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STUDIES ON AMINO ACIDS AND PEPTIDES XII[1]
SYNTHESIS OF THIATED ANALOGUES OF Boc-S-Ala-Aib-S-Ala-OMe AND Ac-S-Ala-Aib-S-Ala-OMe

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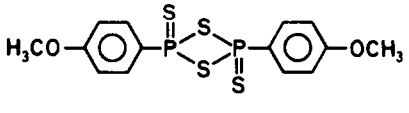
Abstract - The two model peptides Boc-S-Ala-Aib-S-Ala-OMe (1a) and Ac-S-Ala-Aib-S-Ala-OMe (1b) and their monothiated analogues Boc-S(R)-AlaΨ(CSNH)-Aib-S-Ala-OMe (3a), Boc-S-Ala-AibΨ(CSNH)-S-Ala-OMe (4a), AcΨ(CSNH)-S-Ala-Aib-S-Ala-OMe (2b), Ac-S-Ala-AibΨ(CSNH)-S-Ala-OMe (4b), and the dithiated Boc-S-AlaΨ(CSNH)-AibΨ(CSNH)-S-Ala-OMe (5a) are synthesized.[a] Peptide 3a was obtained from the coupling of HCl·H-S-Ala-OMe to S-2-(1-(*tert*-butoxycarbonylamino)ethyl-4,4-dimethyl-1,3-thiazol-5(4*H*)-one (11). The thioamide analogues 4a (together with 5a) and 2b were obtained by regioselective thiation of the respective model peptides 1a and 1b using 2,4-bis(4-methylphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, Lawesson's Reagent (LR). Deprotection of the Boc group of 4a, followed by acetylation of the product, afforded 4b. The magnetic nonequivalence of the *gem*-methyl groups of Aib is discussed.

INTRODUCTION

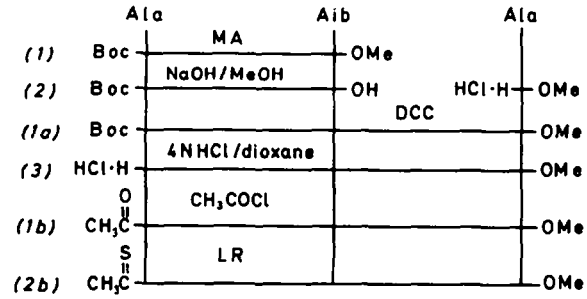
Thioamide analogues of physiologically active peptides are attractive modifications since in receptor interactions they may be more selective and/or potent than their parent compounds;[b] also an enhanced stability against enzymatic hydrolyses can be expected on the basis of previous experience with carboxypeptidase A.[3-5]

Since the discovery in 1958 of the natural occurrence of Aib (α-aminoisobutyric acid) first in the antibiotic I.C.I 13959 [6] and later on in a number of antibiotics, the "peptaibols",[7,8] a class of peptides which, apart from their large contents of Aib residues, are characterized by an amino alcohol C-terminal group, there has been considerable interest in the synthetic, spectroscopic, and conformational aspects of shorter Aib-containing model peptides.[c] The promising results of the selective thiation of the model peptide Boc-Gly-S-Ala-Aib-OMe previously described [12] inspired to a further exploration of the selectivity of LR in thiation reactions. A general method for direct thiation of the peptide function in protected dipeptides demonstrated that LR discriminates between the different carbonyl groups, [13,14] *i.e.* the amide carbonyl is thiated before urethane and ester carbonyls. In this paper it is shown that LR is able to distinguish between different amide groups. Furthermore, we wanted to investigate the possibility of thiating the extremely hindered Aib residue.

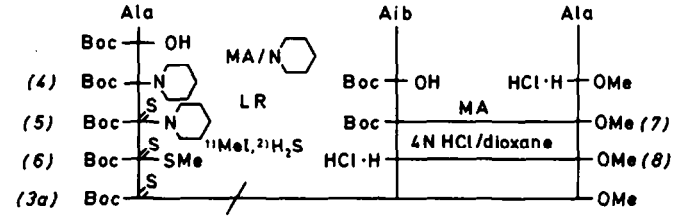


LR

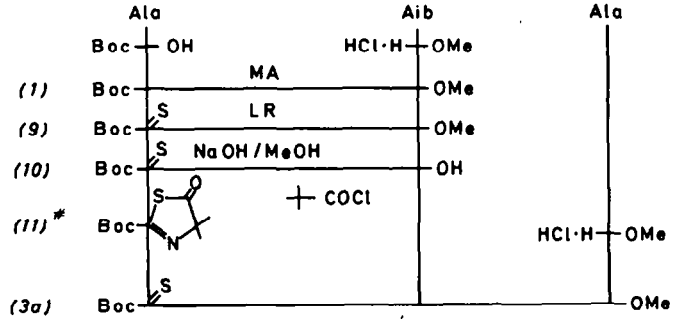
[a] The nomenclature of the compounds is in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, Pure Appl. Chem. 5b, 595 (1984).
[b] For a review see ref.2.
[c] For reviews see refs 9-11.



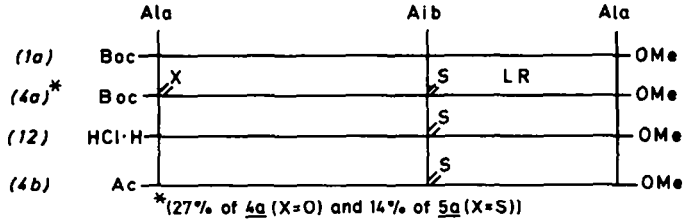
Scheme 1



Scheme 2



Scheme 3



Scheme 4

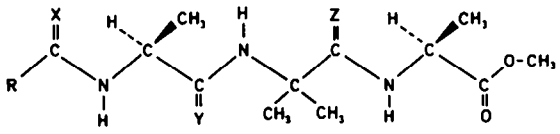
* See Scheme 6

The model peptides chosen, 1a and 1b, provided us with the possibility of direct spectroscopic and structural comparative studies since they have recently been examined by G. Jung and coworkers.[15,16]

Investigation of the crystal structures of the thiamide analogues is a continuation of a more general study of β-turn conformation in monothiated Aib peptide surrogates [12] and crystallographic work on the structures of 2b, 4a, 4b and 5a will be published elsewhere with C. Toniolo *et al.*

RESULTS AND DISCUSSION

Throughout this work mixed anhydride coupling procedures (MA) have been used successfully to prepare dipeptides. The Boc-deprotection was achieved by treatment with 4 N HCl/dioxane.



a) R = ⟊ O. b) R = CH_3

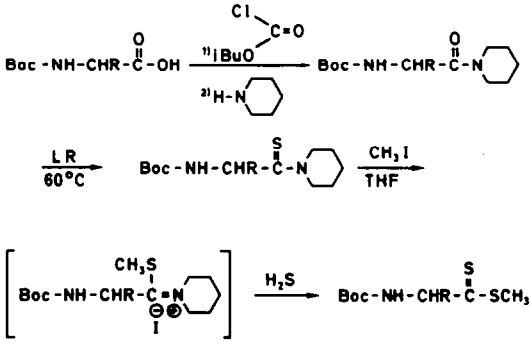
1a,b : X = O, Y = O, Z = O
2b : X = S, Y = O, Z = O
3a : X = O, Y = S, Z = O
4a,b : X = O, Y = O, Z = S
5a : X = O, Y = S, Z = S

Fig. 1

The strategy for the synthesis of the model peptides 1a and 1b is outlined in Scheme 1. The reason for coupling Boc-S-Ala--Aib-OH and HCl·H-S-Ala-OMe instead of coupling Boc-S-Ala-OH and HCl·H-Aib-S-Ala-OMe is that in the latter case prolonged reaction time, and consequently, risk of racemization together with low yields at the tripeptide stage may be expected due to the notoriously hindered amino group of Aib.

