# Improved Synthesis of [4-Alanine]chlamydocin: Cyclization Studies of Tetrapeptides Containing Five α-Substituents[1]

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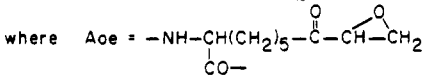
A complete search for the optimal conditions for preparing cyclic tetrapeptide **2** was carried out. In this study all four possible sequences of the linear tetrapeptide precursors were synthesized and cyclized. The results establish that one linear sequence is especially favorable for synthesizing the peptide ring system in chlamydocin (1).

Chlamydocin (1) is a cytostatic cyclic tetrapeptide isolated from culture filtrates of *Diheterospora chlamydosporia* and characterized by Closse and Huguenin in 1974.[2] Chlamydocin was reported to be about 10–100 times more active than actinomycin D, vinblastine, vincristine, amethopterin, and colchicine for inhibition of cell growth in mouse P-815 mastocytoma cells ($ED_{50}$ = 0.36 ng/mL)[3] and to inhibit $C_6$ rat glial tumor cells ($ED_{50}$ = 1 ng/mL) at concentrations that do not cause cell lysis. While these results indicate that chlamydocin is cytostatic, little is known about its site of action.

cyclo (Aibu-L-Phe-D-Pro-L-Aoe) (1)

where Aoe = 

The unusual structure and potent biological activity of this molecule have stimulated our synthetic[4] and conformational[5,6] studies of analogues of chlamydocin. Approaches to the synthesis of the cyclic tetrapeptide ring system found in chlamydocin have been evaluated by using the model cyclic tetrapeptide, [Ala[4]]chlamydocin [*cyclo*(Aib-L-Phe-D-Pro-L-Ala), **2**].[4a,7] Direct cyclization of the linear precursor HCl·Aib-Phe-D-Pro-Ala-OTcp at 90 °C in pyridine gave only trace amounts (~1% yield) of monomeric cyclic tetrapeptide **2**. Cyclization of a linear precursor, H·Aib-Phe[(*Z*)Δ]-D-Pro-L-Ala-OTcp, in which (*Z*)-dehydrophenylalanine (Phe[(*Z*)Δ]) replaced L-phenylalanine, gave *cyclo*(Aib-Phe[(*Z*)Δ]-D-Pro-Ala) which was converted to **2** by stereospecific hydrogenation of the cyclic dehydrophenylalanine residue.[4a] However, the overall yield for the cyclization and reduction reactions was still low (~10%).

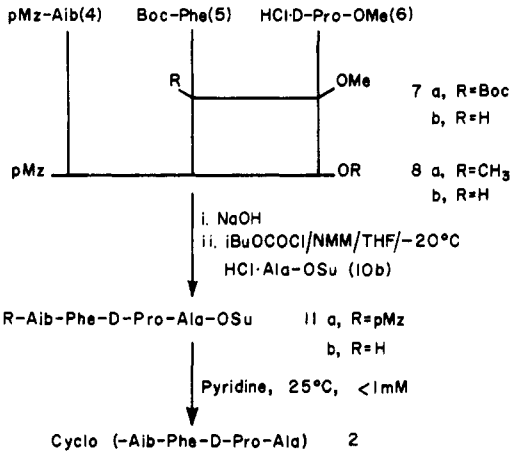
These results prompted us to carry out a complete search for optimal conditions for preparing cyclic tetrapeptide **2**. For this study we synthesized and cyclized all four possible sequences of the linear tetrapeptide precursors. Our results, reported herein, establish that one linear sequence is especially favorable for synthesizing the peptide ring system in chlamydocin.

## Results

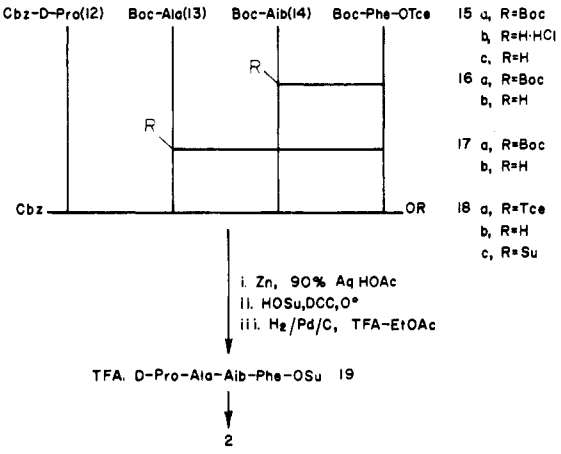
**Synthesis of Linear Tetrapeptides.** The four linear tetrapeptide precursors are shown in Schemes I–IV. Protected sequence Aib-Phe-D-Pro-Ala (**8a**) was synthesized by the route outlined in Scheme I with DCC/HOBt[8] as the coupling reagent. Dipeptide **7a** was obtained in 85% yield and tripeptide **8a** in 87% yield.

Tripeptide **8a** was saponified and coupled to L-alanine *N*-hydroxysuccinimide ester with isobutyl chloroformate according to typical mixed-anhydride activation conditions.[9] Tetrapeptide active ester **11a** was N-deprotected and cyclized immediately via slow addition (8 h) to a large

Scheme I




Scheme II

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(1) Abbreviations used: Aib = α-aminoisobutyric acid; OTcp = 2,4,5-trichlorophenyl ester; OSu = *N*-hydroxysuccinimide ester; DCC = dicyclohexylcarbodiimide; HOBt = 1-hydroxybenzotriazole; pMZ = *p*-methoxybenzyloxycarbonyl; Boc = *tert*-butoxycarbonyl; Cbz = benzyloxycarbonyl; OTce = 2,2,2-trichloroethyl ester; TFA = trifluoroacetic acid; EtOAc = ethyl acetate; EEDQ = *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline; Aoe = L-2-amino-8-oxo-9,10-epoxydecanoic acid; TLC = thin-layer chromatography; MeOH = methyl alcohol; EDC = 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide.

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(3) Stahelin, H.; Trippmacher, A. *Eur. J. Cancer* **1974**, *10*, 801.

(4) (a) Rich, D. H.; Jasensky, R. D. *J. Am. Chem. Soc.* **1979**, *101*, 5412. (b) Rich, D. H.; Jasensky, R. D.; Mueller, G. C.; Anderson, K. E. *J. Med. Chem.* **1981**, *24*, 567. (c) Rich, D. H.; Jasensky, R. D.; Singh, J. "Neurohypophyseal Peptide Hormones and Other Biologically Active Peptides"; Schlesinger, D. H., Ed.; Elsevier/North-Holland: Limerik, Ireland, 1981; pp 49–61.

