5.20 g (97%) of a diastereomeric mixture of the crude sulfoxides **7**. The crude sulfoxides **7** were dissolved in ethanol (120 mL) and then heated with trimethyl phosphite (1.16 g, 9.3 mmol) at 70 °C for 4 h under nitrogen atmosphere. The reaction mixture was concentrated and chromatographed on silica gel column (300 g, eluted with ether-hexane, 2:1) to give the pure allyl alcohol **8** (3.05 g, 74%) from the sulfide **6**. In another batch when the sulfoxides **7** were purified (84% yield) with SiO_2, the diastereomeric mixture rearranged into **8** in 87% yield. **8**: δ ($CDCl_3$) 5.92 (1 H, dd, J = 11, 17.5 Hz), 5.63 (1 H, d, J = 3 Hz) 5.29 (1 H, d, J = 17.5 Hz), 5.24 (1 H, d, J = 11 Hz), 4.30 (2 H, q, J = 7 Hz), 4.25 (1 H, m), 4.15 (2 H, s), 4.09 (1 H, s), 3.25 (2 H, m), 1.88 (2 H, m), 1.45 (18 H, s), 1.31 (3 H, t, J = 7 Hz): ν ($CHCl_3$) 3430, 2950, 1755 (sh), 1738 cm^{-1}. For analytical purpose, the allyl alcohol **8** (100 mg, 0.21 mmol) was acetylated with 1 mL of acetic anhydride and pyridine (1 mL) for 5 h at room temperature. The reaction mixture was dried up in vacuo, and the residue was triturated in petroleum ether to give 90 mg (83%) of the crystalline acetate of **8**. Recrystallization from ether gave analytically pure needle, mp 105-106 °C; Analysis ($C_{27}H_{38}O_{10}$): C, H.

Epoxidation of 8. The allyl alcohol **8** (2.80 g, 5.83 mmol) dissolved in methylene chloride (150 mL) was stirred with 80% *m*-chloroperbenzoic acid (1.50 g, 6.95 mmol) and water (50 mL) for 20 h at room temperature. Additional mCPBA (0.3 g, 1.4 mmol) was then added, and stirring was continued for further 10 h. Excess mCPBA was decomposed with aqueous sodium sulfite. The organic layer was washed with sodium bicarbonate solution and water, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed on silica gel column (280 g, eluted with ether-hexane, 3:1) to give 2.32 g (80.2%) of the epoxide **9**: δ ($CDCl_3$) 5.70 (1 H, dd, J = 11, 17 Hz), 5.26 (1 H, d, J = 17 Hz), 5.20 (1 H, d, J = 11 Hz), 4.30 (2 H, q, J = 7 Hz), 4.18 (2 H, ABq, J = 11 Hz), 4.11 (1 H, s), 4.00 (1 H, m), 3.56 (1 H, d, J = 9.5 Hz), 3.20 (1 H, s), 3.06 (1 H, d, J = 9.5 Hz), 2.47 (1

H, d, J = 9 Hz, O*H*), 1.70 (2 H, m), 1.40 (18 H, s), 1.36 (3 H, t, J = 7 Hz); ν ($CDCl_3$) 3490, 2940, 1755, 1740, 1725 cm^{-1}. For analytical purpose, the hydroxy epoxide **9** (150 mg, 0.30 mmol) was acetylated with 1 mL of acetic anhydride and 1 mL of pyridine for 5 h at room temperature. The reaction mixture was dried up in vacuo, and the residue was chromatographed on silica gel column (eluted with ether-hexane, 2:1) to give 120 mg (74%) of the acetoxy epoxide **29.** Crystallization from ether gave analytically pure **29**, mp 160-161 °C. Analysis ($C_{27}H_{38}O_{11}$): C, H.

