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SYNTHESIS OF BOC-CYS-ALA-OME AND ITS STEREOSELECTIVE ADDITION TO α-METHYLENE- γ-BUTYROLACTONES

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Abstract: The stereoselective addition of enantiomerically pure (as shown by 200 MHz ^{1}H NMR study) dipeptide **Boc-Cys-Ala-OMe 4** to α-methylene-γ-butyrolactones has been studied The stereoselectivity of the addition parallels the enantioselectivity observed in the allergic contact dermatitis induced in guinea pigs

INTRODUCTION

Allergic contact dermatitis (ACD), an inflamation of the skin, is generally provoked by small electrophilic compounds called haptens The hapten penetrates the skin, reacting with cell surface and structural proteins These modified proteins then undergo phagocytosis and proteolysis in the epidermis macrophage, the Langerhans cell The effective antigen which is the hapten bound to a protein fragment is then reexpressed on the cell surface by the Langerhans cell and presented to T-lymphocytes bearing a receptor that fits the antigenic entity This receptor mediated cell-cell interaction triggers the immune system specifically against the hapten and leads, through a complex cascade of biological processes, to hypersensitivity to the chemical

Since the protein itself is a pure enantiomer, conjugates that result from the addition of optically pure haptens are diastereomeric and their properties and particularly the recognition by the T-lymphocyte receptors can be expected to be different It was recently shown in this laboratory[1] that the enantiospecificity observed in ACD was, at least in part, related to the proximity of the chiral center relative to the site of nucleophilic protein attack[1] This site is most probably the α-methylene group in α-methylene-γ-butyrolactones

Establishment of enantiospecificity of ACD is based on the absence of cross-reaction A cross-reaction is an adverse skin reaction to a substance B , different from the primary or initial sensitizer A or allergen There is no cross-reaction when guinea pigs sensitized to one enantiomer are unaffected by the other enantiomer The principle of cross-reaction is commonly used in clinical practice to determine to which family of compounds a patient is allergic Cross-sensitivity or cross-reaction generally occurs when the structure of the primary sensitizer and of the test compound are related From a molecular view point, this means that the "effective antigens" produced *in vivo* with two related haptens are not structurally very different and are recognized by the same T-cell receptor Data from this laboratory have shown, however, that

enantiomers do not cross-react One of the purpose of this study was to find out whether there was some correlation between the expected diastereoselection of a model dipeptide addition to α-methylene-γ-butyrolactones and the enantiospecificity of the observed skin reaction in experimentally sensitized guinea pigs

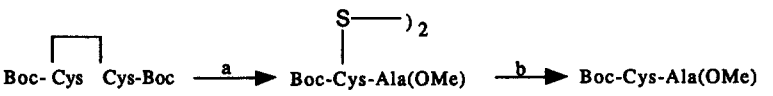
In this paper, we describe the stereochemistry of the addition of a dipeptide Boc-Cys-Ala-OMe **4**, used as a model of protein nucleophilic attack - to enantiomers of natural and synthetic α-methylene-γ-butyrolactones In order to unambiguously establish a possible diastereoselection in the addition reaction, we needed to demonstrate the enantiomeric purity of the dipeptide This was done by NMR We shall first deal with the dipeptide synthesis and the determination of its optical purity and second with the addition of this dipeptide to α-methylene-γ-butyrolactone enantiomers

RESULTS AND DISCUSSION

1 PEPTIDE SYNTHESIS

We have synthesized the following dipeptide Boc-L-Cys-L-Ala-OMe using Boc- protected L-cystine and L-alanine methyl ester, according to the mixed carbonic anhydride method[2,3] Regeneration of the thiol function was accomplished with tri-(1-n-butyl) phosphine[4] (Scheme I)

Scheme I



a N-methyl morpholine, Isobutyl chloroformate, THF-DMF
Ala(OMe), -15°C ⟶ RT

b $P(Bu)_3$, $MeOH/H_2O$

In order to determine whether racemization had occurred or not during these two steps, bis-Boc-(D,L)-cystine[5] and (D,L)-Ala-OMe were also used and compared to the peptides obtained from pure L-amino acids[6] The 200 MHz spectra were recorded on a Brucker 200 MHz spectrometer, the chemical shifts as well as the spin-spin coupling constants are collected in Table I