The saponification of Boc-S-Ala-Aib-OMe (1) in (NaOH/MeOH) was performed at 40 °C for 1.5 h to give good yields of the corresponding free acid. These somewhat forcing conditions, which also turned out to be compatible with the thiated analogue 9, were found by Jung *et al.* to be optimal for the reaction.[17] The thiation of 1b with LR at room temperature in THF afforded the monothiated derivative 2b exclusively in 92% yield; this was expected from previous results [12] with specific thiation with LR and demonstrates the reagent's discrimination between different carbonyl sites in thiation reactions. An alternative route to 2b would be the reaction of methyl dithioacetate with HCl·H-S-Ala-Aib-S-Ala-OMe (3). The preparation of methyl dithioacetate, however, is a three-step synthesis.[18-19]

The dithioester method (Scheme 5) was employed in the original strategy for the preparation of 3a (Scheme 2). The alanine dithioester (6) was treated with HCl·H-Aib-S-Ala-OMe (8). However, the expected product was not formed and the dithioester recovered. A check of the optical purity of the recovered dithioester showed that it had totally racemized during the reaction. That the dithioester method is not always reliable for thioamidation reactions has been seen before in one case.[19] Here the crowded Aib amino terminal of 8 is



Scheme 5

Table 1. ^{13}C NMR Chemical shifts of peptides and intermediate fragments

	Boc/Ac			Ala			Aib			Ala			XMe
	C_q	CH_3	C=X	C_α	C_β	C=Y	C_α	C_β	C=Z	C_α	C_β	C=O	XCH_3
1	79.8	28.1	155.4	49.9	17.9	172.0	56.0	24.6	174.6				52.3
9	80.4	28.2	155.6	59.8	21.0	204.0	56.6	24.0	173.2				52.5
2*	78.0	28.2	154.9	49.4	18.4	171.9	54.8	24.8 24.7	175.5				
10	80.8	28.2	155.8	56.0	21.8	204.1	60.4	23.0	176.7				
1a*	78.1	28.1	151.2	47.8	17.4	172.8	55.6	24.4 25.5	173.9	47.5	17.0	172.1	51.8
3				48.3	16.9	173.0	56.0	24.0 25.7	173.2	47.8	16.7	168.9	51.8
3a	80.6	28.2	155.7	57.9	20.9	104.2	60.3	23.9 24.8	172.0	48.4	17.9	173.2	52.3
4a	80.2	28.1	155.7	53.8	17.4	172.2	62.0	27.6 28.4	206.5	50.8	16.4	172.3	52.3
5a	81.2	28.1	156.0	59.5	20.9	204.0	65.1	25.7 29.8	202.4	54.0	16.5	172.4	52.3
1b*		23.8	169.8	49.1	17.0	173.0	55.7	26.3 27.3	173.9	47.7	16.7	172.3	51.8
2b*		32.6	199.8	54.7	17.2	172.9	55.9	23.7 25.9	173.7	47.7	17.0	170.5	51.8
4b		22.8	170.8	53.8	17.5	172.7	62.5	27.4 28.8	206.5	49.7	16.4	172.3	52.4
4*	77.8	28.2	154.8	45.7	17.8	170.2							
5	79.3	28.3	154.6	52.0	22.1	202.8							
6	80.0	28.3	154.6	61.1	19.2	241.5							24.0
7*	78.0	28.1	154.1				55.5	24.7 25.3	174.2	47.6	17.1	173.0	51.8
11	80.1	28.2	154.9	27.0	19.1	167.6	50.7	24.3 26.4	182.6				

All spectra were recorded in $CDCl_3$ except those marked with * which were recorded in DMSO-d_6

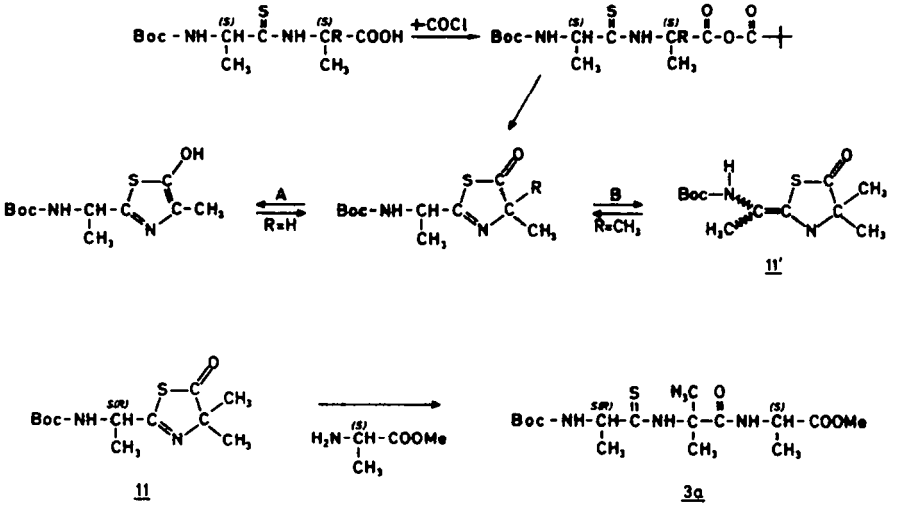
Table 2. ^1H NMR Chemical shifts of peptides and intermediate fragments