(5) (a) Rich, D. H.; Jasensky, R. D. *J. Am. Chem. Soc.* **1980**, *102*, 1112. (b) Rich, D. H.; Jasensky, R. D. "Peptides: Structure and Biological Function"; Gross, E., Meienhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; pp 487–490.

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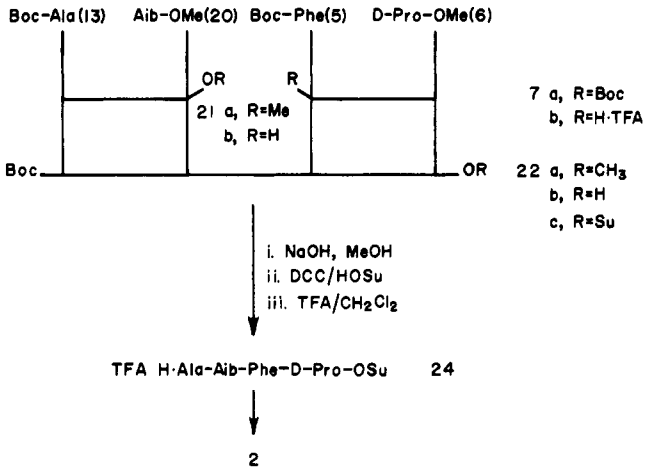
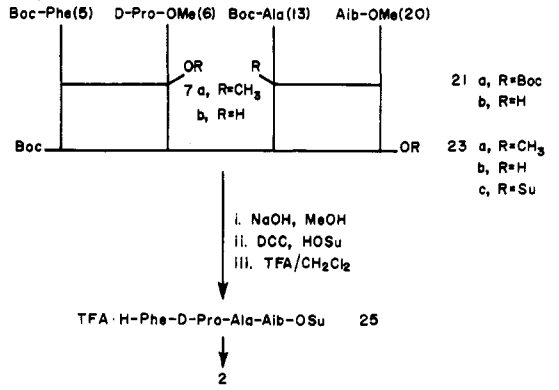
Scheme III




Scheme IV




volume of pyridine under conditions where the maximal possible concentration was less than 1 mM. After isolation and purification by preparative TLC, [Ala$^4$]chlamydocin (**2**) was isolated in 3% yield. This yield was not significantly different from that obtained earlier[4a] by using the 2,4,5-trichlorophenyl ester in pyridine at 90 °C for the cyclization conditions.

The linear sequence D-Pro-Ala-Aib-Phe (**18a**) was synthesized in stepwise fashion by the route outlined in Scheme II. By use of the trichloroethyl ester (see **15a**) as the C-terminal protecting group, dipeptide **16a** was obtained in good yield (81%) by using DCC/HOBt. However, coupling of Boc-Ala to neutralized dipeptide **16b** with DCC/HOBt as the coupling reagent gave only a modest yield (46%) of tripeptide **17a**. This result was not unexpected because the amino group of $\alpha$-aminoisobutyric acid is known to exhibit low reactivity in peptide condensation reactions.[10] To improve the yield for this step, we tried two other coupling procedures. The first employed the "symmetrical anhydride" technique[11] in which 1.5 equiv of Boc-Ala anhydride was generated by reaction of DCC (1.5 equiv) with 3 equiv of Boc-Ala. The symmetrical anhydride was then allowed to react with 1 equiv of free amine dipeptide **16b**, but tripeptide **17a** was isolated only in moderate yield (41%). EDC [ethyl[3-dimethylamino)propyl]carbodiimide] in place of DCC to generate Boc-Ala symmetrical anhydride gave a slightly higher yield (54%) of tripeptide **17a**. In view of the subsequent cyclization results no further attempts to improve this coupling were carried out. The coupling of Cbz-D-Pro to tripeptide **17a** gave linear tetrapeptide **18a** in good yield (91% from protected tripeptide). Tetrapeptide ester **18a** was converted to *N*-hydroxysuccinimide ester **18c** using the conditions outlined in Scheme II. The trichloroethyl ester group was removed by reaction with zinc in aqueous acetic acid and the acid **18b** converted to the *N*-hydroxysuccinimide ester **18c**. The overall yield for these two steps was 86%.

Table I

| compd | sequence[a] | % yield of cyclic tetrapeptide 2[b] |
|---|---|---|
| | X·Aib-Phe-D-Pro-Ala-OTcp | 2 |
| 11 | X·Aib-Phe-D-Pro-Ala-OSu | 3 |
| 19 | X·D-Pro-Ala-Aib-Phe-OSu | 3 |
| 24 | X·Ala-Aib-Phe-D-Pro-OSu | 44 |
| 25 | X·Phe-D-Pro-Ala-Aib-OSu | 2 |

[a] X indicates the TFA salt for 19, 24, and 25 and HCl for 11. [b] Yields are an average of at least two separate cyclizations and were obtained under identical experimental conditions: 7-8-h addition to pyridine, 25 °C, maximal possible concentration <1.0 mM followed by stirring for 14-16 h at 25 °C.

The Cbz group was removed from **18c** by hydrogenolysis of tetrapeptide succinimide ester **18c** over palladium on carbon and the free amine isolated as the trifluoroacetate salt **19**. This material was cyclized to cyclictetrapeptide **2** by using the same reaction conditions as described for sequence **11a**. Cyclic tetrapeptide **2** was isolated in about 3% yield.

Sequences **22a** (Scheme III) and **23a** (Scheme IV) were prepared by a two plus two strategy to take advantage of the common intermediate dipeptides **21a** and **7a**. Dipeptide **21a** was synthesized by using EEDQ [*N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline][12] as the coupling reagent. Dipeptide **21a** was isolated in a higher yield (94%) by using EEDQ than when either DCC/HOBt (~71%) or the *N*-hydroxysuccinimide active ester (73%) methods were used. It is interesting to note that this amide bond is formed in much higher yield in dipeptide **21a** than in tripeptide **17a**.

Both two plus two couplings (Schemes III and IV) were carried out by using DCC/HOBt as the condensing reagent. Reasonable yields of both tetrapeptides (82% for **22a**, 61% for **23a**) were obtained. Linear tetrapeptides **22a** and **23a** were separately converted to the corresponding *N*-hydroxysuccinimide esters (**22c**, **23c**), deprotected, and cyclized under the same conditions employed for tetrapeptide active esters **11** and **19**. Cyclic product **2** was isolated in excellent yield (44%) from tetrapeptide **24** (Scheme III) but in only trace amounts (~2%) from tetrapeptide **25** (Scheme IV). The cyclization results are summarized in Table I.