Opening of the Oxirane Ring in 9 and Intramolecular Conjugate Reduction to 10. A pink solution of the epoxy alcohol **9** (1.94 g, 3.91 mmol) in HMPA (40 mL) was treated with sodium cyanoborohydride (0.30 g, 4.8 mmol) at 30 °C for 3 h under argon atmosphere resulting a red solution, which was diluted with tetrahydrofuran (25 mL) and cooled to −45 °C. To this cold mixture was added 1.0 M diborane-THF solution (25 mL, 25 mmol), and the mixture was stirred for 6 h at that temperature to give a light yellow solution. Cracked ice was added to the reaction mixture (evolution of hydrogen gas) and the mixture was extracted with ether. The extract was washed with water and brine, dried over anhydrous sodium sulfate, and evaporated. The residue was chromatographed on silica gel column (180 g, eluted with ether-hexane, 3:1) to give 1.38 g (73.4%) of the diol **10**, which was triturated in petroleum ether resulting in colorless prisms, mp 120-125 °C. Recrystallization from ether-petroleum ether gave analytically pure **10:** mp 128-129 °C; δ ($CDCl_3$-D_2O) 5.76 (1 H, dd, J = 11, 17.5 Hz), 5.30 (1 H, d, J = 17.5 Hz), 5.16 (1 H, d, J = 11 Hz), 4.17 (2 H, brs), 4.16 (2 H, q, J = 7 Hz), 3.95 (1 H, d, J = 2.5 Hz), 3.84 (1 H, br-ddd, J = 5, 9, 10 Hz), 3.74 (1 H, d, J = 4 Hz), 3.60 (1 H, t, J = 11 Hz), 2.60 (1 H, dd, J = 4, 11 Hz), 2.08 (1 H, dt, J = 2.5, 10 Hz), 1.85 (1 H, dd, J = 5, 14 Hz), 1.60 (1 H, dd, J = 9, 14 Hz), 1.45 (18 H, s), 1.25 (3 H, t, J = 7 Hz); ν (Br) 3500, 3430, 2980, 2930, 1740, 1723, 1640, 1482, 1462, 1430, 1398, 1372, 1343, 1310, 1260, 1165, 1140, 1069, 1049, 1031, 999, 920, 848 cm^{-1}; *m/e* (FD) 499 ($M + 1^+$), (EI) 425, 386, 369, 360, 323, 283, 265 (base); Anal. ($C_{25}H_{38}O_{10}$): C, H.

Isolation of the Ene-Diol Intermediate 31. A pink solution of the epoxide **9** (150 mg, 0.302 mmol) in HMPA (3 mL) was stirred with sodium cyanoborohydride (30 mg, 0.48 mmol) for 1 h at room temperature under nitrogen atmosphere. The reaction mixture was poured into a cold 0.1 N HCl solution and extracted with ether. The extract was washed with sodium bicarbonate solution, water, and brine, dried over anhydrous sodium sulfate, and then evaporated to give 137 mg of the ene-diol **31:** δ ($CDCl_3$-D_2O) 5.92 (1 H, dd, J = 11, 17.5 Hz), 5.32 (1 H, d, J = 17.5 Hz), 5.24 (1 H, d, J = 11 Hz), 4.68 (1 H, dd, J = 6, 8 Hz), 4.52 (1 H, d, J = 4.5 Hz), 4.30 (2 H, q, J = 7 Hz), 4.03 (2 H, ABq, J = 13 Hz), 3.30 (1 H, d, J = 10 Hz), 3.10 (1 H, dd, J = 4.5, 10 Hz), 2.38 (1 H, dd, J = 8, 14 Hz), 1.82 (1 H, dd, J = 6, 14 Hz), 1.53 (9 H, s), 1.51 (9 H, s), 1.33 (3 H, t, J = 7 Hz). The ene-diol **31** (137 mg) was acetylated with acetic anhydride (1.5 mL) and pyridine (1 mL) for 12 h at room temperature. The reaction mixture was dried up in vacuo to give a yellow oil, which was chromatographed on silica gel column (eluted with ether-hexane, 2:1) to afford crude crystals of the diacetate **32** (90 mg, 51.3% from the epoxide **31**). Recrystallization from ether gave analytically pure **32**, mp 141-142 °C. Analysis ($C_{29}H_{40}O_{12}$): C, H.