	Boc/Ac	Ala			Aib		Ala			XMe/OH
	CH_3	NH	$C_\alpha H$	$C_\beta H$	NH	$C_\beta H$	NH	$C_\alpha H$	$C_\beta H$	CH_3/OH
1	1.30	5.33	4.05	1.20	6.90	1.39				3.54
9	1.35	5.26	4.29	1.30	8.49	1.59				3.58
2	1.40	5.20	3.85	1.30	6.88	1.55				-
2*	1.36	6.74	3.95	1.14	7.88	1.33				12.23
10	1.40	5.49	4.59	1.47	8.60	1.74				7.15
11	1.45	5.40	4.55	1.25	-	1.40				-
1a*	1.38	7.57	4.27	1.28	7.96	1.35	7.04	3.88	1.15	3.62
3*		8.31	3.89	1.32	8.65	1.35	7.87	4.25	1.27	3.59
3a	1.45	5.13	4.50	1.43	8.30	1.72	6.53	4.30	1.38	3.70
4a	1.45	5.10	5.08	1.30	7.05	1.69	8.85	4.10	1.56	3.73
5a	1.43	5.15	5.00	1.53	8.51	1.73	8.43	4.34	1.43	3.70
1b*	1.82	8.17	4.09	1.23	8.17	1.35	7.57	4.22	1.16	3.62
2b*	2.40	10.14	4.51	1.32	8.25	1.35	7.43	4.23	1.22	3.60
4b	2.00	6.00	5.01	1.36	7.37	1.68	8.76	4.40	1.47	3.75
4*	1.36	6.86	4.41	1.11						
5*	1.36	6.78	4.72	1.19						
6	1.41	5.26	4.77	1.50						
7*	1.35	-	-	-	6.79	1.29	7.74	4.27	1.24	3.75

All spectra were recorded in $CDCl_3$ except those marked with * which were recorded in DMSO-d_6

believed to be the reason for the absence of a coupling product.[9]

It is known that elongation of thiopeptides by coupling from the C-terminal is generally not feasible. Instead of coupling with the amine the activated thiopeptide is converted to the corresponding thiazlactone [19,20,21] (thiazol-5(4*H*)-one). These thiazlactones are normally not sufficiently reactive to participate in coupling reactions and are readily racemized *via* their keto-enol tautomerism (Scheme 6). In the special case of Boc-S-AlaΨ(CSNH)-Aib-OH (10) where the achiral Aib is the C-terminal amino acid no major problems with racemization would be expected and since Aib bears no α-hydrogen the enol form, in which the activity of the thiazlactone is reduced, is not possible we decided to prepare 3a *via* the thiazlactone (11) which was isolated from the reaction of 10 with pivaloyl chloride [22] according to Scheme 6, with R = CH_3. Subsequently, aminolysis of 11 with HCl·H-S-Ala-OMe yielded 3a. The ^{1}H NMR spectrum of the reaction product revealed the presence of two



Scheme 6

diastereomers* After crystallization the ^{1}H NMR spectrum showed only single peaks, indicating the isolation of one of the diastereomers. It is, to our knowledge, the first example of a thiopeptide prolongation *via* its thiazlactone derivative and of any thiopeptide C-terminal prolongation. And although thiazlactone formation has been suspected as the reason for the lack of amidation product in the coupling reaction [20] this is the first time the postulated thiazlactone has been isolated. Several examples of 2-phenylthiazol-5(4*H*)-ones have been isolated and shown to be weak acylating reagents towards amino acids and peptides.[23]

The strategy for the preparation of 4a and in turn 4b was based on thiation of Boc-Aib-S-Ala-OMe (7) to give Boc-AibΨ(CSNH)-S-Ala-OMe, followed by deprotection of the Boc group and coupling with Boc-S-Ala-OH, but quite unexpectedly the thiation of 1a with LR gave a reasonable yield of 4a, together with a small amount of the dithiated compound Boc-S-AlaΨ(CSNH)-AibΨ(CSNH)-S-Ala-OMe, 5a. Thiation was assumed to be the easiest way to 3a, and hence used in our first attempt to prepare this analogue. We anticipated thiation of Ala^{1}C=O because it seemed that the surroundings of this group were less sterically hindered than those of Aib^{2}C=O with the two adjacent *gem*-methyl groups.

The NMR resonances for the α-, the β-, and the *N*-protons of the thioamides are shifted downfield with respect to the parent amides.[13,14,18] The largest values are found for the *N*-protons for which the change is about 1.7 ppm (Table 2). This observation and the fact that the N-H group signal of the Aib residue appears as a singlet, in contrast to the N-H group signals of Ala which are doublets due to coupling with the C protons, makes simple ^{1}H NMR spectroscopy an efficient tool for the verification of the thiation site(s) in a quantitative way (Fig.2).

Similarly, the ^{13}C signals for the substituents α and β to the thioamide function exhibit