## Discussion

The structure of chlamydocin (**1**) poses two major problems to the synthetic chemist: the synthesis of Aoe,

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(11) (a) Schüssler, H.; Zahn, H. *Chem. Ber.* **1962**, *95*, 1076–1080. (b) Wieland, T.; Kern, W.; Zehring, R. *Justus Liebigs Ann. Chem.* **1950**, *569*, 117–121. (c) Sheehan, J. C.; Frank, V. S. *J. Am. Chem. Soc.* **1950**, *72*, 1312–1316.
(12) (a) Mühlemman, M.; Titov, M. I.; Schwyzer, R.; Rudinger, J. *Helv. Chim. Acta* **1972**, *55*, 2854–2860. (b) Belleav, D.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651–1652.

the amino acid that contains the epoxy ketone functionality (L-2-amino-8-oxo-9,10-epoxydecanoic acid), and the synthesis of the cyclic tetrapeptide backbone which contains five $\alpha$ substituents. Preliminary work directed toward synthesizing the epoxy ketone amino acid Aoe by selective functionalization has been presented elsewhere.[2c] To apply this approach to the synthesis of chlamydocin, it is necessary that an efficient method for synthesizing the cyclic tetrapeptide ring system be found.

To study the approaches to the synthesis of the 12-membered tetrapeptide ring system, we chose the model compound **2** in which the side chain of Aoe has been replaced by a methyl group. This replacement corresponds to replacing the L-Aoe residue in linear precursors with L-alanine. The cyclic analogue thus obtained is designated [Ala[4]]chlamydocin [*cyclo*(Aib-Phe-D-Pro-Ala), **2**). Synthesis of model compound **2** is expected to approximate the synthesis of more highly substituted precursors of chlamydocin because **2** has the same substitution pattern and chirality as chlamydocin and has the same solution conformation in chloroform-*d* and in dimethyl sulfoxide.[6]

The first sequence selected for cyclization in the present study was studied previously and found to cyclize in low yield. The linear precursor (Scheme I) was resynthesized and cyclized in order to compare the *N*-hydroxysuccinimide cyclization method used here with the trichlorophenyl ester method used previously. As shown in Table I the yields of **2** obtained from either ester were comparable and establish that the poor yield obtained earlier was not due entirely to the use of the Tcp ester method.

Formation of cyclic tetrapeptide **2** is greatly favored (Table I) by the linear sequence **24** which gave a 15-fold improvement in yield over the other three sequences. The sequence specificity is remarkable and, as with other sequence-dependent cyclization results reported in the literature for cyclic tetrapeptides[13] and other peptide ring systems,[14] we have no compelling explanation for this phenomenon. Examination of Drieding models of the four linear precursors in Schemes I–IV did not suggest an obvious reason why sequence **24** cyclizes in such high yield. Solution conformation studies on the four linear tetrapeptides are being pursued to provide information that may account for the sequence specificity observed.

Cyclization of sequence **24** does produce the desired product, [Ala[4]]chlamydocin (**2**), in a much higher yield than that obtained from cyclization of the other linear tetrapeptide sequences **11**, **19**, and **25** and in substantially better overall yield than the approaches using dehydrophenylalanine precursors. This result should open the way to the synthesis of chlamydocin and its analogues in good overall yield.

## Experimental Section

**General Methods.** The solvents used in this study were purified according to the following procedures: methylene chloride, distilled from $P_2O_5$; methanol, distilled from magnesium methoxide; ethyl acetate, distilled neat; tetrahydrofuran, distilled from sodium benzophenone ketyl; chloroform was used as supplied. The L amino acids, $\alpha$-aminoisobutyric acid, and all coupling reagents were commercially available and used as supplied. D-Proline was made from L-proline by using previously described procedures.[15] The *tert*-butyloxycarbonyl-protected amino acids were synthesized in good yields (80–98%) by using literature procedures,[16] except for (*tert*-butyloxycarbamoyl)-$\alpha$-aminoisobutyric acid (**12**) which was obtained in 55% yield by using "method A" as described in ref 16.

The $^1$H NMR spectra were recorded on either Varian EM-390 or JEOL FX-90Q instruments. Satisfactory NMR data were obtained for all compounds synthesized, and the data is reported for most of the stable intermediates used. Melting points were determined by using a Fisher-Johns apparatus and are uncorrected. Thin-layer chromatography was performed on commercially prepared glass plates with the following solvent systems: solvent A = hexane/acetone (3:2); solvent B = 10% methanol in $CHCl_3$; solvent C = 5% methanol in $CHCl_3$; solvent D = butanol/acetic acid/$H_2O$ (4:1:1).

**General Procedure A: Removal of the Boc Group. (A-1) Using Trifluoroacetic Acid.** The *tert*-butyloxycarbonyl-protected peptides were dissolved in cold (0 °C) trifluoroacetic acid (at least 10 mL/1 mmol of peptide). The reactions were stirred for 0.5 h at room temperature followed by the removal of the trifluoroacetic acid in vacuo. Two types of workup were then used. (1) In the case of C-terminal active ester peptides (i.e., succinimide) the trifluoroacetate salts were precipitated directly from ethyl acetate/ether. The resulting white solid materials obtained were then dried in a desiccator over potassium hydroxide. (2) In the case of C-terminal methyl ester peptides the residue was reevaporated from methanol twice and then precipitated from ethyl acetate/ether or chloroform/ether.

**(A-2) Using HCl/Dioxane.** An excess of 4 N HCl in *p*-dioxane (20 mL) was added to either the *N*-Boc- or the *N*-pMz-protected amino acid or peptide derivatives (5 mmol), and the solution was stirred at room temperature under anhydrous conditions. The progress of the reaction was monitored by TLC. After completion of the reaction, usually in 30–60 min, the solution was concentrated in vacuo. The residue was reevaporated from anhydrous ether (two or three times) and finally dried in a vacuum desiccator over $P_2O_5$/KOH to yield the hydrochloride salt of the peptide or the amino acid derivative, which was usually used without further purification.

