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Bi- and Tricyclic Products from Tetra- and Penta-peptides of α-Methylalanine

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Tetra- and penta-peptides of α-methylalanine give with PCl_5, $SOCl_2$, *etc.*, intermediates which, with or without loss of amino acids, lead to bi- and tricyclic products containing imidazole and piperazine rings.

During attempts to prepare cyclic peptides from linear peptides of α-methylalanine (α-aminoisobutyric acid), a series of abnormal reactions have been encountered. Although there is no reason to expect that such cyclic products are particularly unstable, the higher members were never obtained and the cyclic dipeptide only with difficulty.

CYCLIZATION OF DIPEPTIDES

The 2,4,5-trichlorophenyl ester of α-methylalanyl-α-methylalanine gave no cyclic product when refluxed in methanol or in benzene for 24 h, and only a 15 % yield when heated at 100° without solvent for 48 h. The otherwise less reactive methyl ester gave under the same conditions better yields, 15 and 90 %, respectively. This general reluctance of the linear dipeptide to form a monocyclic peptide is quite surprising, considering the perfect stability of the product and the presence of two *gem*-dimethyl groups, which are generally observed [1] to promote cyclization. The anomaly is best explained by the difficulty of obtaining the *cis*-amide conformation required for cyclization (Fig. 1).

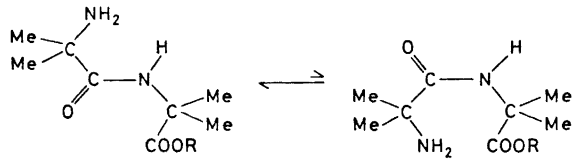


Fig. 1.

The heavy substitution is in fact comparable to that in di-*t*-butyl ethylene where the *cis*-isomer is 9.3 kcal/mol higher in energy than the *trans*-isomer.[2] Also, the bulkiness of the activated ester group explains the more facile reaction with the less "active" but smaller methyl ester group. In cases such as sarcosyl-sarcosine,[3] where *cis*-amide is as likely as *trans*, cyclic dipeptide formation is unavoidable under mild conditions.

ATTEMPTS TO CYCLIZE HIGHER PEPTIDES

Several attempts were made to cyclize the tri- and tetra-peptide of α-methylalanine activated either as the 2,4,5-trichlorophenyl ester,[4] with dicyclohexylcarbodiimide,[5] or with Woodward's reagent K.[6] At most, some polymer was obtained together with unchanged peptide. This is all in accord with the failure of other workers [7] to cyclize these peptides through oxazolone intermediates.

Under conditions used for the formation of the more reactive acid chlorides and for their subsequent cyclization in pyridine solution, two crystalline sublimable substances were obtained both from the tetra- and the penta-peptide, together with polymers and unreacted peptide. One of these, having infrared bands at 1730, 1670, and 1640 cm^{-1}, proved to have the bicyclic imidazolone structure (VI, Fig. 2), formed by loss of one, and two, respectively, amino