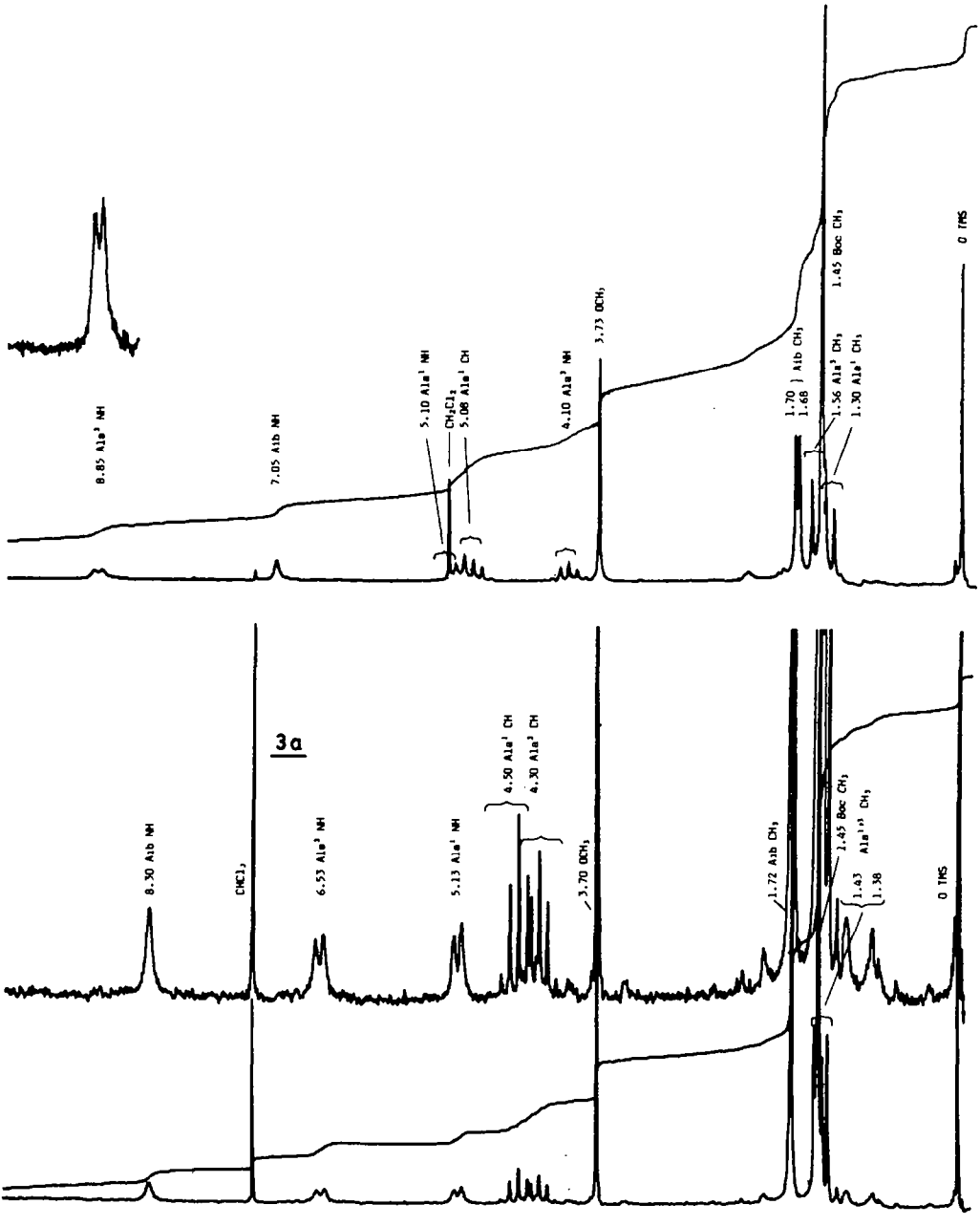


Fig. 2. ^{1}H NMR spectra of Boc-Ala^{1}-Aib$^{2}\Psi$(CSNH)-Ala^{3}-OMe (4a) and Boc-Ala$^{1}\Psi$(CSNH)-Aib^{2}-Ala^{3}-OMe (3a) in $CDCl_3$

downfield shifts (Table 1). The thiocarbonyl carbon resonates at 204 ppm *vs* 173 ppm for the carbonyl carbon.[13,14,18,24] An interesting property of the two model peptides 1a and 1b is the very large magnetic nonequivalence (MNE) of the geminal ß-methyl groups in the Aib residues (Table 1). Generally, protected Aib dipeptides exhibit a small ^{13}C MNE of about 0.5 ppm which is explained by diastereotopic induction from chiral centres of neighbouring residues, whereas in achiral of chiral peptides with less hindered rotation around the N-C_α or C_α-C=O bonds MNE is lacking.[25] Obviously, the large MNE which also occurs in our series of thioamide analogues is brought about by a very restricted backbone conformation in which the torsion angle between Aib C_α and Aib carbonyl oxygen (C_β-C_α-C-O) is strongly twisted and in effect moves one methyl group close to the carbonyl oxygen (or sulfur) which increases the electrostatic interaction; thus, a large downfield shift is caused for the methyl group in question.[16,26]

The change to a greater magnetic anisotropy on substituting oxygen by sulfur [26] in the Aib carbonyl group was expected to enhance the MNE of the *gem*-methyl groups, but no unambigous difference was seen, probably because of a large solvent dependence. The IR absorptions [19] are observed in the regions 3450-3230 cm^{-1} (N-H stretching), 2760-1745 cm^{-1} (ester), 1720-1680 cm^{-1} (urethane), 1670-1630 cm^{-1} (amide I), 1170-1130 cm^{-1} (thioamide I), and 1165 cm^{-1} (dithioester). The thioamide I bands fall in the region of stronger absorptions from the C-O stretching and are of less diagnostic value. The absorptions for the N-H stretch below 3400 cm^{-1} and urethane and/or amide C=O stretch at 1690 and 1660 cm^{-1}, respectively, of the target compounds (a and b series) seem indicative of the existence of hydrogen bonds in the solid state.

The thiocarbonyl group is a characteristic UV chromophore [19] which absorbs strongly at wavelengths in the range 260-307 nm with log ε values from 3.0 to 4.1.

The mass spectra [19] show in most cases $[M]^{+\cdot}$ or $[M+1]^{+}$, together with the characteristic fragments $[M\text{-isobutene}]^{+\cdot}$ and $[M\text{-}tert\text{BuO})]^{+}$. Occasional loss of H_2S and/or HS was observed in the mass spectra of the thioamide analogues.

The IR and UV absorptions of 11, 1630 (C=N), 1710-1720 cm^{-1} (urethane, thiazlactone C=O) and λ_{max} = 241 nm (methanol) are in accordance with those found in the literature for the keto forms of 2,4-disubstituted thiazolin-5-ones.[27]

*Racemization is believed to occur at the *N*-terminal Ala residue *via* route B in Scheme 6, giving rise to two diastereomers of the final product 3a. After completion of this work, use of catalysts which might suppress this epimerization has appeared in the literature.[20a]

EXPERIMENTAL

Instruments

^1H NMR spectra were recorded at 60 MHz on Varian EM-360 or at 80 MHz on Varian CFT-20 spectrometers with $CDCl_3$ as solvent. Samples with DMSO-d_6 as solvent were recorded at 300 MHz on a Varian XL-300 spectrometer. ^{13}C NMR spectra were recorded at 25 MHz on Varian XL-100-15 or at 75.426 MHz on Varian XL-300 spectrometers with $CDCl_3$ or DMSO-d_6 as solvents. Chemical shifts are reported as parts per million on the δ scale, with reference to TMS as 0 ppm (for ^1H spectra), $CDCl_3$ as 76.95 ppm and DMSO-d_6 as 39.5 ppm. Spectra with DMSO-d_6 as solvent were recorded at the Department of Chemistry, University of Louisville, Louisville, KY, USA. UV absorption spectra were recorded on Perkin Elmer 402 and Uvikon 860 (compounds 4a and 5a) spectrophotometers. IR absorption spectra were obtained with a Beckman IR-18 spectrophotometer. Mass spectra and precise mass measurements were recorded on a Micromass 7070F spectrometer operating at 70 eV with direct inlet.

Elemental analyses were carried out by Løvens Kemisk Fabrik, DK-2750 Ballerup (Microanalytical Laboratory). Optical rotations were measured in a 1 dm cell in a Perkin Elmer 241 polarimeter. Silica gel 60 (Merck) 63-200 μm was used for column chromatography and silica gel 60 (Merck) 40-63 μm for flash chromatography. The dimensions for the flash column were 30 x 200 mm, and the flow rate was 2.54 cm/min (N_2 gas). The following systems were used for TLC monitoring (I): butanol/acetic acid/water (4:1:1), (II): chloroform/methanol/acetic acid (85:10:5), (III): butanol/acetic acid/water (3:1:1), (IV): 2-butanol/acetic acid/water (67:10:23). The TLC plates were prepared as described earlier.[12] The ninhydrin spray solution consisted of 0.25% ninhydrin in butanol. For UV monitoring the wavelengths 254 mμ and 350 mμ were used to detect thioamides and amides, respectively. For detection of ninhydrin negative impurities the TLC plates were exposed to iodine vapour. The methyl ester hydrochlorides [28] and *N*-*tert*-butyloxycarbonyl [29] derivatives of S-alanine and α-aminoisobutyric acid were prepared by standard methods although for Aib prolonged reaction times were allowed.