Conversion of 10 into Bisnorvernolepin (36) for Identification with Authentic Sample. The diol **10** (49.8 mg, 0.1 mmol) was heated at 100 °C for 14 h with a mixture of aluminum oxide 150 basic (type T) (0.8 g), dioxane (2 mL), and water (0.03 mL). Aluminum oxide was removed by filtration and thoroughly washed with ether. The filtrate was concentrated and the residue was chromatographed on silica gel column (4 g, eluted with ether-hexane, 3:1) to give 20.4 mg (48%) of the decarboethoxylated diol **35:** δ ($CDCl_3$-D_2O) 6.78 (1 H, dd, J = 11, 18 Hz), 6.27 (1 H, d, J = 11 Hz), 6.27 (1 H, d, J = 18 Hz), 4.18 (2 H, s), 4.05 (1 H, d, J = 2 Hz), 3.81 (1 H, td, J = 5, 11 Hz), 3.61 (1 H, t, J = 11 Hz), 3.01 (1 H, dd, J = 1, 19 Hz), 2.60 (1 H, dd, J = 7, 19 Hz), 1.90 (4 H, m), 1.48 (18 H, s). The decarboethoxylated diol **35** (6 mg) was treated with aqueous trifluoroacetic acid (0.05 mL, TFA-water, 9:1) for 15 min at room temperature. The reaction mixture was dried up in vacuo, and the residue was taken up with benzene. After removal of the solvent, the residue was heated in 0.5 mL of *o*-dichlorobenzene at 160 °C for 30 min. The reaction mixture was dried up in vacuo and the residue was purified on silica gel TLC to afford 1 mg of pure bisnorvernolepin (**36**) and 0.5 mg of bisnorvernomenin (**37**), whose R_f values on three different solvent systems were coincidental with those of authentic bisnorvernolepin derived from **38** kindly furnished by Professor Danishefsky. The NMR spectrum of bisnorvernolepin (**36**) was also identical with that reported:[4] (**36**) δ ($CDCl_3$) 5.80 (1 H, dd, J = 9.5, 18 Hz), 5.3[5] (1 H, d, J = 18 Hz), 5.36 (1 H, d, J = 9.5 Hz), 4.32 (2 H, s), 3.99 (1 H, t, J = 11 Hz), 3.90 (1 H, m), 2.0-2.9 (6 H, m), 1.78 (2 H, dABq, J = 5, 10, 14 Hz): *m/e* 252 (M^+).

Preparation of Vernolepin. The diol **10** (40 mg, 0.080 mmol) was dissolved in methanol (2 mL) and then stirred with Amberlite IRA400 (0.3 mL, activated with 10% NaOH and thoroughly washed with water) for 30 min at room temperature and then the reaction mixture was packed into a small glass column. An eluate with 1 mL of aqueous trifluoroacetic acid (TFA-water, 1:1) was allowed to stand for 30 min and then dried up in vacuo. The residue (22 mg) was treated successively with 0.2 mL of diethylamine and 30% formalin (0.4 mL) at room temperature for 15 min and then at 100 °C for 30 min, and with 40 mg of sodium acetate and acetic acid (0.4 mL) at 100 °C for 30 min. The reaction mixture was extracted with methylene chloride and the extract was washed with a sodium bicarbonate solution and water, dried over anhydrous sodium sulfate, and evaporated to give 7.2 mg of the crude product. It was chromatographed on silica gel TLC (AcOEt-ether, 1:2) affording 4.8 mg (22%) of (±)-vernolepin (**1**), crystallization from chloroform afforded colorless prisms: mp 206 °C; δ ($CDCl_3$) 6.75 (1 H, t, J = 1.0 Hz), 6.25 (1 H, d, J = 3 Hz), 6.03 (1 H, d, J = 3 Hz), 5.95 (1 H, d, J = 1.0 Hz), 5.75 (1 H, dd, J = 10, 18 Hz), 5.30 (1 H, d, J = 10 Hz), 5.27 (1 H, d, J = 18 Hz), 4.32 (1 H, d, J = 12 Hz), 4.20 (1 H, dd, J = 1.5, 12 Hz), 4.08 (1 H, dt, J = 5, 10 Hz), 3.94 (1 H, t, J = 11 Hz), 2.98 (1 H, brd, J = 11 Hz), 2.68 (1 H, tt, J = 3, 10 Hz), 2.00 (1 H, dd, J = 5, 14 Hz), 1.65 (1 H, dd, J = 10, 14 Hz); ν ($CHCl_3$) 1775, 1727 cm^{-1}; *m/e* 276 (M^+), 258, 246, 228 (base peak); exact mass, found *m/e* 276.1008, calcd 276.0998 for $C_{15}H_{16}O_5$. Analysis ($C_{15}H_{16}O_5 \cdot 1/3H_2O$): C, H.