Table I Boc-protected dipeptide methyl esters

Dipeptide	Anal.data calcd/(found) C	H	N	^{1}H-NMR of -OMe and -CH_2S- group in $CDCl_3$[a]
Disulfide dimer of Boc-L-Cys-L-Ala-OMe **1**	47 20 (47 42)	6 93 (7 01)	9 17 (8 99)	-OCH_3 s 3 71 -CH_2S- AB p of an ABX δ_a 2 89 δ_b 3 08 J_{ab} 14 7 J_{ax} 11 1 J_{bx} 3 8
Disulfide dimer of Boc-(D,L)-Cys-L-Ala-OMe **2**	47 20 (47 29)	6 93 (6 82)	9 17 (9 12)	-OCH_3 four s 3 76 3 75 3 74 3 71 -CH_2S- complex m
Disulfide dimer of Boc-L-Cys-(D,L)-Ala-OMe **3**	47 20 (47 04)	6 93 (7 04)	9 17 (9 08)	-OCH_3 three s 3 74 3 73 3 70 -CH_2S- complex m
Boc-L-Cys-L-Ala-OMe **4**	47 04 (46 93)	7 24 (7 29)	9 14 (8 90)	-OCH_3 s 3 76 -CH_2SH AB p of an ABXY δ_a 2 79 δ_b 3 10 J_{ab} 13 9 J_{ax} 10 1 J_{bx} 7 9
Boc-(D,L)-Cys-L-Ala-OMe **5**		nd[b]		-OCH_3 s 3 75 -CH_2SH two AB p of an ABXY from the (L- L) compound (same data as **4**)
Boc-L-Cys-(D,L)-Ala-OMe **6**	47 04 (47 04)	7 24 (7 22)	9 14 (8 92)	-OCH_3 s 3 74 -CH_2SH identical to **5**

a chemical shifts are reported in δ ppm with respect to TMS as internal standard Coupling constants (J) are expressed in hertz Multiplicities are indicated by s (singlet) and m (multiplet) Other domains of the NMR spectra (concerning the H_αCys, H_αAla) show significant changes in racemic peptides but are not discussed here

b nd = not done since **5** is a racemic mixture corresponding to **4**

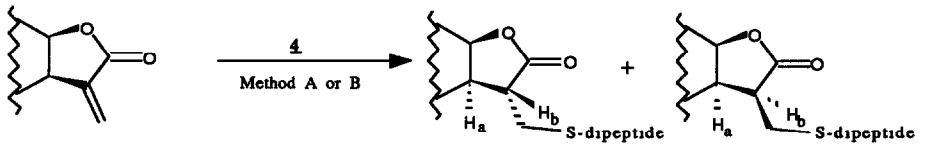
2 . HAPTEN - DIPEPTIDE COUPLING

Two general methods were used for the preparation of the hapten-dipeptide derivatives

In a procedure that resembles physiological conditions (method A) the reaction was run in 0 2M sodium phosphate buffer, at pH 7 4 containing absolute ethanol to dissolve the dipeptide and the lactone[7]

Alternatively (method B), the dipeptide was solubilized in dry methanol, to which a catalytic amount of sodium methoxide (0 3 eq) was added A solution of the lactone in dry methanol was then added dropwise The reaction was completed in 10 minutes and quenched by the addition of 1N HCl The product composition thus obtained is summarized in Table II

Table II Results of dipeptide addition to lactones 7 to 11 and of skin tests in guinea pigs



H_a - H_b ANTI adduct H_a - H_b SYN adduct

Lactone (sensitizer[a])	Anti/syn ratio[b] of adducts		Average skin reaction[d] Tests with enantiomers	
	A[c]	B[c]	(+)-	(-)-
(+)-β-methyl-α-methylene-γ-butyrolactone (+)-7	nd	6 1	0 9	0[1]
(-)-β-methyl-α-methylene-γ-butyrolactone (-)-7	9 5 1	8 1	0 2	1 7[1]
(+)-γ-methyl-α-methylene-γ-butyrolactone (+)-8	6 1	2 1	1 3	0 4[8]
(-)-γ-methyl-α-methylene-γ-butyrolactone (-)-8	2 1	2 1	0 8	0 9[8]
(+)-β,γ-dimethyl-α-methylene γ-butyrolactone (+)-9	anti[e]	anti[e]	0 4	0 5[1]
(+)-Frullanolide (+)-10	anti[e]	anti[e]	2 0	0 2[9]
(-)-Frullanolide (-)-10	anti[e]	anti[e]	0 3	1 8[9]
(+)-Alantolactone (+)-11	anti[e]	4 1	2 0	NA[f]