N-tert-*Butyloxycarbonyl-S-alanyl-α-aminoisobutyric acid methyl ester (Boc-S-Ala-Aib-OMe,* 1).[17,30] Boc-S-Ala-OH; 37.84 g (0.200 mol), was dissolved in a mixture of 20.23 g (0.200 mol) *N*-methylmorpholine and 182 ml dry tetrahydrofuran. After cooling to - 20 °C, 25.45 g (0.19 mol) isobutyl chloroformate was added dropwise, keeping the temperature below - 10 °C. The mixed anhydride was allowed to form in 15 min of activation time; then a precooled mixture (- 10 °C) of 72.7 g (0.180 mol) HCl-H-Aib-OMe, 18.2 g (0.180 mol) *N*-methylmorpholine, and 113 ml tetrahydrofuran was added over a period of 15 min. The reaction proceeded for 0.5 hr at - 10 °C, then for 1 hr at 0 °C and finally 1 hr at room temperature, after which the temperature again was lowered to 0 °C and 70 ml of saturated $KHCO_3$ solution was added to destroy any unreacted mixed anhydride. After 0.5 hr the organic solvent was removed *in vacuo*. The aqueous slurry was extracted with 100 ml and 2 x 50 ml ethyl acetate. The combined organic phases were washed with 1 N HCl (3 x 50 ml), water (3 x 50 ml), 5% $KHCO_3$ (3 x 50 ml) and 10 ml water, then dried over $MgSO_4$. The solvent was evaporated under vacuum to yield 49.79 g of 1 as a colourless oil. R_f = 0.6 in ethyl acetate $[\alpha]_D^{22}$ = - 33.6° (c = 1 in methanol). The spectroscopic data correspond well with those found in the literature.

N-tert-*Butyloxycarbonyl-S-thioalanyl-α-aminoisobutyric acid methyl ester (Boc-S-AlaΨ(CSNH)-Aib-OMe,* 9).[12] Boc-S-Ala-Aib-OMe (1), (7.55 g, 0.0262 mol) and LR (5.82 g, 0.0144 mol) was refluxed in dry toluene (15 ml) for 0.5 hr. The purification follows the same procedure as applied for 5. The dimensions of the column were 64 x 240 mm. The yield of 9 was 0.8 g as a slightly yellow oil. R_f = 0.52 in 10% diethyl ether/dichloromethane. All data correspond well with those previously found.

N-tert-*Butyloxycarbonyl-S-alanyl-α-aminoisobutyric acid (Boc-S-Ala-Aib-OH,* 2).[17,30] Boc-S-Ala-Aib-OMe

(1), 28.7 g (0.10 mol) was dissolved in 100 ml of methanol and 150 ml of 1 N NaOH added. As indicated by TLC the saponification was complete after 1.5 hr at 40 °C. The mixture was acidified and evaporated in vacuum to half its volume and then extracted with ethyl acetate (4 x 150 ml). After washing of the combined ethyl acetate phases with saturated NaCl solution and drying with $MgSO_4$, the solvent was evaporated *in vacuo*, yielding 26.0 g (91%) of 2 as a colourless solid. Mp. 175° (lit.[17,30] 178 and 170 °C). R_f = 0.82 in I, R_f = 0.66 in II. $[\alpha]_D^{22}$ = - 33.5° (c = 0.2 in methanol), (lit.[17] $[\alpha]_D^{22}$ = 35° (c = 0.2 in methanol). IR (KBr)= 3350 (N-H stretch), 1700 (urethane), 1530 cm^{-1} (amide I). UV (ethanol): λ_{max} (log ε) = 202 nm (3.5). MS: *m/z* = 276 $[M+2]^+$, 275 $[M+1]^+$, 220 [M+2-i-sobutene]$^+$, 219 [M+1-isobutene]$^+$, 203 [M+2-*tert*BuO]$^+$, 202 [M+1-*tert*BuO]$^+$, 201 [M-*tert*BuO]$^+$, 174 [M+1-*tert*BuOCO]$^+$, 173 [M-*tert*BuOCO]$^+$.

N-tert-*Butoxycarbonyl-S-thioalanyl-α-aminoisobutyric acid (Boc-S-AlaΨ(CSNH)-Aib-OH,* 10). Boc-S-AlaΨ(CSNH)-Aib-OMe (9), (0.98 g, 0.003 mol) was dissolved in 17 ml of methanol and 3.5 ml of 1 N NaOH was added. The mixture was stirred at 40 °C for 2 hrs at which point the colour of the solution turned from yellow to orange. After evaporation of the solvents the residue was taken up in 10 ml of water and 5 ml of 1 N NaOH and extracted with ethyl acetate (4 x 10 ml). The orange coloured by-product was extracted into the organic phase. The aqueous phase was acidified to pH 2 and extracted with ethyl acetate (4 x 10 ml). The combined organic phases were dried with $MgSO_4$ and evaporated under reduced pressure. The yield was 0.65 g (70%) as a colourless solid. Mp. 137-38 °C. R_f = 0.70 in II, R_f = 0.88 in IV. $[\alpha]_D^{22}$ = - 24.1°, (c = 0.2 in ethyl acetate). IR (KBr): 3320 (N-H stretch), 1720 (urethane), UV (ethanol): λ_{max} (log ε) = 200 (3.6), 267 nm (3.9). MS: *m/z* = 292 $[M+2]^+$, 291 $[M+2]^+$, 290 $[M]^+$, 257 $[M-HS]^+$, 235 [M+1-isobutene]$^+$, 234 [M+2-isobutene]$^+$, 217 [M-*tert*BuO]$^+$, 201 [M+1-isobutene-H_2S]$^+$, 155 [M-isobutene-H_2S-COOH]$^+$, 146 [M-Boc-NHCH(Me)]$^+$. Precise mass measurement: Calc. *m/z* = 290.1300, found *m/z* = 290.1300 $[M]^+$.

*S-2-(1-(*tert*-Butyloxycarbonylamino)-ethyl)-4,4-dimethyl-1,3-thiazol-5(4H)-one* (11). A suspension of Boc-S-AlaΨ(CSNH)-Aib-OH (0.50 g, 0.00172 mol) and *N*-methylmorpholine (0.26 g, 0.0026 mol) in 10 ml of dry toluene was cooled to - 5 °C and pivaloyl chloride (0.31 g, 0.0026 mol) added. The mixture was stirred at - 5 °C for 2 hrs and then at room temperature for 1 hr. The filtrate was evaporated *in vacuo*. The remaining product was purified by flash chromatography with 30% diethyl ether/dichloromethane as eluent. On evaporation of the solvents the product (11) solidified as a colourless wax with mp. 71-73 °C in quantitative yield. R_f = 0.63 in 30% diethyl ether/dichloromethane. $[\alpha]_D^{22}$= - 1.8°, (c = 0.2 in methanol) and - 1.3°, (c = 1.0 in ethyl acetate). IR (KBr): N-H stretch), 1710-1720 (urethane, thiazolone C=O), 1630 cm^{-1} (C=N). UV (ethanol): λ_{max} (log ε) = 241 nm (3.4). MS: *m/z* = 272 $[M]^+$, 200 [M-1-*tert*BuO]$^+$, 171 [M-*tert*BuOCO]$^+$, 144 [Boc-NHCH(Me)]$^+$. Precise mass measurement: Calc. *m/z* = 273.1272 $[M+1]^+$, found *m/z* = 273.1272 $[M+1]^+$.