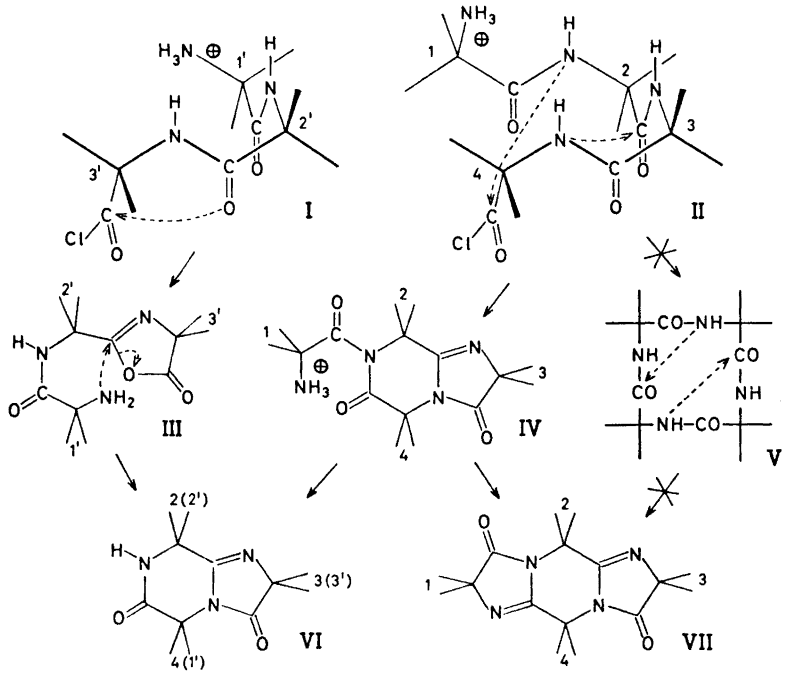


Fig. 2.

acids. It was identical with a product reported earlier by Kenner's group [8] to be formed from the tripeptide oxazolone (III). The second crystalline product had infrared bands at 1720 and 1630 cm^{-1} and was shown to possess a tricyclic bis-imidazolone structure (VII, Fig. 2); in the case of the penta-peptide it was formed by loss of one amino acid.

That the bis-imidazolone (VII) did not arise from initially formed monocyclic tetrapeptide (V) by subsequent double transannular cyclol formation [9] and dehydration was shown by the observation that replacement of one of the amino acids of the tetrapeptide by a fully methyl-deuterated amino acid did not lead to the required equal distribution of deuterium on all positions of VII, but to specific deuteration of only one (see below).

The following additional arguments point to partial ring-formation already in the chlorination step:

1. Identical products are formed from the tetra- and penta-peptide.

2. Infrared bands for the amide group at 1670 and 1525 cm^{-1} are absent in the precipitated intermediate obtained from the tetra- and penta-peptide with PCl_5 in acetyl chloride, while three new bands are present at 1790, 1730, and 1590 cm^{-1}. Of these, the 1730 cm^{-1} band may be the same as found in the product, while the 1790 cm^{-1} band must be due to the acid chloride. The intermediate obtained from the tripeptide with $SOCl_2$ has no band at 1790 cm^{-1}, but instead a band at 1820 cm^{-1} typical of all oxazolones used as intermediates in the synthesis [7,10] of the linear peptides, and so must be in fact the oxazolone.

3. The authentic oxazolone of the tetrapeptide does not give the bi- and tricyclic products on attempted cyclization.

4. The bi- and tricyclic products (VI and VII) are formed also by direct sublimation of the chlorinated intermediate at reduced pressure.

To gain insight into the mechanism of this reaction, one residue of α-methylalanine fully deuterated in both methyl groups was introduced systematically by synthesis in various positions of the tri-, tetra-, and penta-peptide, and the bi- and tricyclic products examined by NMR-spectroscopy in $CDCl_3$ (Fig. 3). Earlier ^{14}C labelling experiments by Kenner's group [8] on the tripeptide oxazolone (III) showed that the last amino acid ended up in the imidazolone ring; hence the methyl groups in this ring give rise to the line at 1.35 δ. For the tricyclic product, the lines at 1.33 and 1.89 δ are by inference assigned to the methyl groups in the five- and six-membered rings, respectively.

On the basis of these deuteration experiments a reaction mechanism II→IV→VI+VII can be proposed for the case of the tetrapeptide (Fig. 2.) The assumption is made that any intermediate chloroimine double bond, as well as the parent amide group, is exclusively in *trans*,* and that chain-folding therefore can only occur at the α-carbon to give part of a 4-helix (I and II). The amino-end being protected as the hydrochloride, the acid chloride can only be attacked by the 2-carbonyl oxygen in the tripeptide (I) to give the

* Although the figures have been drawn with amide groups in all positions, it is understood that the chloroimine ($-\overset{\overset{Cl}{|}}{C}=N-$) and the amide ($-\overset{\overset{O}{\|}}{C}-NH-$) are comparable both sterically and mechanistically, carbon having similar electrophilic and nitrogen similar nucleophilic properties in the two cases.