[a] Guinea pigs were sensitized (made allergic) with the mentioned lactones by the FCAT method[10]

[b] The anti/syn ratio was determined by 200 and 400 MHz NMR

[c] Method A Phosphate buffer/ethanol, method B, sodium methoxide/methanol

[d] Guinea pigs were tested on their flanks with non-irritant 1 % ethanolic solutions of lactones The *average skin reaction* was obtained by adding the intensities of the individual tests and dividing the result by the number of guinea pigs used The intensity of the skin test was ranked according to the following scale

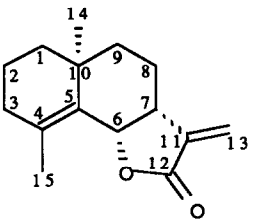
0 = no reaction,
0 5 = discrete erythema not covering the test area;
1 = erythema covering the test area,
2 = erythema + swelling on the test area,
3 = erythema and swelling going well beyond the test area

[e] No syn could be detected neither by 200 nor by 400 MHz NMR spectroscopy

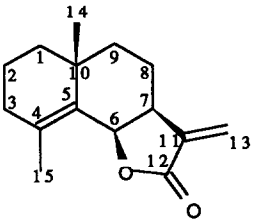
[f] The other enantiomer of alantolactone is not available

The induction of contact sensitivity requires coupling of the hapten to a skin protein Most strong haptens are electrophilic (like α–methylene-γ-butyrolactones) and undergo nucleophilic attack by free amino or thiol groups of polypeptides Derivatization of proteins by α,β-unsaturated lactones alters their structure and makes them antigenic These antigenic protein-lactone conjugates are diastereomeric since both proteins and the lactone used for sensitization are optically pure The antigenic structures obtained with one or the other isomer of the hapten are expected to be different and would not be recognized by the same receptors on T-cells The results obtained in this study relate the skin reaction to the absolute configuration of the chiral center in position β– and γ– of the γ-butyrolactone It can also be expected that, the closer the chiral center to the α-methylene group (the site of Michael addition of the protein nucleophile), the more specific should be the biological response

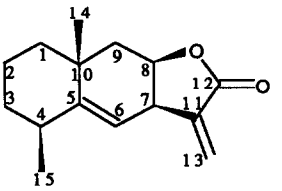
With both (+)- and (-)- frullanolides (+)-**10** and (-)-**10** only one diastereomer (isomer H_7,H_{11} Anti) was observed when the dipeptide **4** was added under the two reaction conditions This "total" asymmetric induction (within the limit of 400 MHz NMR detection >98%) is parallel to the observed high enantioselectivity of the allergic skin reaction in the guinea pig almost no cross-reaction (average 0 2) to the (+)- enantiomer was observed in (-)- enantiomer sensitized animals and conversely[9]



(+)- **10**



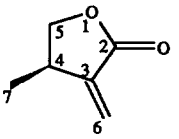
(-)- **10**



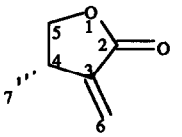
(+)- **11**

With alantolactone **11** (only one enantiomer is occurring naturally), 100 % asymetric induction (again within the limit of 400 MHz NMR detection) was observed when coupling was performed in phosphate buffer-ethanol but an anti/syn ratio of 4/1 was observed in sodium methoxide/methanol Diastereoselectivity was always higher when coupling was effected in phosphate buffer as compared to the sodium methoxide method In sodium methoxide/methanol condition, the attacking species is the dipeptide-S^- anion while in phosphate buffer (pH 7 4), the nucleophile is probably the non ionized thiol due to the higher pKa of the SH group Discrepancy in the two reaction conditions is therefore not unexpected In this case, it was not possible to compare the results with the *in vivo* data since the (-)-enantiomer is not available