Elemental analysis: $C_{12}H_{20}N_2O_3S$ (272.1). Calc.: C 52.92, H 7.40, N 10.29, S 11.77
Found: C 53.09, H 7.49, N 10.08, S 11.52.

N-tert-*Butyloxocarbonyl-S(R)-thioalanyl-α-aminoisobutyryl-S-alanine methyl ester (Boc-S(R)-AlaΨ-(CSNH)-Aib-S-Ala-OMe,* 3a). A solution of 11 (0.30 g, 0.0011 mol) in 1,2-dimethoxyethane was added to a suspension of HCl•H-S-Ala-OMe (0.77 g, 0.0055 mol) and *N*-methylmorpholine (0.56 g, 0.0055 mol) in 1,2-dimethoxyethane. The reaction proceeded at 60 °C for 3 hrs and 48 hrs at room temperature. After evaporation of the solvents the residue was extracted with ethyl acetate (2 x 50 ml) and washed with 1 N HCl, water, and 5% $NaHCO_3$, three times each once with a saturated NaCl solution for neutralization. The products were separated on preparative TLC plates with 30% diethyl ether/dichloromethane as eluent, yielding 0,2 g (50%) of 3a as a colourless solid, m.p. 130-140 °C, R_f = 0.62 in 10% methanol/chloroform and 0.07 g (23%) of recovered 11. After recrystallization from methanol/hexane: $[\alpha]_D^{22}$ = + 1.0° (c = 0.4 in methanol). IR (KBr): 3400-3260 (N-H stretch), 1750 (ester), 1700 (urethane), 1650 cm^{-1} (amide). UV (methanol): λ_{max} (log ε) = 270 nm (3.7). MS: *m/z* = 375 $[M]^+$, 302 [M-*tert*BuO]$^+$, 231 [M-Boc-NH-$CHCH_3$]$^+$. Precise mass measurements: Calc. *m/z* = 375.1829, found *m/z* = 375.1828 $[M]^+$.

N-tert-*Butyloxycarbonyl-S-alanyl-α-aminoisobutyryl-S-alanine methyl ester (Boc-S-Ala-Aib-S-Ala-OMe,* 1a).[17] The dipeptide 2 (22.0 g, 0.08 mol and HCl•S-Ala-OMe (11.2 g, 0.08 mol) were dissolved in a mixture of N-methylmorpholine (8.1 g, 0.08 mol) and 110 ml dichloromethane. After cooling to - 15 °C DCC (18.1 g, 0.088 mol) was added. The mixture was stirred overnight at room temp. The precipitate was filtered off at 0 °C and washed with dichloromethane. During filtration the filtrate formed a gel which solidified upon evaporation of the solvent *in vacuo*. The residue was taken up in ethyl acetate and successively washed three times with each of the following solutions: 1 N HCl, water, 5% $KHCO_3$, and once with saturated NaCl. After drying with $MgSO_4$ and evaporation of the solvent 18.0 g (63%) of 1a was obtained as a colourless solid which recrystallized from dichloromethane/hexane. M.p. 168-169 °C (lit.[17] mp. 171 °C). R_f = 0.73 in I, R_f = 0.51 in 10% diethyl ether/dichloromethane: $[\alpha]_D^{22}$ = - 34°, (c = 0.1 in methanol), lit.[17] $[\alpha]_D^{25}$ = - 32° (c = 0.1 in methanol). IR (KBr): 3400, 3320, 3280 (N-H stretch), 1750 (ester), 1700 (urethane), 1650 cm^{-1} (amide I). UV ethanol): λ_{max} (log ε) = 202 nm (3.6). MS: *m/z* = 360 $[M+1]^+$, 359 $[M]^+$, 304 [M+1-isobutene]$^+$, 286 [M-*tert*BuO]$^+$, 260 [M+2-*tert*BuOCO]$^+$, 257 [M-Ala-OMe]$^+$, 229 [M-CO-Ala-OMe]$^+$, 215 [M-Boc-NHCH(Me)]$^+$, 144 [Boc-NHCH(Me)]$^+$.

N-tert-*Butyloxycarbonyl-S-alanyl-α-aminoisothiobutyryl-S-alanine methyl ester (Boc-S-Ala-AibΨ(CSNH)-S-Ala-OMe,* 4a) and N-tert-*Butyloxycarbonyl-S-thioalanyl-α-aminoisothiobutyryl-S-alanine methyl ester (Boc-S-AlaΨ(CSNH)-AibΨ(CSNH)-S-Ala-OMe,* 5a). The tripeptide 1a (10.0 g, 0.028 mol and LR (5.6 g, 0.014 mol) were suspended in 70 ml of toluene and stirred at 100 °C for 45 min after which the solvent was removed *in vacuo*. The remaining product was chromatographed on a column (70 x 230 mm) with 10-20% diethyl ether/dichloromethane as eluent systems, starting with the 10% mixture and gradually increasing the contents of ether. Three compounds were produced: the "P,S" by-product [31,32] with R_f = 0.9 in front, then 5a with R_f = 0.72, and 4a with R_f = 0.60 as the last to leave the column. The R_f values were measured in 30% diethyl ether/dichloromethane. The yield of 4a was 2.8 g

(27%) which was recrystallized to give long colourless needle-shaped crystals. Mp. 114-115 °C. $[\alpha]_D^{22} = -31.3°$ (c = 0.2 in methanol). IR (KBr): 3310 (N-H stretch, 1750 (ester), 1690 (urethane), 1650 cm^{-1} (amide I). UV (methanol): λ_{max} (log ε): 202 (3.9), 266 nm (4.0). MS: *m/z* = 375 $[M]^+$, 319 $[M-isobutene]^+$, 302 $[M-tertBuO]^+$, 288 $[M-CH(Me)COOMe]^+$, 231 $[M-Boc-NHCH(Me)]^+$, 229 $[Boc-Ala-NHC(Me)_2]^+$, 187 $[M-((Me)_2CCS-Ala-OMe)]^+$. Precise mass measurement: Calc. *m/z* = 375.1828, found: *m/z* = 375.1828 $[M]^+$. The yield of compound 5a was 1.5 g (14%) which on recrystallization gave colourless long needle-shaped crystals with m.p. 110-111 °C. $[\alpha]_D^{22} = +25.9°$ (c = 1.6 in methanol). IR (KBr): 3380-3270 (N-H stretch), 1740 cm^{-1} (ester). UV (methanol): λ_{max} (log ε) = 216 (3.3), 268 nm (3.4). MS: *m/z* = 391 $[M]^+$, 357 $[M-H_2S]^+$, 318 $[M-tertBuO]^+$, 301 $[M-H_2S-isobutene]^+$, 284 $[M-H_2S-tertBuO]^+$, 274, 247 $[M-Boc-NHCHMe]^+$. Precise mass measurement: Calc. *m/z* = 391.1599, found *m/z* = 391.1599 $[M]^+$.