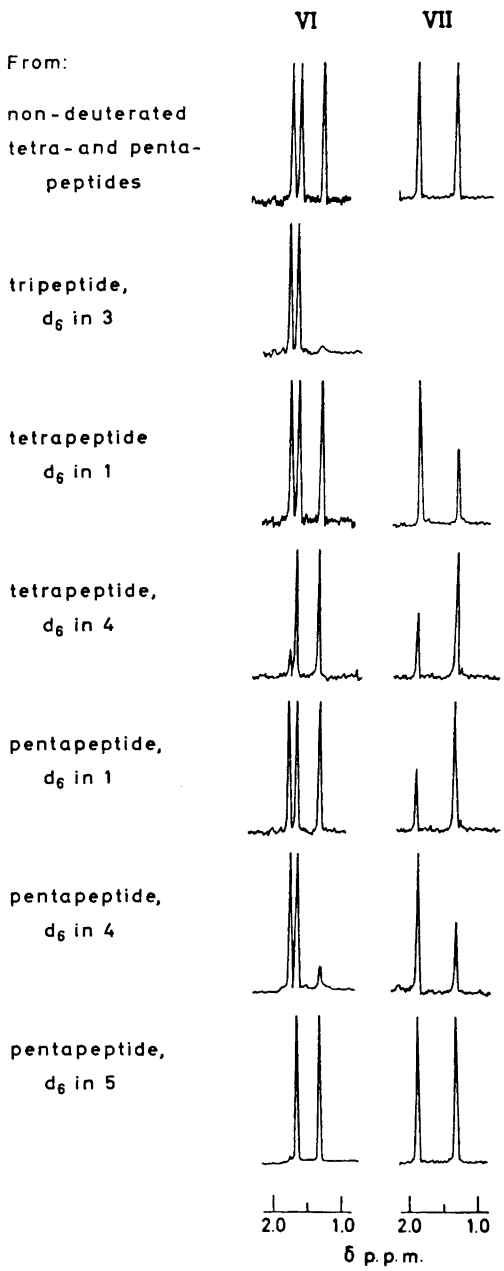


Fig. 3. NMR-spectra at 60 MHz in $CDCl_3$-solution of bicyclic imidazolone (VI) and tricyclic bis-imidazolone (VII) formed from non-deuterated peptides (top) and from various deuterated peptides (lower curves).

oxazolone (III), whereas in the tetrapeptide (II) imidazolone-formation seems likely (*cf.* hippuric amide [11] using PCl_5, and benzoyl-α-methylalanine amide [12] by simple heating) and presumably synchronized with fused piperazine formation as the acid chloride group is drawn closer to the remaining amide group. Elimination of the side-chain of the resulting intermediate (IV) gives the bicyclic imidazolone (VI), and cyclization of the side-chain when the amino group is liberated affords the tricyclic bis-imidazolone (VII).

A corresponding scheme for the pentapeptide is also in full agreement with the deuteration results (Fig. 3) for the bicyclic imidazolone assuming elimination of a dipeptide side-chain on the amino end. However, it does not explain the deuteration results for the tricyclic bis-imidazolone, which show that now the C-terminal residue is eliminated. Possibly, cyclization of the dipeptide side-

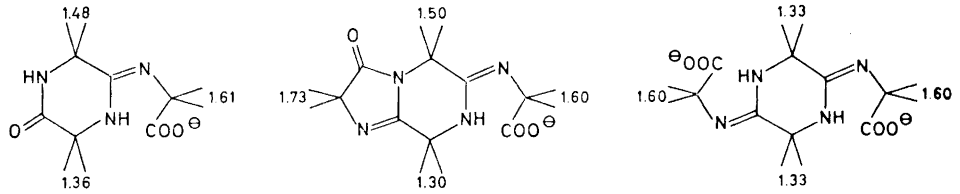
Table 1. Properties of bicyclic imidazolone (VI) and tricyclic bis-imidazolone (VII).

	VI	VII
M.p.	255°	253°
Mol. ion (mass spectrometry)	237	304
Yield from tripeptide	11 %	–
tetrapeptide	26 %	10 %
pentapeptide	33 %	15 %
Double bond abs. in IR	1730, 1670, 1640 cm^{-1}	1720, 1630 cm^{-1}
NMR shifts (ppm) in $CDCl_3$	1.78, 1.68, 1.35	1.89, 1.33

chain is now unlikely since an 8-membered ring would be formed. A different mechanism seems in this case to lead to the same bis-imidazolone in even better yield (Table 1). Initial imidazolone formation at the 2-residue might lead to this substance by final elimination of a side-chain consisting now of the 5-residue.