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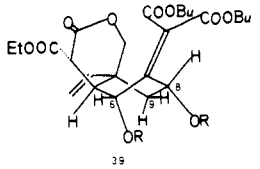
Supplementary Material Available: 100-MHz ^1H NMR charts of **1, 4, 5, 6, 8, 9, 10, 29, 31**, and **32:** IR charts of **1, 5, 6, 8, 9, 10, 29,** and **32;** and data of ^1H NMR and/or ^{13}C NMR of **6, 9, 10, 12, 18, 25, 27, 28**, and **31** (23 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Synthesis of Sesquiterpene Antitumor Lactones. 5; for the preceding paper 4, "Total Synthesis of Vernolepin I. Synthesis of the Key Intermediate", see reference 9.
(2) S. M. Kupchan, R. J. Hemingway, D. Werner, A. Karim, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.*, **90**, 3596 (1968); S. M. Kupchan, M. Eakin, and A. Thomas, *J. Med. Chem.*, **14**, 1147 (1971).
(3) P. A. Grieco, M. Nishizawa, S. D. Burke, and N. Marionvic, *J. Am. Chem. Soc.*, **98**, 1612 (1976); P. A. Grieco, M. Nishizawa, T. Oguri, S. D. Burke, and N. Marionvic, *ibid.*, **99**, 5773 (1977).
(4) S. Danishefsky, T. Kitahara, P. F. Schuda, and S. J. Etheredge, *J. Am. Chem. Soc.*, **98**, 3029 (1976); S. Danishefsky, T. Kitahara, R. McKee, and P. F. Schuda, *ibid.*, **98**, 6715 (1976); S. Danishefsky, P. F. Schuda, T. Kitahara, and S. J. Etheredge, *ibid.*, **99**, 6066 (1977).
(5) M. Isobe, H. Iio, T. Kawai, and T. Goto, *J. Am. Chem. Soc.*, **100**, 1940 (1978).
(6) G. R. Kieczykowski and R. H. Schlessinger, *J. Am. Chem. Soc.*, **100**, 1938 (1978).
(7) P. M. Wege, R. D. Clark, and C. H. Heathcock, *J. Org. Chem.*, **41**, 3144 (1976), and their work referenced therein; S. Torii, T. Okamoto, and S. Kadono, *Chem. Lett.*, 495 (1977); F. Zutterman, P. DeClercq, and M. Vandewalle, *Tetrahedron Lett.*, 3191 (1977).
(8) M. Isobe, H. Iio, T. Kawai, and T. Goto, *Tetrahedron Lett.*, 703 (1977).
(9) H. Iio, M. Isobe, T. Kawai, and T. Goto, *Tetrahedron*, **35**, 941 (1979).
(10) F. Johnson and S. K. Malhotra, *J. Am. Chem. Soc.*, **87**, 5492 (1965); S. K. Malhotra and F. Johnson, *ibid.*, **87**, 5493 (1965).
(11) W. Lehnert, *Tetrahedron*, **29**, 635 (1973).
(12) S. Danishefsky and G. Rovnyak, *J. Chem. Soc., Chem. Commun.*, 820 (1972).
(13) R. Tang and K. Mislow, *J. Am. Chem. Soc.*, **92**, 2100 (1970); D. A. Evans and G. C. Andrews, *Acc. Chem. Res.*, 7, 147 (1974).
(14) M. Isobe, H. Iio, M. Kitamura, and T. Goto, *Chemistry Lett.*, 541 (1978).
(15) K. B. Sharpless, R. F. Lauer, and A. Y. Teranishi, *J. Am. Chem. Soc.*, **95**, 6136 (1973).
(16) R. F. Borch, M. D. Bernstein, and H. D. Drust, *J. Am. Chem. Soc.*, **93**, 2879 (1971); R. O. Hutchins and D. Kandasamy, *J. Org. Chem.*, **40**, 2530 (1975); R. O. Hutchins, B. E. Maryanoff, and C. A. Milewski, *J. Chem. Soc., Chem.*

Commun., 1097 (1971); C. V. Grudzinshas and M. J. Weiss, *Tetrahedron Lett.*, 141 (1973).
(17) Actual conformation of the diacetate (**32**) [δ 2.19 ($2H_9$, d, J = 8.5 Hz), 5.63 ppm (H_8, t, J = 8.5 Hz)] could be the one inbetween IV and **39**, since A_2X-spin system in H-99–H-8 specifies only a twisted form in B ring around C-8 and C-9.



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(18) A. E. Green, A. Cruz, and P. Crabbe, *Tetrahedron Lett.*, 2707 (1976).
(19) J. Martin, P. C. Watts, and F. Johnson, *J. Chem. Soc., Chem. Commun.*, 27 (1970); P. Grieco and K. Hiroi, *ibid.*, 500 (1973).
(20) Melting points were determined on a hot stage apparatus (uncorrected). IR spectra were recorded on JASCO IR-G. ^{1}H NMR spectra were measured with JEOL MH-100 or FX-100 spectrometer, reporting chemical shifts in δ (ppm) by using Me_4Si as an internal standard. Low resolution electron impact (EI) mass spectra were recorded on JEOL D-100 instrument by using direct probe insertion. High resolution and field desorption (FD) mass spectra were determined on JEOL 01SG2 instrument. Tlc was performed on 0.25-mm pre-coated silica gel PF_{254} plates supplied by E. Merck (Art no. 5715). Preparative TLC separations were made on plates prepared with a 2-mm layer of silica gel PF_{254} obtained from E. Merck (Art no. 7747). Column chromatography was conducted on silica gel supplied by also E. Merck (Art no. 7734).