**General Procedure B-1. Saponification of Methyl Esters.** The fully protected peptides were dissolved in methanol or ethanol for tetrapeptides (2–5 mL/1 mmol of peptide), and 1 N sodium hydroxide was added (1.1–1.5 mL/1 mmol of peptide). The reactions were stirred for 1–2 h as required by TLC analysis. The methanol was then removed in vacuo, and the remaining aqueous solution was diluted with water (5–10× by volume). This aqueous solution was washed with EtOAc, acidified to pH ~3 with potassium bisulfate, and extracted three times with ethyl acetate (equal volumes). The ethyl acetate washes were combined, washed with water, dried over magnesium sulfate, and evaporated to dryness.

**General Procedure B-2.** The methyl ester (10 mmol) was dissolved in 50 mL of acetone and treated with 11–15 mL of 1 N aqueous NaOH solution in a 22 °C water bath. The progress of the reaction was monitored by TLC, and it was complete in 1–2 h. The reaction mixture was diluted with 20 mL of water and concentrated in vacuo to about one-third its volume. The aqueous alkaline solution was washed with ethyl acetate (2×). The aqueous layer was acidified to pH ~3 with solid citric acid (or 1 N citric acid solution), saturated with solid sodium chloride, and extracted with ethyl acetate (3×). The organic layer was washed with water and saturated brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to yield a white foam, which was dried in a vacuum desiccator over $P_2O_5$/KOH.

**General Procedure C. DCC/HOBt[8] Coupling.** The amine hydrochloride (or amine trifluoroacetate) salt (10 mmol) was dissolved in 50 mL of methylene chloride, cooled to 0 °C, and neutralized with (10 mmol) triethylamine. The N-protected amino acid or peptide acid (10 mmol) solution in 50 mL of methylene chloride and solid 1-hydroxybenzotriazole (HOBt) were added to the chilled solution. The mixture was stirred for 5 min in an ice bath. A solution of DCC (10 mmol) in 25 mL of methylene chloride was added, and the reaction mixture was stirred at 0 °C for 4 h and overnight at room temperature. The reaction mixture was cooled in dry ice, and the white solid was filtered. The filtrate

(13) Faulstich, H.; Trischmann, H.; Dabrowski, J. In "Peptides 1978"; Siemion, I. Z., Kupryszewski, G., Eds.; Wroclaw University Press: 1979; pp 305–310. These authors found H-Pro-Phe-Pro-Ala-OH cyclized in 54% yield; H-Ala-Pro-Phe-Pro-OH gave only trace amounts.

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was concentrated in vacuo, and the residue was taken up in ethyl acetate. The organic layer was washed with water (2×), 1 N HCl or 1 N citric acid solution (2×), saturated $NaHCO_3$ (2×), and saturated brine until the aqueous layer was neutral. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to yield a white foam. If required, the product was purified by column chromatography on silica gel.

**General Procedure D. Synthesis of Succinimide Esters.** The N-protected amino acid or peptide acid (10 mmol) was dissolved in 10 mL of $CH_2Cl_2$ and cooled in an ice bath. Solid *N*-hydroxysuccinimide (15 mmol) was added, and the mixture was stirred for 10–15 min, when a clear solution was obtained. A solution of DCC (10 mmol) in 10 mL of $CH_2Cl_2$ was added, and the reaction mixture was stirred overnight at 4 °C. The reaction mixture was cooled in dry ice and the solid was removed by filtration. The filtrate was concentrated in vacuo, and the residue was partitioned between water and ethyl acetate. The organic layer was washed with water (1×) and with saturated brine until the aqueous layer was neutral. The ethyl acetate layer was dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to yield a white foam, which was thoroughly dried in a vacuum desiccator over $P_2O_5$/KOH.

**General Procedure E. Cyclization Procedure.** The trifluoroacetate or hydrochloride salt of the peptide active esters (succinimide) was dissolved in 10 mL of dimethylformamide or ethyl acetate. These solutions were added via syringe pump to a large volume of pyridine (~150 mL/0.1 mmol of peptide) with stirring. The addition took place over a period of 7–8 h, and the reactions were continued at room temperature for a period of 10–12 h after the addition was complete. The pyridine was removed in vacuo, and the crude residue was dried in a vacuum desiccator over phosphorus pentoxide. This crude material was purified by preparative TLC (2 mm thickness, Merck precoated silica gel 60 F-254) with Skelly B/acetone (3:2) for the solvent system. The appropriate band (UV positive) was collected and extracted with 10% methanol in chloroform. The silica gel was then removed by filtration, and the filtrate was evaporated to yield the purified product.

**D-Proline Methyl Ester Hydrochloride (6).** The title compound was prepared by reaction of D-proline (2.3 g, 20 mmol) in 20 mL of methanol with thionyl chloride (4.76 g, 2.90 mL, 40 mmol) at −10 °C for 2 h at room temperature. Crystallization from ethanol/ether gave the product: 2.55 g (77%); mp 73 °C; TLC $R_f$ 0.25 (solvent A); $[\alpha]^{20}_D$ +30.8° (*c* 2, methanol).

***N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl-D-proline Methyl Ester (7) via DCC/HOBt.** The title compound was prepared from Boc-L-Phe (29.15 g, 110 mmol) and HCl·D-Pro-OMe (16.6 g, 100 mmol) by general procedure C to yield 32.0 g (85%) of the dipeptide **7** as a white foam: $R_f$ 0.70 (solvent C); $[\alpha]^{23}_D$ +59.6° (*c* 1.19, methanol). Anal. Calcd for $C_{20}H_{28}N_2O_5$: C, 63.81; H, 7.50; N, 7.44. Found: C, 64.08; H, 7.61; N, 7.70.

***N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl-D-proline Methyl Ester (7) via EEDQ.** D-Proline methyl ester hydrochloride (**6**; 3.31 g, 20 mmol) was dissolved in 20 mL of water, and saturated sodium bicarbonate solution was added until the pH reached ~10. This aqueous solution was extracted with ethyl acetate (3 × 20 mL). The ethyl acetate layers were combined, dried ($MgSO_4$), and evaporated. The oil obtained was used directly in the following coupling reaction.

A solution containing (*tert*-butyloxycarbonyl)-L-phenylalanine [**5**; 6.63 g in ethyl acetate (~100 mL), 25 mmol] was added to the flask containing the oil and chilled to 0 °C in an ice bath. Then *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (6.2 g, 25 mmol, 97% EEDQ) was added. The reaction mixture was stirred for 3 h at 0 °C, followed by overnight stirring at room temperature. The reaction mixture was worked up as described in procedure C to yield 6.85 g (91%) of **7**.