S-Alanyl-α-aminoisobutyryl-S-alanine methyl ester hydrochloride (HCl·H-S-Ala-Aib-S-Ala-OMe 3*).*[17] Boc-S-Ala-Aib-S-Ala-OMe, 1a (7.0 g, 0.02 mol) was dissolved in 35.5 ml of 5 N HCl/dioxane. The deprotection was monitored by TLC (10% ethanol/dichloromethane) and showed completion within 0.5 hr. The solvent was evaporated under reduced pressure at 30-40 °C and the residue recrystallized from methanol/diethyl ether and dried overnight *in vacuo*. The product showed a single ninhydrin positive spot. R_f = 0.26 in I, R_f = 0.10 in II. The yield was 3.7 g (74%) with m.p. 235 °C (lit.[17] m.p. 240 °C), $[\alpha]_D^{22}$ = -10° (c = 0.2 in methanol). IR (KBr): 3380-3270 (N-H stretch), 1745 (ester), 1650 cm^{-1} (amide I). UV (ethanol): λ_{max} (log ε) = 204 nm (4.0). MS: *m/z* = 260 $[M-Cl]^+$, 173 $[M-Cl-CH(Me)COOMe]^+$, 157 $[M-HCl-(HN-Ala-OMe)]^+$, 129 $[M-HCl-(CO-Ala-OMe)]^+$.

S-Alanyl-α-aminoisothiobutyryl-S-alanine methyl ester hydrochloride (HCl·H-S-Ala-Aib Ψ (CSNH)-S-Ala-OMe, 12*).* The thiopeptide 4a was deprotected as described for 3. After evaporation of the solvent the residue was extracted with diethyl ether (3 x 50 ml), decanted and dried *in vacuo* over NaOH pellets and blue silica gel. The foam which was obtained in quantitative yield was used directly in the next step. R_f = 0.55 in I and R_f = 0.51 in II. Single ninhydrin positive spot.

N-*Acetyl-S-alanyl-α-aminoisobutyryl-S-alanine methyl ester (Ac-S-Ala-Aib-S-Ala-OMe,* 1b*).*[16] The hydrochloride salt 3 (2.96 g, 0.01 mol) was stirred in a mixture of 20 ml dichloromethane and 1.01 g (0.01 mol) triethylamine for 1.5 hrs. Then 1.01 g (0.01 mol) triethylamine was added, followed by 0.785 g (0.01 mol) acetyl chloride, the temp. being kept below - 10 °C. The reaction mixture was stirred for 3 hrs, while the temp. slowly rose to room temp. The precipitated triethylammonium chloride was filtered off and the filtrate extracted with water several times. The combined water extracts were washed with chloroform and extracted with ethyl acetate and an ethyl acetate/butanol (1:1) mixture. Evaporation of the combined organic extracts yielded 2.11 g (70%) of 1b as a colourless solid with m.p. 152-153 °C. R_f = 0.46 in I and R_f = 0.50 in II. $[\alpha]_D^{22} = -45.7$ °C (c = 1.0 in methanol). IR (KBr): 3304 (N-H stretch, 1760 (ester), 1660 cm^{-1} (amide I). UV (ethanol): λ_{max} (log ε) = 201 nm (3.0). MS: *m/z* = 301 $[M+1]^+$, 215 $[M-(Ac-NHCH(Me))]^+$, 199 $[M-Ala-OMe]^+$, 171 $[Ac-Ala-NHCH(Me)]^+$.

N-*Acetyl-S-thioalanyl-α-aminoisobutyryl-S-alanine methyl ester (Ac Ψ (CSNH)-S-Ala-Aib-S-Ala-OMe),* 2b. Ac-S-Ala-Aib-S-Ala-OMe (1b), 0.94 g (0.0031 mol) and 0.63 g (0.0016 mol) of LR were suspended in 8 ml of anhydrous tetrahydrofuran. The mixture was stirred overnight at room temp. The crystals, which precipitated from the reaction mixture, were purified on a column (20 x 200 mm) with 20% methanol/dichloromethane as eluent. The oily product was crystallized from methanol/hexane, producing 0.91 g (92%) of colourless crystals with m.p. 192-195 °C. R_f = 0.73 in 20% methanol/dichloromethane. $[\alpha]_D^{22}$ = - 132.6° (c = 0.6 in methanol). IR (KBr): 3360-3230 (N-H stretch), 1760-40 (ester), 1660 cm^{-1} (amide I). UV (methanol: λ_{max} (log ε) = 207 (3.6), 266 nm (3.7). MS: *m/z* = 317 $[M]^+$, 302 $[M-CH_3]^+$, 284 $[M-HS]^+$, 215 $[M-Ala-OMe]^+$. Precise mass measurement: Calc. *m/z* = 317.1408, found *m/z* = 317.1408 $[M]^+$.

N-*Acetyl-S-alanyl-α-aminoisothiobutyryl-S-alanine methyl ester (Ac-S-Ala-Aib Ψ (CSNH)-S-Ala-OMe,* 4b*).* The thiopeptide hydrochloride methyl ester 12 (0.43 g, 0.0014 mol) was suspended in a mixture of 10 ml of dioxane and 10 ml 10% $NaHCO_3$. Acetic anhydride (0.55 ml) was added, followed after 10 min by 5 ml of 10% $NaHCO_3$. The reaction mixture was stirred for 2 hrs. After a work-up procedure as for 1. the product was further purified by flash chromatography with 20% methanol/dichloromethane as eluent system. This yielded 4b quantitatively as an oil which was crystallized from a dichloromethane/diethyl ether/hexane solution by slow evaporation. Colourless crystals with m.p. 105-106 °C were obtained. R_f = 0.72 in III, R_f = 0.67 in IV. $[\alpha]_D^{22} = -29.6°$ (c = 1.3 in methanol). IR (KBr): 3304 (N-H stretch), 1745 (ester), 1660 cm^{-1} (amide I). UV (methanol): λ_{max} (log ε) = 255 nm (3.06). MS: *m/z* = 317 $[M]^+$, 286 $[M-OMe]^+$, 258 $[M-COOMe]^+$, 231 $[M-(Ac-NHCH(Me))]^+$, 171 $[Ac-Ala-NHC(Me)_2]^+$, 114 $[M-Ac-NHCH(Me)CO]^+$, 86 $[Ac-NHCH(Me)]^+$. Precise mass measurement: Calc. *m/z* = 317.1408, found *m/z* = 317.1409 $[M]^+$.