RING OPENING REACTIONS

Certain acylamidines of bicyclic type are known to undergo reversible conversion to mono-cyclic amides.[9] The possibility that our mono- and bis-imidazolones (VI and VII) might in alkaline medium be hydrated to cyclols, and then converted to cyclic tri- and tetrapeptides, was therefore examined. In alkaline water/methanol solution the imidazolone (VI) was slowly converted to the salt of the amidino acid (VIII, Fig. 4), whose structure was subsequently confirmed by NMR-spectroscopic comparison with the authentic substance.[8] The bis-imidazolone (VII) under the same conditions gave the salts of the mono-amidino acid (IX) and the bis-amidino acid (X). This was concluded by following the NMR-spectral changes of the solutions as well as from a study of



VIII IX X

Fig. 4.

partially deuterated amidino acids (VIII) which made possible the assignments of NMR-signals given in Fig. 4.

All these amidino acids reverted to the parent imidazolone (VI or VII) under acidic or even neutral conditions. No further change, apart from a low-field shift of the NMR-signals, could be observed in strongly acidic water/methanol solutions.

SYNTHETIC PATHS

The linear peptides were built up according to the methods developed by Kenner's group [7,10] using benzyloxycarbonyl protection at the amino end and *t*-butyl ester protection at the carboxyl end. Methyl ester protection could be used only for the dipeptide; thus, alkaline hydrolysis of the methyl ester of benzyloxycarbonyl dipeptide in aqueous dioxan [13] gave the acid in 85 % yield, whereas the tripeptide gave only 41 % (*cf.* Ref. 7). Oxazolone activation [7] was employed for coupling the tri- and higher peptides. This method was usefully modified to combine oligopeptide oxazolones with amino-esters containing more than one amino acid residue, although a longer reaction time (> 96 h) was needed. The individual operations necessary to prepare a penta-peptide were thus reduced by four steps (overall yield 50 %).

α-Methylalanine-d_6 was prepared from acetone-d_6 *via* the hydantoin derivative.[14,15] The isotopic purity of the labelled residue in labelled tri-, tetra-, and penta-peptides was 96 – 98 % as determined by NMR-spectroscopy.

EXPERIMENTAL

Derivatives and oligomers of α-methylalanine were prepared according to Kenner *et al.*,[7,10] except when otherwise stated. Evaporations were under reduced pressure. Neutral products were isolated by washing in ethyl acetate solution successively with 0.1 N HCl, water, 0.5 M $NaHCO_3$, water, and finally dried over $MgSO_4$ and evaporated.

Benzyloxycarbonyl-(α-methylalanyl)$_3$-α-methylalanine t-butyl ester. α-Methylalanyl-α-methylalanine *t*-butyl ester (2.2 g = 9 mmol), prepared by catalytic hydrogenation of the corresponding benzyloxycarbonyl derivative, was dissolved in dry acetonitrile (40 ml), 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone [10] (2.74 g = 9 mmol) added, and the solution heated under reflux for 96 h. The acetonitrile was evaporated and the neutral tetrapeptide isolated in the usual way and recrystallized from ethyl acetate-light petroleum (4.5 g = 91 %), m.p. 178°.[7]

Benzyloxycarbonyl-(α-methylalanyl)$_4$-α-methylalanine t-butyl ester. (a) A solution of 2-(1′-benzyloxycarbonyl-α-methylalanylamino-1′-methyl)ethyl 4,4-dimethyloxazolone [7] (0.98 g = 2.5 mmol) and α-methylalanyl-α-methylalanine *t*-butyl ester (0.73 g = 3 mmol) in dry acetonitrile (20 ml) was heated under reflux for 7 days. The neutral pentapeptide was isolated in the usual way and recrystallized from ethyl acetate-light petroleum or acetonitrile (1.1 g = 70 %), m.p. 236° (decomp.).[7]