**L-Phenylalanyl-D-proline Methyl Ester (7b) Hydrochloride.** The title compound was prepared from the dipeptide **7a** (14.3 g, 38 mmol) by general procedure A-2, and the product was recrystallized from methanol–ether to yield 11.4 g (95%) of the hydrochloride **7b** as a white solid; mp 141–142 °C.

***N*-(*p*-Methoxybenzyloxycarbonyl)-α-aminoisobutyryl-L-phenylalanyl-D-proline Methyl Ester (8a).** The title compound was prepared from pMz-Aib (1.35 g, 5.0 mmol) and HCl·L-Phe-D-Pro-OMe (**7b**; 1.312 g, 4.2 mmol) by general procedure C and purified by column chromatography (2% MeOH–$CHCl_3$) to give 1.92 g (87.1%) of the tripeptide **8a** as a white foam: $[\alpha]^{25}_D$ +37.34° (*c* 0.665, MeOH); $R_f$ 0.56 (solvent C), 0.70 (solvent B). Anal. Calcd for $C_{28}H_{35}N_3O_7$: C, 63.98; H, 6.71; N, 7.99. Found: C, 63.71; H, 6.83; N, 7.98.

***N*-(*p*-Methoxybenzyloxycarbonyl)-α-aminoisobutyryl-L-phenylalanyl-D-proline (8b).** The title compound was prepared from the methyl ester **8a** (1.92 g, 3.66 mmol) with 10 mL of 1 N NaOH solution in 30 mL of water by general procedure B-1 to give 1.70 g (91.4%) of the acid **8b** as a white amorphous solid: $R_f$ 0.12 (0.5% HOAc in solvent C). Anal. Calcd for $C_{27}H_{33}N_3O_7 \cdot H_2O$: C, 61.19; H, 6.65; N, 7.93. Found: C, 60.94; H, 6.25; N, 8.01.

***N*-Succinimidyl Diphenylphosphate (SDPP, 9).** The title compound was prepared by modification of the literature procedure.[17] Diphenyl chlorophosphate (13.4 g, 50 mmol) in 50 mL of anhydrous methylene chloride was added to a three-necked flask fitted with a dropping funnel and a thermometer. The solution was cooled to −5 °C in a salt–ice freezing mixture, and a solution of *N*-hydroxysuccinimide (5.75 g, 50 mmol) in 75 mL of methylene chloride was added in one portion. A solution of triethylamine (6.06 g, 60 mmol) in 25 mL of methylene chloride was added dropwise over a 40-min period to the vigorously stirred solution. The reaction mixture was stirred at 0 °C for another 1 h and then at room temperature for 1 h. The solution was washed with brine (3 × 100 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give **9** as a white solid. Recrystallization from EtoAc–Skelly B gave 14.0 g of **9** as a white crystalline solid, mp 86–87 °C (lit.[17] mp 88–90 °C).

***N*-Hydroxysuccinimide Ester of (*tert*-Butyloxycarbonyl)-L-alanine (10a).** The title compound was prepared from Boc-L-Ala (2.08 g, 11 mmol) and SDPP (**9**; 3.47 g, 10 mmol) to give 2.767 g (96.7%) of the *N*-hydroxysuccinimide ester **10a** as a white solid. A small sample was recrystallized from 2-propanol to give a white solid: mp 166–197 °C (lit.[18] mp 167 °C); $R_f$ 0.55 (solvent C); $^1H$ NMR ($CDCl_3$) δ 1.46 (9 H, s), 1.56 (3 H, d, $J$ = 7.3 Hz), 2.84 (4 H, s), 4.71 (1 H, br s), 5.08 (1 H, br s).

***N*-Hydroxysuccinimide Ester of (*p*-Methoxybenzyloxycarbonyl)-α-aminoisobutyryl-L-phenylalanyl-D-prolyl-L-alanine (8c).** A solution of the tripeptide acid **8b** (765 mg, 1.5 mmol) in 30 mL of tetrahydrofuran was stirred and chilled to −20 °C by using a dry ice $CCl_4$ bath. *N*-Methylmorpholine (152 mg, 1.5 mmol) was added followed by isobutyl chlorocarbonate (205 mg, 1.5 mmol), producing a white precipitate. After 3 min a mixture of the *N*-hydroxysuccinimide ester of L-alanine hydrochloride (**10b**, (prepared from 430 mg of the Boc derivative **10a** by general procedure A-2) and *N*-methylmorpholine (152 mg, 1.5 mmol) in 30 mL of tetrahydrofuran was added. Stirring was continued for 1 h at −20 °C and 1 h at 0 °C, and then the mixture was allowed to warm to room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, 1 N citric acid, water, and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The tetrapeptide derivative **8c** (848 mg, 83%) was obtained as a white foam and was used in the cyclization reaction without further purification; $R_f$ 0.25 (solvent C).

***cyclo*(α-Aminoisobutyryl-L-phenylalanyl-D-prolyl-L-alanyl) (2) from Sequence 11.** The linear tetrapeptide active ester **8c** (810 mg) was treated with 4 N HCl/dioxane according to general procedure A-2. The corresponding hydrochloride salt **11** was dissolved in 5 mL of anhydrous DMF and cyclized in pyridine at room temperature according to the general procedure E. The crude product obtained from the cyclization reaction was purified by TLC (10% MeOH–$CHCl_3$) to yield 13.8 mg (2.9%) of the cyclic tetrapeptide **2** as a white film: $R_f$ 0.64 (solvent B); $^1H$ NMR ($CDCl_3$) δ 1.34 (3 H, d, $J$ = 6 Hz), 1.37 (3 H, s), 1.81 (3 H, s), 1.97–2.4 (4 H, complex), 2.84–3.44 (3 H, m), 3.94 (1 H, m), 4.49 (1 H, dd, $J$ = 6, 9 Hz), 4.47 (1 H, m), 5.34 (1 H, m), 5.34 (1 H, ddd, $J$ = 6, 9.8, 11.0 Hz), 6.18 (1 H, s), 7.35 (1 H, d, $J$ = 11 Hz), 7.42 (5 H, s), 7.71 (1 H, d, $J$ = 11.3 Hz).