With (+)- and (-)-β-methyl-α-methylene-γ-butyrolactones (+)-**7** and (-)-**7**, a high stereoselection was observed, whether the reaction was run in phosphate buffer or in sodium methoxide/methanol Reaction at pH 7 4 in the phosphate buffer is closer to *in vivo* conditions In both cases however, a selectivity of 6 1 was observed This parallels the animal experiment little, if any (0 2 average skin reaction) cross-reaction to the other enantiomer was observed[1]

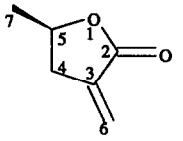


(+)- **7**

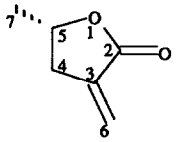


(-)- **7**

The case of the γ-methyl-α-methylene-γ-butyrolactones **8** is an interesting one since both (+)- and (-)- enantiomers behave differently in *in vivo* experimental sensitization (+)-γ-Methyl-α-methylene-γ-butyrolactone (+)-**8** is a better sensitizer than its (-)- enantiomer (-)-**8** Guinea pigs sensitized with the (+)-compound gave a very weak skin reaction with the (-)-γ-methyl-α-methylene-γ-butyrolactone (-)-**8** In contrast however, when guinea pigs were sensitized to the (-)- enantiomer and skin tested with the (+)-enantiomer, the allergic reaction was comparable to the response obtained with the primary sensitizer Thus (+)- and (-)- γ-methyl-α-methylene-γ-butyrolactones do cross-react on (-)-sensitized guinea pigs While the absence of cross-reaction could be confirmed both ways with β-methyl lactones and frullanolides, γ-methyl-α-methylene-γ-butyrolactone enantiomers did not behave identically in a two way skin test These *in vivo* findings are parallel to the diastereoselectivity observed in the formation of γ-methyl lactone - dipeptide adduct An anti/syn ratio of 6 1 was measured in the addition of dipeptide **4** to the (+)- γ-methyl-α-methylene-γ-butyrolactone in phosphate buffer (2 1 anti/syn ratio in sodium methoxide), while a ratio of 2 1 only was found when **4** was added to the (-)- enantiomer in phosphate buffer The fact that enantiospecificity of the allergic reaction was observed in (+)-**8**, in contrast to (-)-**8** suggests that proximity of the chiral center with the site of attack is not the only factor that influence the enantiospecificity of the immune response



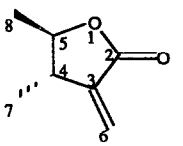
(+)- **8**



(-)- **8**

One case however did not follow the general observation No parallelism could be observed between enantiospecificity of the allergic reaction and diastereoselectivity of the peptide addition with (+)- β,γ-dimethyl α-methylene γ-butyrolactone **9** One single isomer was formed when peptide **4** was added to the lactone 100 % enantiomeric selectivity was noted in both phosphate buffer or sodium methoxide coupling conditions The (+)- β,γ- dimethyl α-methylene γ-butyrolactone is a weak allergen (0 4 average skin reaction)

which means that this hapten is less reactive in comparison with the other lactones tested The reaction between (+)-**2** and **4** was slow indicating that (+)-**2** is not as electrophilic as the other haptens tested A less reactive hapten however may produce fewer antigenic sites on skin proteins and may also be cleared more rapidly from the circulation This could diminish the affinity maturation of immunocompetent cells - which require constant antigenic stimulation - thereby preventing the production of high affinity T cell clones



(+)- **2**

CONCLUSION

A high level of diastereoselection of the addition of a nucleophilic dipeptide with enantiomers of α-methylene γ-butyrolactones could be noted Also, a parallelism between the diastereoselectivity of dipeptide addition and the enantioselectivity of allergic contact dermatitis in six out of eight enantiomeric lactones was observed

