Peptide-bond Formation, Chemoselective Acylation of Amino Acids, and Crosslinking Reaction between Amino Acids Utilizing a Functional Five-membered Heterocycle, 1,3-Thiazolidine-2-thione†

Yoshimitsu Nagao, Tadayo Miyasaka, Kaoru Seno, and Eiichi Fujita*
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan
Daisuke Shibata and Etsushiro Doi
Research Institute for Food Science, Kyoto University, Uji, Kyoto 611, Japan

The monitored aminolysis of 3-acyl-1,3-thiazolidine-2-thiones has been extended to the peptide-bond formation, the chemoselective acylation of amino acids having multifunctional groups, and the crosslinking reaction between amino acids.

Our recent research interests have been focused on the development of new reactions utilizing functional five-membered heterocycles.[1] We reported previously a new method for amide preparation by the monitored aminolysis of 3-acyl-1,3-thiazolidine-2-thiones (ATTs)[2] and its application to the synthesis of macrocyclic spermidine alkaloids.[3] During these studies, we found some remarkable features in the aminolysis of ATTs. (1) The end-point of the reaction can be judged conveniently by the disappearance of the original yellow colour of the ATT. (2) ATTs can be used to detect weak intramolecular five- or six-membered hydrogen bonding between an amino group and an imino group; when an ATT was treated with a diamine, triamine, or tetra-amine, including compounds with both amino and imino groups in the molecule, only the amide formed with the amino group was exclusively obtained in high yield. (3) ATTs show a high chemoselectivity to amines. When an ATT was allowed to react with an amino alcohol, amino phenol, or amino thiol, respectively, only the corresponding amide was obtained. (4) ATTs are fairly stable in aqueous solvents.

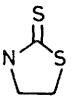
These chemical aspects of ATTs seemed to be very useful for peptide synthesis. Thus, we applied this ATT method to the amide-bond formation of amino acids and their derivatives, as communicated already.[4] We report here in full details of the peptide-bond formation, the chemoselective acylation of the multifunctional amino acids, and the crosslinking reaction between amino acids or their derivatives.

Monitored Peptide-bond Formation.—We synthesized several kinds of Z(or Boc)-peptides according to the sequence which is shown in Scheme 1. All the results are summarized in Tables 1 and 2. For a typical procedure for preparation of a 1,3-thiazolidine-2-thione (TT) amide (**3**) from the corresponding Z(or Boc)-amino acid, the reader is referred to the Experimental section. Some data on the TT-amides (**3a—e**) are shown in Table 1. All of the TT-amides are yellow crystals.

The monitored aminolysis of (**3**) with an equimolar amount of amino acid in aqueous tetrahydrofuran (THF) medium was carried out to afford the corresponding Z(or Boc)-dipeptide (**4**) in high yield. Z(or Boc)-Tripeptides (**6**) were prepared in the same way; a Z(or Boc)-dipeptide (**4**) was deprotected under acidic conditions as shown in Scheme 1. The resulting dipeptide hydrobromide (**5**) was converted into the free-base form and the second monitored aminolysis of (**3**) with this free dipeptide gave the Z(or Boc)-tripeptide (**6**) in high yield. The Z(or Boc)-tetrapeptides (**7**) and Z(or Boc)-pentapeptides (**8**) were prepared by similar procedures. Although many methods for peptide-bond formation have been reported,[5] such a conveniently monitored method as ours has not previously been described.

Table 1. Synthesis of 1,3-thiazolidine-2-thione amides (**3**)

Amide		Yield (%)	M.p. (°C)	$[\alpha]_D^{t\,a}$	t (°C)
Z-L-Ala-X[b]	(**3a**)	61	163—165	−120.0°	17
Z-L-Met-X	(**3b**)	64	99—101	−97.2°	17
Z-L-Leu-X	(**3c**)	83	78—79	−98.4°	21
Boc-L-Phe-X	(**3d**)	77	168.5—170.5	−29.8°	19
Z-L-Lys(Boc)-X	(**3e**)	53	102—103	−66.3°[c]	22

[a] Determined in $CHCl_3$ (c 2.0). [b] X =  [c] Determined in $CHCl_3$ (c 1.0).

Compounds (**4j—m**), (**6b**), (**7a**), and (**8a**) were synthesized for the study on the action of benzoyl-L-arginine *p*-nitroaniline hydrolase (a proteolytic enzyme in rice seeds).[6]

Although the racemisation test on the synthetic peptides was not performed, the Young test[7] of Bz-L-Leu-Gly-OEt (**11**), which was prepared according to our method (see Scheme 2), showed it to be 95% L-isomer in comparison with the same compound[7] derived by the azide procedure. This result means that more than 5% racemisation does not occur even with the use of the *N*-benzoyl derivative (**11**) which readily causes racemisation. In our general procedure utilizing Z- or Boc-amino acid derivatives, significant racemisation has never been recognized.