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***N*-(*tert*-Butyloxycarbonyl)-L-phenylalanine 2,2,2-Trichloroethyl Ester (15a).** A solution containing (*tert*-butyloxycarbonyl)-L-phenylalanine (2.0 g, 7.5 mmol), 2,2,2-trichloroethanol (1.2 g, 8.0 mmol), and 4-(dimethylamino)pyridine (90 mg, ~0.1 equiv) in methylene chloride (30 mL) was chilled to 0 °C in an ice bath. The reaction was initiated with the addition of dicyclohexylcarbodiimide (DCC; 1.65 g, 8.0 mmol) and stirred for 3 h at 0 °C. After an additional 1 h of stirring at room temperature, the reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate (50 mL) and worked up as described in procedure C. The white residue was recrystallized from diethyl ether/Skelly B, producing 2.77 g (93%) of **15a** as a white crystalline solid: mp 67–69 °C; $[\alpha]^{23}_D$ −1.55° (*c* 1.29, MeOH). Anal. Calcd for $C_{16}H_{20}Cl_3NO_4$: C, 48.45; H, 5.08; N, 3.53. Found: C, 48.68; H, 5.04; N, 3.61.

***N*-(*tert*-Butyloxycarbonyl)-α-aminoisobutyryl-L-phenylalanine 2,2,2-Trichloroethyl Ester (16a).** *N*-(*tert*-Butyloxycarbonyl)-L-phenylalanine 2,2,2-trichloroethyl ester (**15a**; 2.1 g, 5.3 mmol) was deprotected according to general procedure A-1. The resulting trifluoroacetate salt **15b** was neutralized as follows. After evaporation of solvent, the residue was dissolved in ethyl acetate (40 mL) and chilled to 0 °C. Saturated $NaHCO_3$ was added until no further bubbling occurred upon addition of more bicarbonate. The ethyl acetate layer was then separated and washed with more saturated $NaHCO_3$ (50 mL) and saturated NaCl (50 mL). The organic phase was dried over $Na_2SO_4$ and concentrated to an oil (**15c**; yield 1.5 g, 96%) which was used directly in the following coupling reaction.

Ester **15c** (1.5 g, 5.1 mmol) was dissolved in methylene chloride and reacted with (*tert*-butyloxycarbonyl)-α-aminoisobutyric acid (**14**; 1.05 g, 5.17 mmol) according to general procedure C. After the workup, the resulting oil was triturated with Skelly B. A white solid was collected by filtration and recrystallized from ether/Skelly B: yield 1.98 g (81%); mp 102–103 °C; $[\alpha]^{23}_D$ −9.17° (*c* 1.45, methanol). Anal. Calcd for $C_{20}H_{27}Cl_3N_2O_5$: C, 49.86; H, 5.65; N, 5.81. Found: C, 50.00; H, 5.79; N, 5.91.

***N*-(*tert*-Butyloxycarbonyl)-L-alanyl-α-aminoisobutyryl-L-phenylalanine Trichloroethyl Ester (17a).** Compound **16a** (630 mg, 1.31 mmol) was deprotected according to general procedure A-1. The trifluoroacetate salt was dissolved in methylene chloride (10 mL), chilled to 0 °C, and neutralized with triethylamine. This solution of neutralized amine dipeptide **16b** was used directly in the following coupling reaction.

A solution containing (*tert*-butyloxycarbonyl)-L-alanine (**13**; 0.56 g, 3 mmol) in methylene chloride was chilled to 0 °C in an ice bath. Ethyl[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC; 0.30 g, 1.5 mmol) was added and the solution was stirred at 0 °C for 0.5 h. The free amine dipeptide solution (vide supra) **16b** was added dropwise over a 10-min period. The reaction mixture was stirred for 4 h at 0 °C followed by 16 h at room temperature. The reaction mixture was evaporated to dryness. The residue was dissolved in ethyl acetate (30 mL) and worked up as described in procedure C. The resulting solid was recrystallized from ether/Skelly B to yield **17a**: 402 mg (55% from protected dipeptide); mp 158–159 °C; TLC (solvent A) $R_f$ 0.33; $[\alpha]^{23}_D$ −20.2° (*c* 2.53, methanol). Anal. Calcd for $C_{23}H_{32}Cl_3N_3O_6$: C, 49.97; H, 5.83; N, 7.60. Found: C, 50.23; H, 5.95; N, 7.51.

***N*-(Benzyloxycarbonyl)-D-prolyl-L-alanyl-α-aminoisobutyryl-L-phenylalanine 2,2,2-Trichloroethyl Ester (18a).** Tripeptide **17a** (0.70 g, 1.26 mmol) was treated with TFA/$CH_2Cl_2$ according to general procedure A-1. The resulting oil was dissolved in methylene chloride (25 mL), chilled (0 °C), and neutralized with triethylamine (0.13 g). This chilled solution containing free amine tripeptide **17b** was coupled directly with *N*-(benzyloxycarbonyl)-D-proline (**12**; 0.38 g, 1.52 mmol) according to general procedure C (0.31 g of DCC, 0.23 g of HOBt).

The oil obtained from the coupling reaction was purified by column chromatography over silica gel 60 by eluting with Skelly B/acetone (4:1). The appropriate fractions were pooled and evaporated to dryness to yield **18a**: 0.785 g (91%); $[\alpha]^{23}_D$ −5.87° (*c* 1.09, pyridine); TLC (solvent A) $R_f$ 0.25; NMR ($CDCl_3$) δ 1.28 (d, 3 H, *J* = 8.4 Hz), 1.44 (s, 6 H), 1.76–2.32 (complex, 4 H), 3.08–3.28 (m, 2 H), 3.48–3.67 (m, 2 H), 3.88–4.54 (complex, 2 H), 4.74 (d, 2 H, *J* = 10.5 Hz), 4.83–4.96 (m, 1 H), 5.11 (d, 2 H, *J* = 6.6 Hz), 6.15 (br s, 1 H), 6.90–7.04 (br, 1 H), 5.11 (d, 2 H, *J* = 6.6 Hz), 6.15 (br s, 1 H), 6.90–7.04 (br, 1 H), 7.25 (s, 5 H), 7.35 (s, 5 H), 7.21–7.42 (br, 1 H). Anal. Calcd for $C_{31}H_{37}Cl_3N_4O_7$: C, 54.43; H, 5.45; N, 8.19. Found: C, 54.27; H, 5.54; N, 8.00.