EXPERIMENTAL SECTION

General methods

Proton NMR spectra of samples were recorded on 200- or 400-MHz Brucker spectrometers in $CDCl_3$ or C_6D_6 Chemical shifts (δ) are indicated in ppm with respect to TMS as internal standard Infrared spectra were obtained on a Beckman Acculab spectrometer using $CHCl_3$ solutions Mass spectra were recorded on a LKB 9000 S spectrometer

Dry solvents were freshly distilled before use Tetrahydrofuran (THF) was distilled from sodium benzophenone Dimethylformamide (DMF) was stored over 4A molecular sieves All air and moisture sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon The chromatographic purifications were conducted at normal pressure on silica gel columns (D,L) alanine-OMe was purchased from Sigma and used without any further purification Bis-Boc-(D,L)-cystine was prepared from (D,L)-cystine according to Itoh's procedure[5]

(+)- and (-)-Frullanolides were extracted respectively from *Frullania dilatata* (L) Dum ssp nisquallensis and *F tamarisci* (L) Dum ssp tamarisci as described[9] Alantolactone and isoalantolactone were obtained as reported[11] from Helenin® (Sigma), an extract of *Inula helenium* (L) The α-methylene lactones **7** to **9** have been synthesized as described[1,8]

The peptide-lactone adducts **12** to **16** are rather unstable Nevertheless we tried up to four times to obtain a correct elemental analysis When we could not obtain a satisfactory elemental analysis we characterized the products by mass spectrometry

PEPTIDE SYNTHESIS

Disulfide dimer of Boc-Cys-Ala-OMe 1
A solution of N,N'-di Boc-Cystine (1 0 g, 2 3 mmol) in dry THF (10 mL) under argon was cooled to -15°C (CCl_4, dry ice) with stirring, 2 1 eq (0 54 ml) of N-Methyl-morpholine, followed by 2 2 eq (0 66 ml) of isobutyl chloroformate were added After five minutes, a solution of alanine methyl ester (2 eq) (642 mg) in DMF was added The CCl_4 - dry ice bath was removed 5 minutes later and the solution was allowed to stand for 20 h at room temperature The solution was concentrated under vacuum, the residue was taken up in ethyl acetate and water After extraction, the aqueous phase was discarded and the organic phase was washed successively with 10 mL of a saturated sodium bicarbonate solution, 10 mL of water, 10 mL of 1N hydrochloric acid and 10 mL of water The solution was dried over dry magnesium sulfate, filtered and then concentrated under vacuum The peptide was recrystallized in ethyl acetate Two crops of crystals (828 mg) of peptide 1 were obtained
Yield 60% (See Table I)

Disulfide dimer of Boc-(D,L)-Cys-Ala-OMe 2
The reaction was performed as above
Yield 65% (See Table I)

Disulfide dimer of Boc-Cys-(D,L)-Ala-OMe. 3
The reaction was performed as above
Yield 70% (See Table I)

Boc-Cys-Ala-OMe: 4
Reduction of 1 to 6 was essentially complete after reaction of peptide 1 (See Table I) with 0 2 M $P(Bu)_3$ (2 eq) in 9/1 (v/v) MeOH / water for 2h at 25°C The crude peptide was dissolved in ether and purified by column chromatography on silica gel (Eluent hexane / AcOEt 2/1)
Yield 85% (See Table I)

Boc-(D,L)-Cys-Ala-OMe: 5
The reaction was performed in the same way as for 4
Yield 85% (See Table I)

Boc-Cys-(D,L)-Ala-OMe: 6
The reaction was performed in the same way as for 4
Yield 85% (See Table I)

General procedure for the preparation of peptide-lactone adducts:
Procedure A. formation of adducts in phosphate buffer.
To a mixture of absolute ethanol (1 mL) and phosphate buffer (0 2 M, pH 7 4, 0 2 mL) was added 1 mmol of lactone and 1 mmol of dipeptide Boc-Cys-Ala-OMe 4 A stream of argon was bubbled through the reaction mixture for 1 h The flask was stored in the dark at room temperature for 5 days The reaction mixture was then poured into 5 mL ether The layers were separated and the organic phase was dried over magnesium sulfate and concentrated under vacuum
The adducts were purified by chromatography on silica gel
Procedure B. formation of adducts by using MeO^-Na^+/MeOH
To 5 mL of absolute methanol was added 1 mmol of dipeptide Boc-Cys-Ala-OMe 4 and 0 3 eq of sodium hydride at 0°C under argon The lactone (1 mmol, in 3 mL absolute methanol) was added dropwise and the mixture stirred for 10 min and quenched with 2 mL of 1N HCl The reaction mixture was then concentrated under vacuum, the residue taken up in ether and water After extraction, the organic layer was dried over magnesium sulfate The adducts were purified by chromatography on silica gel