Studies on Polypeptides. 54. The Synthesis of a Peptide Corresponding to Positions 24–104 of the Peptide Chain of Ribonuclease T_1

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Abstract: A synthesis is described of a partially (*S*-cysteine and N^{ϵ}-lysine) protected peptide containing 81 amino acid residues corresponding to positions 24–104 of the peptide chain of the enzyme ribonuclease T_1. The peptide was assembled by condensing suitably protected fragments in a sequential manner by azide couplings. Every intermediate was purified, characterized, and analyzed. Small intermediates were characterized by thin-layer chromatography, elemental analysis, optical rotation, and amino acid analyses of acid and in some instances aminopeptidase M digests. Large fragments were characterized by thin-layer chromatography, amino acid analyses of acid, and aminopeptidase M digests (except for insoluble compounds) and dansyl end-group determinations. Certain sparingly soluble fragments obtained by azide coupling of acyl components not C-terminating in glycine or proline were acid hydrolyzed and the digests exposed to L-amino acid oxidase to assess the stereochemical homogeneity of certain amino acid residues. Based on the method of synthesis and the results of extensive analytical evaluation, it is concluded that the final peptide does not contain failure sequences and major backbone imperfections. However, the analytical methods employed are not sensitive enough to exclude some racemization.

In previous communications,[1-6] we have described syntheses of various fragments corresponding to sections of the polypeptide chain of the enzyme ribonuclease T_1 [ribonucleate guanine nucleotido-2′-transferase (cyclizing), EC 3.1.4.8] which served as intermediates in fragment condensation studies aimed at total synthesis of the entire peptide chain of the enzyme. Initially we subdivided the T_1 sequence into seven fragments (Figure 1) which were designated by the letters A to G.[3,7] These fragments were assembled from smaller subfragments designated as E_1, E_2, F_1, F_2, and G_1, G_2, G_3, etc. The larger fragments with the exception of A and G were N-protected by the benzyloxycarbonyl group and terminated in a Boc-hydrazide function. This combination of protecting groups made possible the selective deprotection of either the H_2N terminus or the HOOC terminus to generate fragments that could be coupled by the azide method to form larger peptide chains.[8] Using this approach, we succeeded in building up such sections of the T_1 chain as fragments ABCD,[4] BCD,[2] EF,[3] and FG.[6] In these studies we protected the sulfhydryl function of cysteine-103 by an ethylcarbamoyl group[9] and the carboxyl of threonine-104 by an amide function. The ϵ-amino group of lysine-41 was permanently protected by a formyl group.[10] This selection was based on results of chemical modifications of the N-terminal and the ϵ-amino group of lysine-41 which led to the conclusion that these groups are not essential for the activity of the enzyme. However, more recently it was shown that treatment of the enzyme with *cis*-aconitic anhydride brings about irreversible inactivation.[11]

As we gained more experience it became apparent that the original combination of backbone protecting groups was not adequate for the construction of the entire peptide chain of the enzyme and consequently had to be modified. Hydrogenolysis, the method of choice for removing benzyloxycarbonyl groups, was not applicable to sections of the T_1 chain containing cysteine residues, i.e., fragments A and G. Here decarbobenzoxylation was effected by acidolytic cleavage with HBr/TFA. This technique was not likely to succeed with fragments incorporating the acid sensitive single tryptophan residue of the enzyme, i.e., those containing fragment E.

Assembly of Peptide H-CDEFG-OH. In the present communication, we describe experiments which enabled us to assemble a large section of the peptide chain of the enzyme (fragment H-CDEFG-OH (Figure 2) via the following steps:

N-Boc-E-azide + H-FG-OH

$$\rightarrow N\text{-Boc-EFG-OH} \xrightarrow{H^+} \text{H-EFG-OH}$$

N-Msc-CD-azide + H-EFG-OH

$$\rightarrow N\text{-Msc-CDEFG-OH} \xrightarrow{OH^-} \text{H-CDEFG-OH}$$

The amino acid sequences of these fragments are illustrated on Figure 3.

Since Boc groups can be cleaved under conditions that do not result in tryptophan destruction, *N*-Boc-E-hydrazide was

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