(b) (α-Methylalanyl)$_2$-α-methylalanine *t*-butyl ester (0.82 g = 2.5 mmol), prepared by catalytic hydrogenation of the corresponding benzyloxycarbonyl derivative,[7] was dissolved in dry acetonitrile (15 ml), 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyl-oxazolone [10] (0.6 g = 2 mmol) added, and the mixture heated under reflux for 7 days. The pentapeptide ester was isolated and recrystallized as above (0.8 g = 63 %), m.p. 236° (decomp.).[7]

Imidazolone VI from (α-methylalanyl)$_2$-α-methylalanine. Benzyloxycarbonyl-(α-methylalanyl)$_2$-α-methylalanine [7] (1.26 g = 3 mmol) was hydrogenated in methanol (50 ml) containing 5 % Pd-C catalyst (0.4 g) until evolution of CO_2 ceased, then filtered and evaporated. The resulting (α-methylalanyl)$_2$-α-methylalanine (0.82 g = 3 mmol) was refluxed with thionyl chloride (25 ml) for 1 h and evaporated. Traces of $SOCl_2$ were removed by evaporation with benzene. The product (0.9 g), showing ν_{max} at 1820 cm^{-1}, was dissolved in dry dimethylformamide (25 ml) and added during 1 h to dry pyridine (700 ml) at 70° with stirring. Heating and stirring was continued for 5 h. After evaporation the residue was taken into methanol (10 ml) and kept overnight at 0°. A precipitate of polymers (0.28 g) was filtered off and unreacted tripeptide (0.3 g) precipitated with ether from the filtrate. The filtered solution was evaporated, the residue dissolved in chloroform and washed with water to remove remaining traces of unreacted tripeptide and polymers. The chloroform solution was dried over $MgSO_4$ and evaporated to afford the imidazolone VI (0.075 g = 11 %), purified by sublimation (90 – 110°/0.02 mmHg), m.p. 255° (sealed tube).[8]

Imidazolone VI and bis-imidazolone VII. A. *From (α-methylalanyl)$_3$-α-methylalanine.* Benzyloxycarbonyl-(α-methylalanyl)$_3$-α-methylalanine (2.5 g = 5 mmol), m.p. 244°, prepared by hydrolysis of the corresponding *t*-butyl ester in trifluoroacetic acid,[7] was hydrogenated in methanol (100 ml) using 5 % Pd-C catalyst (0.7 g). After filtration, the solution was evaporated to afford the tetrapeptide, which was reprecipitated from acetone-ether (1.7 g = 95 %).

(a) (α-Methylalanyl)$_3$-α-methylalanine (0.72 g = 2 mmol) was refluxed with thionyl chloride (20 ml) for 1 h and evaporated. Traces of thionyl chloride were removed by repeating the evaporation with benzene. The residue (0.8 g), showing ν_{max} at 1790 cm^{-1}, was dissolved in dry dimethylformamide (25 ml) and added during 1 h to dry pyridine (700 ml) at 70° with stirring; the mixture was kept at this temperature for 4 h. The residue after evaporation was taken into methanol (10 ml) and left overnight at 0°. Some bis-imidazolone VII crystallized along with a precipitation of polymers. The crystals (0.02 g) were picked out manually and the polymers (0.2 g) filtered off. The filtrate was evaporated and the residue taken into water and extracted with chloroform. The aqueous solution contained the polymers, unreacted peptide and α-methylalanine. The chloroform solution was dried over $MgSO_4$ and evaporated, and the imidazolone VI and more bis-imidazolone VII were fractionally crystallized from methanol and further purified by sublimation. Total yields and properties were: VI (0.068 g = 14 %), subl. at 90 – 110°/0.02 mmHg, m.p. 255° (sealed tube);[8] VII (0.31 g = 5 %, subl. at 100 – 120°/0.02 mmHg, m.p. 253° (sealed tube). (Found: C 63.12; H 7.77; N 18.60. $C_{16}H_{24}N_4O_2$ requires C 63.16; H 7.95; N 18.42.)