***N*-(Benzyloxycarbonyl)-D-prolyl-L-alanyl-α-aminoisobutyryl-L-phenylalanine Succinimide Ester (18c).** The tetrapeptide trichloroethyl ester **18a** (0.13 g, 0.19 mmol) was deprotected with zinc (0.6 g, 40 equiv) in 90% aqueous acetic acid (10 mL). The reaction was stirred for 1.5 h at room temperature and filtered. The filtrate was diluted with $H_2O$ to a volume of 30 mL and extracted with ethyl acetate (2 × 20 mL). The ethyl acetate washes were combined, washed with 1 N HCl, $H_2O$, and saturated NaCl (40 mL each), dried ($MgSO_4$), and evaporated to dryness. The acid **18b** was dried in a vacuum desiccator over KOH and used immediately in the following reaction.

Free acid **18b** (0.10 g) was dissolved in $CH_2Cl_2$ and cooled to 0 °C. *N*-Hydroxysuccinimide (26.5 mg, 0.23 mmol) and dicyclohexylcarbodiimide (45 mg, 0.22 mmol) were added, and the reaction was carried out according to general procedure D. The resulting clear oil **18c** was used directly without further purification.

***cyclo*(-D-Prolyl-L-alanyl-α-aminoisobutyryl-L-phenylalanyl) (2) from Sequence 18c.** The tetrapeptide succinimide ester **18c** was dissolved in ethyl acetate (15 mL), and 10% paladium on carbon (25 mg) and trifluoroacetic acid (0.5 mL) were added. Hydrogen gas was bubbled through the reaction mixture for 5 h with stirring. The reaction mixture was flushed with $N_2$ and then filtered. The filtrate was evaporated to dryness. The oil obtained was dissolved in ether (~5 mL), and the TFA salt **18c** was precipitated with Skelly B.

Ester **19** was dissolved in ethyl acetate (15 mL) and added to pyridine (500 mL) according to general procedure E. After the workup and purification (according to procedure E), 2.3 mg (3% yield) of product **2** was obtained. The TLC and $^1H$ NMR were same as those for **2** obtained from compound **11**. Anal. Calcd for $C_{21}H_{28}N_4O_4$: C, 62,98; H, 7.05; N, 13.99. Found: C, 62.84; H, 7.16; N, 13.74.

**α-Aminoisobutyric Acid Methyl Ester Hydrochloride (20).** α-Aminoisobutyric acid (20.6 g, 0.2 mmol) was suspended in 160 mL of methanol and chilled to −10 °C in an ice bath with salt (brine). Thionyl chloride (17.4 mL, 0.24 mmol) was added dropwise with stirring while the temperature was maintained below 0 °C. After the addition was completed, the reaction mixture was allowed to warm to room temperature and stirred at room temperature for 3 days. The reaction mixture was evaporated to a small volume (~30 mL) and 100 mL of anhydrous ether was added. The crystalline product was collected by filtration and washed with anhydrous ether. The crude product was dried in a desiccator over potassium hydroxide (crude yield 29.5 g, 96%). The product was recrystallized from methanol/ether to yield **20**: 28.3 g (92.1%); mp ~140 °C (sublimes).

***(tert*-Butyloxycarbonyl)-L-alanyl-α-aminoisobutyric Acid Methyl Ester (21a).** α-Aminoisobutyric acid methyl ester hydrochloride **20** (11.4 g, 74.2 mmol) was dissolved in ethyl acetate (500 mL), and triethylamine (10.3 mL, 74.2 mmol) was added. A solution containing (*tert*-butyloxycarbonyl)-L-alanine (**13**; 14.2 g, 75 mmol) in 50 mL of ethyl acetate was added, and the reaction mixture was chilled to 0 °C in an ice bath. *N*-(Ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoine (19.1 g, 75 mmol, 97% EEDQ) was dissolved in cold ethyl acetate (50 mL) and added to the reaction mixture. The reaction mixture was stirred at 0 °C for 3 h and then overnight at room temperature. The solution was filtered (to remove triethylamine hydrochloride) and worked up as described for procedure C to give 20.2 g (94.3%) of **21a** as an oil: $[\alpha]^{25}_D$ −49.5° (*c* 2, dioxane); $[\alpha]^{23}_D$ −34.51 (*c* 1.13, methanol). Anal. Calcd for $C_{13}H_{24}N_2O_5$: C, 54.15; H, 8.39; N, 9.71. Found: C, 54.22; H, 8.52; N, 9.62.

***(tert*-Butyloxycarbonyl)-L-alanyl-α-aminoisobutyryl-L-phenylalanyl-D-proline Methyl Ester (22a).** Dipeptide **21a** was saponified according to general procedure B-1. The resulting free acid **21b** was crystallized from ether: yield 92%; mp 175 °C; TLC $R_f$ 0.7 (solvent D); $[\alpha]^{25}_D$ −27.3 (*c* 1, ethanol).

(*tert*-Butyloxycarbonyl)-L-phenylalanyl-D-proline methyl ester (**7a**) was deprotected according to general procedure A-1. The resulting trifluoroacetate salt (**7be**) was recrystallized from chloroform/ether: yield 79%; mp 134 °C; TLC $R_f$ 0.3 (solvent D). These appropriately deprotected dipeptides were used directly in the following fragment coupling procedure.

L-Phenylalanyl-D-proline methyl ester trifluoroacetate (**7b**; 1.17 g, 3.0 mmol) was dissolved in 30 mL of chloroform and chilled to 0 °C. Triethylamine (0.42 mL, 3.0 mmol) and (*tert*-butyloxycarbonyl)-L-alanyl-α-aminoisobutyric acid (**21b**; 0.9 g, 3.3 mmol, in 10 mL DMF) were added to the solution. 1-Hydroxybenzotriazole monohydrate (0.50 g, 3.3 mmol, in 5 mL of DMF) was added followed by a solution containing dicyclohexylcarbodiimide (0.68 g, 3.3 mmol, in 5 mL of chloroform). The reaction was stirred for 2 h at 0 °C, and stirring was continued at room temperature for 2 days. The reaction mixture was then chilled and filtered, and the filtrate was worked up as described in procedure C. The resulting oil was purified by column chromatography (gravity, silica gel 60) with 3% methanol in chloroform. The appropriate fractions were pooled and evaporated to yield **22a** as amorphous solid: 1.31 g (82%); TLC $R_f$ 0.3 (solvent C); $[\alpha]^{23}_D$ +20.2° (*c* 1.73, methanol); $^1$H NMR ($CDCl_3$) δ 1.33 (d, 3 H, *J* = 7.5 Hz), 1.43 (s, 9 H), 1.50 (s, 6 H), 1.71–2.04 (complex, 4 H), 2.88–3.08 (m, 2 H), 3.41–3.63 (m, 2 H), 3.67 (s, 3 H), 3.94–4.42 (complex, 2 H), 4.76–5.04 (m, 1 H), 5.23 (d, 1 H, *J* = 8.4 Hz), 6.74 (br s, 1 H), 6.95 (d, 1 H, *J* = 10.8 Hz), 7.18 (br s, 5 H). Anal. Calcd for $C_{27}H_{40}N_4O_7$: C, 60.88; H, 7.57; N, 10.53. Found: C, 60.94; H, 7.79; N, 10.36.