(+)- β-Methyl- α-methylene- γ-butyrolactone-Cys(Boc)-Ala(OMe) Adduct 12a
Purified by chromatography, eluent hexane / AcOEt 1/1 (oil)
Method A Yield 95%
Method B Yield 90%
IR (cm^{-1}) 1790, 1770, 1745 , 1710, 1670
NMR (200 MHz) 7 15-7 00 (1H, m, NH-Ala), 5 50-5 40 (1H, m, NH-Cys), 4 57 (1H, dq J=7 1, Hα-Ala), 4 48-4 30 (2H, m, Hα-Cys + 1H of CH_2-1), 3 83-3 71 (1H, m, 1H of CH_2-1), 3 75 (3H, s, OMe), 3 09-2 85 (4H, m, CH_2SCH_2), 2 65-2 50(1H, m, H-4), 2 47-2 35 (1H, m, H-3), 1 47 (9H, s, t-Bu), 1 43 (3H, d $J_{Me-Ala,H\alpha-Ala}$=7 3, Me-Ala), 1 20 (6/7 of 3H, d $J_{Me-7,H-4}$=6 3, Me-7) and 1 08 (1/7 of 3H, d $J_{Me-7,H-4}$=7 0, Me-7)
Anal Calcd for $C_{18}H_{30}N_2O_7S$ C, 51 66, H, 7 22, Found C, 51 92, H, 7 24,
MS *m/e* 418 (M^+)

(-)- β-Methyl- α-methylene -γ-butyrolactone-Cys(Boc)-Ala(OMe) Adduct **12b**
Purified by chromatography, eluent hexane / AcOEt 1/1 (oil)
Method A Yield 93%
Method B Yield 90%
IR(cm^{-1}) 1790, 1770, 1745, 1710, 1670
NMR (400 MHz) 7 03-7 00 (1H, m, NH-Ala), 5 46-5 45 (1H, m, NH-Cys), 4 54 (1H, dq J=7 1, Ha-Ala), 4 47-4 43 (2H, m, Ha-Cys + 1H of CH_2-5), 3 81-3 74 (1H, m, 1H of CH_2-5), 3 75 (3H, s, OMe), 3 01-2 84 (4H, m, CH_2SCH_2), 2 68-2 62 (1H, m, H-4), 2 54-2 50(1H, m, H-3), 1 46 (9H, s, t-Bu), 1 45(3H, d $J_{Me\text{-}Ala,Ha\text{-}Ala}$= 7 3, Me-Ala), 1 19 (8/9 of 3H, d $J_{Me\text{-}7,H\text{-}4}$=9 0, Me-7) and 1 08 (1/9 of 3H, d $J_{Me\text{-}7,H\text{-}4}$=6 4, Me-7)
$C_{18}H_{30}N_2O_7S$ MS *m/e* 418 (M^+)

(+)- γ-Methyl- α-methylene- γ-butyrolactone-Cys(Boc)-Ala(OMe) Adduct **13a**
Purified by chromatography, eluent hexane / AcOEt 1/1 (oil)
Method A Yield 96%
Method B Yield 91%
IR (cm^{-1}) 1790, 1770, 1740 , 1710, 1675
NMR (200 MHz , C_6D_6) 7 15-7 05 (1H, m, NH-Ala), 5 70-5 60 (1H, m, NH-Cys), 4 72-4 56 (2H, m, Hα-Cys + Hα-Ala), 3 76-3 65 (1H, m, H-5), 3 28 (3H, s, OMe), 2 98-2 72 (4H, m, CH_2SCH_2), 2 41-2 28 (1H, m, H-3), 1 49 (9H, s, t-Bu), 1 27 (3H, d $J_{Me\text{-}Ala,H\alpha\text{-}Ala}$=7 1, Me-Ala), 0 95 (6/7 of 3H, d $J_{Me\text{-}7,H\text{-}5}$=6 1, Me-7) and 0 78 (1/7 of 3H, d $J_{Me\text{-}7,H\text{-}5}$=6 5, Me-7)
$C_{18}H_{30}N_2O_7S$ MS *m/e* 418 (M^+)
No satisfactory elemental analysis could be obtained