(b) (α-Methylalanyl)$_3$-α-methylalanine (0.1 g = 0.27 mmol) was suspended in freshly distilled acetyl chloride (10 ml), cooled to 0° and PCl_5 (0.25 g) added. A clear solution was formed after 3 min, and this was stirred for 20 h at room temp., when a white precipitate formed. The solution was decanted and the precipitate washed with a little acetyl chloride. The solid showed ν_{max} (KBr) at 1790, 1730, 1590 cm^{-1}.

A portion of the precipitate, dissolved in dry dimethylformamide (25 ml), was added to pyridine (500 ml) as described above. The residue after evaporation was dissolved in chloroform and washed with water. The water layer contained polymers, unreacted peptide and α-methylalanine. Evaporation of the chloroform solution afforded a mixture of VI and VII, finally separated and purified as in the preceding experiment to give pure VI (0.017 g) and VII (0.007 g).

B. *From (α-methylalanyl)$_4$-α-methylalanine.* Hydrogenolysis of benzyloxycarbonyl-(α-methylalanyl)$_4$-α-methylalanine (1.15 g = 2 mmol) in methanol (50 ml) containing Pd-C catalyst (0.5 g) yielded (α-methylalanyl)$_4$-α-methylalanine, precipitated from acetone-ether (0.83 g = 95 %).

This pentapeptide (0.22 g = 0.5 mmol) was suspended in freshly distilled acetyl chloride (20 ml), cooled to 0° and PCl_5 (0.5 g) added. The clear solution which appeared after few minutes was stirred at room temp. for 20 h, when a white substance precipitated. Both the precipitate and the filtrate after evaporation showed absorption at ν_{max} 1790, 1730, and 1690 cm^{-1}. The combined products in dry dimethylformamide (25 ml) were added to pyridine (700 ml) as in the preceding experiment. After evaporation, the residue was dissolved in chloroform and washed with water. The imidazolone VI (0.024 g = 20 %) and bis-imidazolone VII (0.006 g = 4 %) were isolated in the usual way.

α-*Methylalanine*-d_6. Acetone-cyanohydrin-d_6 was prepared from hexadeuterioacetone (16.5 g = 0.25 mol) and converted into 5,5-dimethylhydantoin-d_6 by the method of Wagner and Simons,[14] recrystallized from water, m.p. 175 – 178°. The resulting hydantoin (13 g = 0.1 mol), $Ba(OH)_2.8H_2O$ (45 g) and water (180 ml) were placed in an autoclave and heated for ½ h at 160 – 170°. After cooling, the solution was filtered and treated with $(NH_4)_2CO_3$ to precipitate the excess of barium, heated again to expel excess of $(NH_4)_2CO_3$, and filtered. The filtrate was concentrated until crystals appeared, and crystallization was completed by addition of methanol. The α-methylalanine-d_6 (7.2 g = 66 %) sublimed [15] at 280°, *m/e* 109.

Benzyloxycarbonyl-α-methylalanine-d_6. α-Methylalanine-d_6 (10.9 g = 0.1 mol) was reacted with benzyl chloroformate,[10] and formed benzyloxycarbonyl-α-methylalanine-d_6 recrystallized from ethyl acetate-light petroleum (20.5 g = 85 %), m.p. 74 – 75°. The integrated NMR-spectrum showed that 98 % of the α-methyl protons were deuterated.

Benzyloxycarbonyl-α-methylalanyl-d_6-*α-methylalanine.* Benzyloxycarbonyl-α-methylalanine-d_6 (8.5 g = 25 mmol) and α-methylalanine methyl ester hydrochloride (3.2 g = 27 mmol) in acetonitrile (150 ml) containing triethylamine (2.5g) was cooled to 0° and dicyclohexylcarbodiimide (5.8 g = 28 mmol) added. The mixture was stirred at room temp. (24 h) and evaporated. The neutral product was isolated in the usual manner and recrystallized from ethyl acetate-light petroleum (5.1 g = 15 mmol), m.p. 110°. Hydrolysis [13] gave benzyloxycarbonyl-α-methylalanyl-d_6-α-methylalanine, recrystallized from aqueous methanol (4.1 g = 85 %), m.p. 161°.