**(*tert*-Butyloxycarbonyl)-L-phenylalanyl-D-prolyl-L-alanyl-α-aminoisobutyric Acid Methyl Ester (23a).** (*tert*-Butyloxycarbonyl)-L-phenylalanyl-D-proline methyl ester **7a** was saponified according to general procedure B-1. The free acid **7b** was crystallized from ether: yield 73%; mp 170–172 °C.

(*tert*-Butyloxycarbonyl)-L-alanyl-α-aminoisobutyric acid methyl ester (**21a**) was deprotected according to general procedure A-1. The resulting trifluoroacetate salt (**21b**) of the dipeptide was crystallized from ether: yield 85%; mp 176–177 °C; $[\alpha]^{25}_D$ +15.0° (*c* 2, methanol). These appropriately deprotected dipeptides were then used directly in the following fragment coupling procedure.

L-Alanyl-α-aminoisobutyric acid methyl ester trifluoroacetate (**21b**; 1.0 g, 3.3 mmol) was dissolved in 25 mL of dimethylformamide and chilled to −10 °C in a salted ice bath. Triethylamine (0.46 mL, 3.3 mmol), (*tert*-butyloxycarbonyl)-L-phenylalanyl-D-proline (**7b**; 1.08 g, 3 mmol), 1-hydroxybenzotriazole monohydrate (0.50 g, 3.3 mmol), and dicyclohexylcarbodiimide (0.68 g, 3.3 mmol) were added in the order given. The reaction mixture was stirred at −10 °C for 1 h, and stirring was continued at room temperature for 2 days. The reaction mixture was filtered, and the filtrate was diluted with 250 mL of ethyl acetate and worked up as described in procedure C to give **23a** as a white crystalline solid, 1.32 g (82.6%). Recrystallization from chloroform/Skelly B gave the product: 1.05 g (66%); mp 212–213 °C. This material contained a small amount of impurity (probably DCU), so it was further purified by column chromatography (gravity, silica gel 60) with 3% methanol in chloroform to give the product: 0.98 g (61%); mp 214 °C; TLC $R_f$ 0.25 (solvent C); $[\alpha]^{23}_D$ +12.8° (*c* 1.06, methanol); $^1$H NMR ($CDCl_3$) δ 1.35 (d, 3 H, *J* = 6.6 Hz), 1.41 (s, 9 H), 1.52 (s, 6 H), 1.62–2.18 (complex, 4 H), 3.01 (d, 2 H, *J* = 6.9 Hz), 3.45–3.63 (m, 2 H), 3.71 (s, 3 H), 4.17–4.69 (complex, 3 H), 5.22 (d, 1 H, *J* = 7.5 Hz), 6.83 (br s, 1 H), 7.02 (d, 1 H, *J* = 6.6 Hz), 7.25 (br s, 5 H). Anal. Calcd for $C_{27}H_{40}N_4O_7$: C, 60.88; H, 7.57; N, 10.52. Found: C, 60.97; H, 7.65; N, 10.51.

**Synthesis of Succinimide Esters 22c and 23c.** Peptides **22a** and **23a** were saponified according to general procedure B-1 by using 2 equiv of sodium hydroxide. The yields of free acid tetrapeptides were 77.5% **22b** and 98% **23b**. The TLC $R_f$ was 0.55 (solvent E) for peptide **22** and 0.45 (solvent B) for peptide **23**.

The free acid tetrapeptides were dissolved in an appropriate amount of methylene chloride and chilled to 0 °C. *N*-Hydroxysuccinimide (1.1 equiv, 3 equiv for Pro C-terminal) and dicyclohexylcarbodiimide (1.2 equiv) were added. The reaction mixtures were stirred at room temperature for 4 h. The reaction mixtures were then chilled to 0 °C and filtered to remove the dicyclohexylurea. The filtrates were evaporated to dryness. The resulting material was not further purified but was deprotected directly to yield the trifluoroacetate salts according to general procedure A-1. Peptide **22c** (TFA salt of OSu ester): yield 81.6%; mp 153–155 °C; TLC $R_f$ 0.2 (solvent E). Peptide **23c** (TFA salt of OSu ester): yield 40%.

***cyclo*(L-Alanyl-α-aminoisobutyryl-L-phenylalanyl-D-prolyl) (2) from 22c and 23c.** The TFA salts of **22c** and **23c** were dissolved in dimethylformamide/ethyl acetate (~1:3, ~10-mL total volume) and cyclized according to general procedure E. After purification by preparative TLC as described, the products were isolated: yield 44% from **22c** and 2% from **23c**; $^1$H NMR and TLC identical with those obtained for compound **2** from sequences **11** and **19**; $[\alpha]^{23}_D$ −90.9° (*c* 0.77, methanol).

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# Rearrangements of Oxygen-Functionalized Cyclopropylcarbinyl Substrates: An Approach to Oxygenated α-Methylene-γ-butyrolactones

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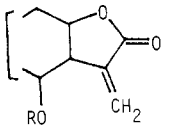
Keto diester **6** (prepared from 2-bromocyclohexenone, dimethyl malonate, and KH) was converted to the keto cyclopropylcarbinyl substrates **10–13** and the methoxycyclopropylcarbinyl substrates **17–20**. The keto substrates were relatively unreactive to rearrangement; however, under some conditions, lactone **23** and diene **24** were formed. Rearrangement of the methoxy substrates gave lactone **25**.

A large number of sesquiterpenes and other naturally occurring compounds possess the α-methylene-γ-butyrolactone ring. Many of these compounds have biological activity, and some have tumor-inhibiting activity; consequently there has been considerable work on the synthesis of α-methylene-γ-butyrolactones.[1] About half of the naturally occurring α-methylene-γ-butyrolactones, including most of those with tumor-inhibiting activity, have an additional oxygen function at the homoallylic position as shown in partial structure **1** (R = H or acyl).[2]




**1**

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