N-(tert-*Butyloxycarbonyl-alanyl)-piperidine (Boc-S-Ala-*$\overline{N\text{-}(CH_2)_4\text{-}CH_2}$, 4*).* A solution of Boc-S-Ala-OH (8.40 g, 0.044 mol) and N-methylmorpholine (5.0 ml, 0.044 mol) in 40 ml of tetrahydrofuran was cooled to - 20 °C, then 5.6 g (0.041 mol) of isobutyl chloroformate was added slowly and after 2-3 min of activation time, followed by addition of a precooled (- 20 °C) solution of piperidine (3.4 g, 0.04 mol) in tetrahydrofuran (25 ml). The coupling proceeded for 1 h at -20 - -10 °C, 0.5 h at 0°C and 1 h at room temp. Destruction of remaining anhydride was accomplished with 15 ml of saturated $NaHCO_3$ solution at 0 °C. After usual work-up, see 1, 10.10 g (99%) of 4 as a colourless oil was obtained. The oil solidified on standing, m.p. 63.5-64.0 °C. R_f = 0.55 in 30% diethyl ether/dichloromethane. $[\alpha]_D^{22} = -25.9°$ (c = 0.6 in methanol). IR (KBr): 3440 (N-H-stretch), 1710 (urethane), 1640 cm^{-1} (amide I). MS: *m/z* = 256 $[M]^+$, 200 $[M-isobutene]^+$, 183 $[M-tert-BuO]^+$.

Elemental analysis: $C_{13}H_{24}N_2O_3$ (256.3) Calc.: C 60.91, H 9.44, N 10.93
Found: C 60.64, H 9.56, N 10.59

N-tert-*Butyloxycarbonyl-S-thioalanyl)-piperidine (Boc-S-Ala*Ψ*(CS)-*$\overline{N\text{-}(CH_2)_4\text{-}CH_2}$, 5*).* Piperidide 4 (4.21 g, 0.016 mol) and LR (3.31 g, 0.008 mol) were suspended in 15 ml of anhydrous 1,2-dimethoxyethane (DME) and stirred for 0.5 h at 60-70 °C. The reaction mixture was evaporated under reduced pressure

with added silica gel, and chromatographed on a column (20 x 200 mm) with dichloromethane until the "P,S" by-product (R_f = 0.88 in 30% diethyl ether/dichloromethane) was eluted. The thiopiperidide was then eluted with 30% diethyl ether/dichloromethane. Evaporation of the collected fractions yielded 2.74 g (63%) of 5 as a colourless powder with m.p. 85.0-85.5 °C. R_f = 0.73 in 30% diethyl ether/dichloromethane. $[\alpha]_D^{22}$ = + 19.2° (c = 0.5 in ethyl acetate). IR (KBr): 3340 (N-H stretch), 1710 cm^{-1} (urethane). UV (diethyl ether): λ_{max} (log ε) = 280 nm (4.1). MS: *m/z* = 272 $[M]^+$, 239 $[M-HS]^+$, 216 $[M-isobutene]^+$, 199 [M-*tert*-BuO]$^+$, 183 $[M-isobutene-HS]^+$, 155 $[M-N(C_5H_{10})HS]^+$, 128 $[M-CSN(C_5H_{10})]^+$.

Elemental analysis: $C_{13}H_{24}N_2O_2S$ (272.2) Calc.: C 57.32, H 8.88, N 10.28, S 11.75
Found: C 57.08, H 8.87, N 9.85, S 11.68

N-tert-*Butyloxycarbonyl-S-dithioalanine methyl ester (Boc-S-Ala Ψ(CS)SMe,* 6). The thiopiperidide 5 (2.55 g, 0.0094 mol) and iodomethane (3.0 ml, 0.0094 mol) in 8 ml of tetrahydrofuran were allowed to react under anhydrous conditions for 24 hrs, whereafter the mixture was evaporated *in vacuo*. The remaining slurry was taken up in 5 ml of methanol and a stream of H_2S gas was bubbled through the solution for 20 min. The solution was allowed to stand for another 20 min before evaporation. The oily product was purified on a column with diethyl ether yielding 1.40 g (60%) of 6 as an oil which was crystallized from pentane to give yellow crystals. M.p. 73.0-73.5 °C. R_f = 0.79 in diethyl ether. $[\alpha]_D^{22}$ = - 52.3° (c = 0.2 in ethyl acetate). IR (KBr): 3310 (N-H stretch), 1700 (urethane), 1165 cm^{-1} (dithioester. UV (diethyl ether): λ_{max} (log ε) = 300 nm (4.0). MS: *m/z* = 235 $[M]^+$, 179 [M-isobutene]$^+$, 120 [M-*tert*-BuO]$^+$, 144 [Boc-NHCH(Me)]$^+$. Precise mass measurement: Calc. *m/z* 235.0700, found *m/z* = 235.0700 $[M]^+$.

N-tert-*Butoxycarbonyl-α-aminoisobutyryl-S-alanine methyl ester (Boc-Aib-S-Ala-OMe,* 7). Boc-Aib-OH (8.12 g, 0.04 mol) and HCl•H-S-Ala-OMe (5.03 g, 0.036 mol) were coupled by the mixed anhydride method described for 1 except for the activation time which was 20 min. The yield was 6.2 g (74%) of a colourless solid which was recrystallized from ethyl acetate/light petroleum ether (b.p.40 °C), m.p. 95.0-95.5 °C. R_f = 0.55 in diethyl ether/dichloromethane. $[\alpha]_D^{22}$ = - 18.7° (c = 0.4 in methanol). IR (KBr): 3410-3280 (N-H stretch), 1745 (ester), 1680 (urethane), 1670 cm^{-1} (amide I). UV (ethanol): λ_{max} (log ε) = 201 nm (3.2). MS: *m/z* = 289 $[M+1]^+$, 233 [M+1-isobutene]$^+$, 189 [M+2-*tert*-BuOCO]$^+$, 158 $[Boc-NHC(Me)_2]^+$, 102 [M-(Ala-OMe)]$^+$.

Elemental analysis: $C_{13}H_{24}N_2O_5$ (288.3) Calc.: C 54.15, H 8.39, N 9.72
Found: C 54.46, H 8.23, N 9.77

α-*Aminoisobutyryl-S-alanine methyl ester hydrochloride (HCl•H-Aib-S-Ala-OMe,* 8). Boc-Aib-S-Ala-OMe, 7 (3.00 g, 0.0104 mol) was deprotected with 52 ml of 1 N HCl/dioxane for 40 min. Evaporation of the solvent yielded 2.03 g (89%) of 8 as a hygroscopic foam. R_f = 0.50 in III, R_f = 0.45 in IV. $[\alpha]_D^{22}$ = - 23.3° (c = 2.6 in methanol). UV (ethanol): λ_{max} (log ε) = 201 nm (3.2).

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