α-*Methylalanine*-d_6 *t-butyl ester.* Benzyloxycarbonyl-α-methylalanine-d_6 *t*-butyl ester (12.7 g = 87 %), m.p. 61°, was prepared from benzyloxycarbonyl-α-methylalanine-d_6 (12.15 g = 50 mmol).[7] Hydrogenolysis of the benzyloxycarbonyl group afforded α-methylalanine-d_6 *t*-butyl ester [7] (94 %).

Deuterated imidazolone VI from (α-methylalanyl)$_2$-α-methylalanine-d_6 via *the oxazolone.* α-Methylalanine-d_6 *t*-butyl ester (0.5 g = 3 mmol) was reacted with 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone [10] (0.92 g = 3 mmol) to yield the protected tripeptide (0.9 g = 64 %), m.p. 167°. The *t*-butyl ester was cleaved in TFA [7] to the acid (0.75 g), m.p. 202°. Treatment of the acid with acetic anhydride gave 2-(1′-benzyloxycarbonyl-α-methylalanylamino-1′-methyl)ethyl-4,4-dideuteromethyloxazolone (0.72 g), m.p. 125°. The oxazolone derivative (0.72 g = 1.8 mmol) was hydrogenated in dry ethyl acetate and set aside for 3 days at room temp.[8] The precipitation of amidine-d_6 was collected and recrystallized from ethanol-ether (0.4 g), δ_{TMS} 1.58, 1.66 in $CDCl_3$, integration ratio 1:1. Sublimation (90 – 110°/0.02 mmHg) gave dehydration to the imidazolone VI-d_6 (0.38 g = 88 %) m.p. 255° (sealed tube), *m/e* 243 (Fig. 3).

Deuterated imidazolone VI and bis-imidazolone VII. (a) *From α-methylalanyl*-d_6-(α-*methylalanyl)$_2$-α-methylalanine.* Benzyloxycarbonyl-α-methylalanyl-d_6-α-methylalanine (2.6 g = 8 mmol) was dehydrated to the corresponding oxazolone [10] (2.3 g = 93 %), m.p. 126°. Part of it (1.1 g = 3.5 mmol) was reacted with dipeptide *t*-butyl ester (0.9 g = 3.5 mmol) to yield benzyloxycarbonyl-α-methylalanyl-d_6-(α-methylalanyl)$_2$-α-methylalanine *t*-butyl ester (1.7 g = 88 %), m.p. 178°. Cleavage of the *t*-butyl ester in TFA [7] yielded benzyloxycarbonyl-tetrapeptide (1.4 g = 93 %), m.p. 245°. Hydrogenolysis in acetic acid (200 ml) using Pd-C catalyst (0.6 g) afforded α-methylalanyl-d_6-(α-methylalanyl)$_2$-α-methylalanine (1 g = 99 %). This tetrapeptide (0.36 g = 1 mmol) was treated with PCl_5 (0.55 g) in acetyl chloride (30 ml) at 0° and stirred at room temp. for 20 h. After evaporation, the residue (showing ν_{max} 1790 cm^{-1}) was dissolved in dimethylformamide (20 ml) and added during ½ h into pyridine (500 ml) at 70° with stirring. After 4½ h at 70° the solution

was evaporated and the residue treated with chloroform-water. The cyclic compounds were isolated from chloroform and purified in the usual way; imidazolone VI (0.06 g = 25 %), *m/e* 237, bis-imidazolone VII-d_6 (0.03 g = 10 %), *m/e* 310 (Fig. 3).

(b) *From (α-methylalanyl)$_3$-α-methylalanine*-d$_6$. α-Methylalanine-d_6 *t*-butyl ester (1.5 g = 9 mmol) was reacted with 2-(1′-benzyloxycarbonyl-α-methylalanylamino-1′-methyl)-ethyl-4,4-dimethyloxazolone [7] (3.2 g = 8 mmol). The resulting protected tetrapeptide (3.4 g = 77 %), m.p. 178°, was hydrolyzed in TFA [7] to the corresponding acid (2.8 g = 93 %), m.p. 245°, and a part of it (1.5 g = 3 mmol) hydrogenated in acetic acid (300 ml) to (α-methylalanyl)$_3$-α-methylalanine-d_6 (1 g = 98 %). This tetrapeptide-d_6 (0.5 g = 1.4 mmol) was reacted with PCl_5 (1.1 g) in acetyl chloride (30 ml), and after evaporation cyclized in pyridine as described. The imidazolone VI-d_6 (0.013 g = 3.4 %), *m/e* 243, and the bis-imidazolone VII-d_6 (0.008 g = 1.8 %), *m/e* 310, were isolated (Fig. 3).