(-)- γ-Methyl α-methylene -γ-butyrolactone-Cys(Boc)-Ala(OMe) Adduct **13b**
Purified by chromatography, eluent hexane / AcOEt 1/1 (oil)
Method A Yield 97%
Method B Yield 90%
IR(cm^{-1}) 1790, 1770, 1740 , 1710, 1675
NMR (200 MHz , C_6D_6) 7 45-7 35 (1H, m, NH-Ala), 5 98-5 89 (1H, m, NH-Cys), 4 89-4 65 (2H, m, Hα-Cys + Hα-Ala), 4 22-4 12 (1/3 of 1H, m, H-5) and 3 90-3 79 (2/3 of 1H, m, H-5), 3 38 (3H, s, OMe), 3 12-2 67 (4H, m, CH_2SCH_2), 2 63-2 42 (1H, m, H-3), 1 54 (9H, s, t-Bu), 1 36 (3H, dd Me-Ala), 1 05 (2/3 of 3H, d $J_{Me\text{-}7,H\text{-}5}$=6 1, Me-7) and 0 87 (1/3 of 3H, d $J_{Me\text{-}7,H\text{-}5}$=6 4, Me-7)
$C_{18}H_{30}N_2O_7S$ MS *m/e* 418 (M^+)
No satisfactory elemental analysis could be obtained

(+)- β,γ-Methyl α-methylene γ-butyrolactone-Cys(Boc)-Ala(OMe) Adduct **14**
Purified by chromatography, eluent hexane / AcOEt 1/1 (oil)
Method A Yield 97%
Method B Yield 97%
IR (cm^{-1}) 1790, 1775, 1740 , 1715, 1680
NMR (200 MHz , C_6D_6) 7 40-7 30 (1H, m, NH-Ala), 5 90 (1H, d $J_{NH\text{-}Cys,H\alpha\text{-}Cys}$=7 9, NH-Cys), 4 95-4 78 (1H, m, Hα-Cys), 4 72 (1H, dq J=7 2, Hα-Ala), 3 48-3 40 (1H, m, H-5), 3 37 (3H, s, OMe), 3 11-2 72 (4H, m, CH_2SCH_2), 2 06-1 98 (1H, m, H-3), 1 92-1 84(1H, m, H-4), 1 55(9H, s, t-Bu), 1 35 (3H, d $J_{Me\text{-}Ala,H\alpha\text{-}Ala}$=7 2, Me-Ala), 1 04 (3H, d $J_{Mc\text{-}8,H\text{-}5}$=6 1, Me-8), 0 82 (3H, d $J_{Me\text{-}7,H\text{-}4}$=6 3, Me-7)
$C_{19}H_{32}N_2O_7S$ MS *m/e* 432 (M^+)
No satisfactory elemental analysis could be obtained

(+)- Frullanolide-Cys(Boc)-Ala(OMe) Adduct **15a**
Purified by chromatography, Eluent hexane / AcOEt 1/1 (oil)
Method A Yield 95%
Method B Yield 95%
IR (cm^{-1}) 1760, 1740 , 1710, 1670
NMR (400 MHz) 7 04-6 95 (1H, m, NH-Ala) , 5 5-5 3 (1H, m, NH-Cys), 5 22 (1H, d, $J_{H6,H7}$=4 3, H-6), 4 57 (1H, dq J=7 2 , Hα-Ala), 4 40-4 30 (1H, m, Hα-Cys), 3 75 (3H, s, OMe), 3 12-3 03 (3H, m, CH_2-13 + 1H of CH_2-Cys), 2 91-2 86 (1H, m, 1H of CH_2-Cys), 2 67-2 60 (1H, m $J_{H11,H7}$=0, H-11), 2 50-2 46 (1H, m $J_{H7,H11}$=0, H-7), 2 14-2 07 (2H, m, CH_2-3), 1 76 (3H, s, Me-15), 1 47 (9H, s, t-Bu), 1 44 (3H, d $J_{Me\text{-}Ala,H\alpha\text{-}Ala}$=4 8, Me -Ala), 1 03 (3H, s, Me-14)
Spin-spin decoupling shows that $J_{H7,H11}$=0 which corresponds to the product with an H-7,H-11 anti arrangement[12]
$[\alpha]_D^{20}$= +31 (c = 1 60 $CHCl_3$)