(c) *From α-methylalanyl*-d$_6$-*(α-methylalanyl)$_3$-α-methylalanine*. The oxazolone of benzyl-oxycarbonyl-α-methylalanyl-d_6-α-methylalanine (0.93 g = 3 mmol) was reacted with the tripeptide *t*-butyl ester (1 g = 3 mmol) to give the protected pentapeptide (1.7 g = 89 %), m.p. 236°. The *t*-butyl ester was hydrolyzed to the acid (1.5 g = 96 %), m.p. 256°, and hydrogenated in acetic acid (200 ml) to α-methylalanyl-d_6-(α-methylalanyl)$_3$-α-methyl-alanine (1.1 g = 97 %). This pentapeptide (0.3 g = 0.67 mmol) was reacted with PCl_5 (0.6 g) in acetyl chloride (30 ml) and cyclized in pyridine as usual. The imidazolone VI (0.048 g = 30 %), *m/e* 237, and the bis-imidazolone VII-d_6 (0.006 g = 3 %), *m/e* 310, were isolated (Fig. 3).

(d) *From (α-methylalanyl)$_3$-α-methylalanyl*-d$_6$-*α-methylalanine*. Benzyloxycarbonyl-(α-methylalanyl)$_3$-α-methylalanine-d_6 (1 g = 2 mmol) was dehydrated to the oxazolone [7] (0.95 g = 2 mmol), m.p. 158° and reacted with α-methylalanine *t*-butyl ester (0.3 g = 2 mmol) to give the protected pentapeptide [7] (1 g = 78 %), m.p. 236°. This was hydrolyzed to the acid (0.8 g = 92 %), m.p. 256°, and then hydrogenated in acetic acid (200 ml) to (α-methylalanyl)$_3$-α-methylalanyl-d_6-α-methylalanine (0.6 g = 97 %). This pentapeptide (0.3 g = 0.67 mmol) was reacted with PCl_5 (0.55 g) in acetyl chloride (15 ml), and after evaporation of solvents cyclized in pyridine to give the imidazolone VI-d_6 (0.051 g = 31 %), *m/e* 243, and the bis-imidazolone VII-d_6 (0.015 g = 7 %), *m/e* 310 (Fig. 3).

(e) *From (α-methylalanyl)$_4$-α-methylalanine*-d$_6$. α-Methylalanine-d_6 *t*-butyl ester (0.7 g = 4.2 mmol) was reacted with 2-(1′-benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-amino-1′-methyl)ethyl-4,4-dimethyloxazolone [7] (1.9 g = 4 mmol) to give the protected pentapeptide (2 g = 74 %), m.p. 236°. The *t*-butyl ester was hydrolyzed to the acid (1.7 g = 98 %), m.p. 256°, and part of it (0.9 g = 1.5 mmol) hydrogenated to (α-methylalanyl)$_4$-α-methylalanine-d_6 (0.65 g = 98 %). The pentapeptide (0.2 g = 0.45 mmol) was reacted with PCl_5 (0.4 g) in acetyl chloride (20 ml), and cyclized in pyridine to give the imidazolone VI-d_6 (0.035 g = 32 %), *m/e* 243, and the bis-imidazolone VII (0.02 g = 15 %), *m/e* 304 (Fig. 3).

Ring opening reactions. These were followed directly in the NMR-tubes in a mixture of 20 % NaOD in D_2O (0.2 ml) and CD_3OD (0.5 ml). The final spectrum starting from non-deuterated imidazolone (VI) had δ 1.61, 1.48, and 1.36, while the same compound deuterated in the five-membered ring had δ 1.48 and 1.36, and in the six-membered ring δ 1.61 and 1.48.

Experiments in acid medium were conducted in a mixture of 36 % DCl in D_2O 0.1 ml) and CD_3OD (0.5 ml).

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