$C_{27}H_{42}N_2O_7S$ MS *m/e* 538 (M^+)
No satisfactory elemental analysis could be obtained

(-)- Frullanolide-Cys(Boc)-Ala(OMe) Adduct **15b**
Purified by chromatography, Eluent hexane / AcOEt 1/1 (oil)
Method A Yield 95%
Method B Yield 95%
IR (cm^{-1}) 1760, 1740 , 1710, 1670
NMR (400 MHz) 7 09 (1H, d $J_{NH\text{-}Ala,H\alpha\text{-}Ala}$=6 9, NH-Ala) , 5 45-5 35 (1H, m, NH-Cys), 5 22 (1H, d $J_{H6,H7}$=4 3, H-6), 4 56 (1H, dq J=7 2 , Hα-Ala), 4 40-4 30 (1H, m, Hα-Cys), 3 74 (3H, s, OMe), 3 08-2 99 (2H, m, CH_2-Cys), 2 96-2 95 (2H, m, CH_2-13), 2 70-2 65 (1H, m, $J_{H11,H7}$=0, H-11), 2 50-2 45 (1H, m, $J_{H7,H11}$=0, H-7), 2 08-2 07 (2H, m, CH_2-3), 1 76 (3H, s, Me-15), 1 46(9H, s, t-Bu), 1 43 (3H, d $J_{Me\text{-}Ala,H\text{-}Ala}$=6 8, Me -Ala), 1 04 (3H, s, Me-14)

Spin-spin decoupling shows that $J_{H7,H11}$=0 which corresponds to the product with an H-7,H-11 anti arrangement[12]

$[\alpha]_D^{20}$=-22 (c = 1 71 $CHCl_3$)

$C_{27}H_{42}N_2O_7S$ MS *m/e* 538 (M^+)
No satisfactory elemental analysis could be obtained

Alantolactone-Cys(Boc)-Ala(OMe) Adduct **16**
Purified by chromatography, Eluent hexane / AcOEt 5/2 (oil)
Method A Yield 96%
Method B Yield 96%
IR(cm^{-1}) 1785, 1770, 1745 , 1710, 1680
NMR (200 MHz) 7 05-6 95 (1H, m, NH-Ala) , 5 50-5 40 (1H, m, NH-Cys), 5 26 (1H, d $J_{H6,H7}$=4 0, H-6), 4 80-4 75 (1H, m, H-8), 4 57 (1H, dq J=7 1, Hα-Ala), 4 40-4 30 (1H, m, Hα-Cys), 3 75 (3H, s, OMe), 3 2-2 8 (3H, m, 3H of CH_2SCH_2), 2 93 2 89 2 86 2 82 (1H, part A of an ABX J_{AB}=15 0 J_{AX}=8 0, 1H of CH_2S), 2 70-2 40 (2H, m, H-11 + H-7), 2 16 2 14 2 09 2 07 (1H, part A of an ABX J_{AB}=13 0 J_{AX}=4 0, 1H of CH_2-9), 1 9-1 4 (7H, m, CH_2), 1 48 (9H, s, t-Bu), 1 44 (3H, d $J_{Me\text{-}Ala,H\alpha\text{-}Ala}$=7 4, Me-Ala), 1 23 (3H, s, Me-14), 1 13 (3H, d J=7 6, Me-15)

$[\alpha]_D^{20}$=+39 (c=1 22 $CHCl_3$)

Anal Calcd for $C_{27}H_{42}N_2O_7S$ C, 60 20, H, 7 86, N, 5 20, Found C, 60 07, H, 7 69, N, 4 93,

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