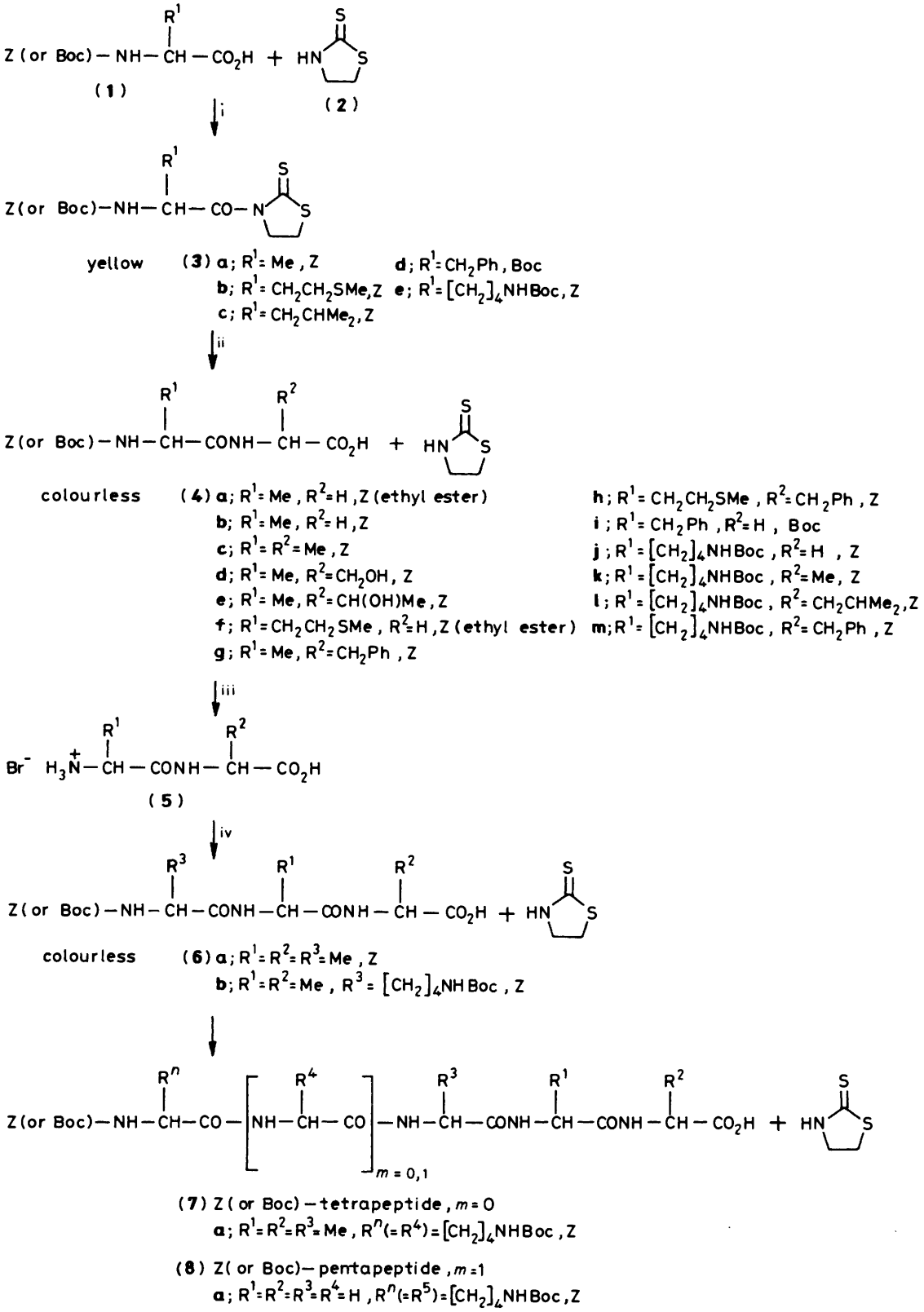
Through these sequential reactions, it was recognized that the TT-amide bond was perfectly stable even under strongly acidic conditions [in a solution in HBr–AcOH (1:3)].

Chemoselective Acylation of Amino Acids.—Chemoselective acylations of amino acid, protein, and enzyme are very attractive from the viewpoints of peptide synthesis, transformation of the physicochemical properties of protein, and chemical modification of enzyme molecules.[8]

Thus, 3-benzoyl-1,3-thiazolidine-2-thione (BzTT) (**12**) was treated with L-arginine, L-cysteine methyl ester, L-serine, or L-lysine in THF–water (or ethanol) to afford chemoselectively the corresponding benzoyl amides (**13**)—(**16**) in good yield (Scheme 3).[9,10] 3-Benzyloxycarbonyl-1,3-thiazolidine-2-thione (ZTT) (**18**)[11] was treated with L-histidine methyl ester hydrochloride or L-lysine methyl ester dihydrochloride in the presence of Et_3N to give the corresponding Z-amide (**19**) or (**20**) in good yield (Scheme 4).

The position of benzoylation or benzyloxycarbonylation of all products was confirmed by their H^1 n.m.r. and i.r. spectra (the absorption band due to the amide bond) and a chemical

† This paper forms Part 6 of the series 'Utilization of Sulphur-containing Leaving Group.' Part 5, Y. Nagao, T. Inoue, E. Fujita, S. Terada, and M. Shiro, *Tetrahedron*, Symposia-in-print, 1984, **40**, 1215.

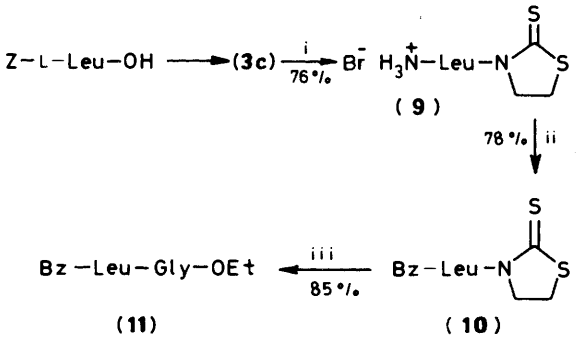


Scheme 1. *Reagents:* i, DCC, CH_2Cl_2, 0 °C; ii, $H_3\overset{+}{N}$–CH(R^2)–CO_2^-, Et_3N, THF–water (1:1); iii, HBr–AcOH (1:3), anisole; iv, (3), Et_3N, THF–water (1:1)

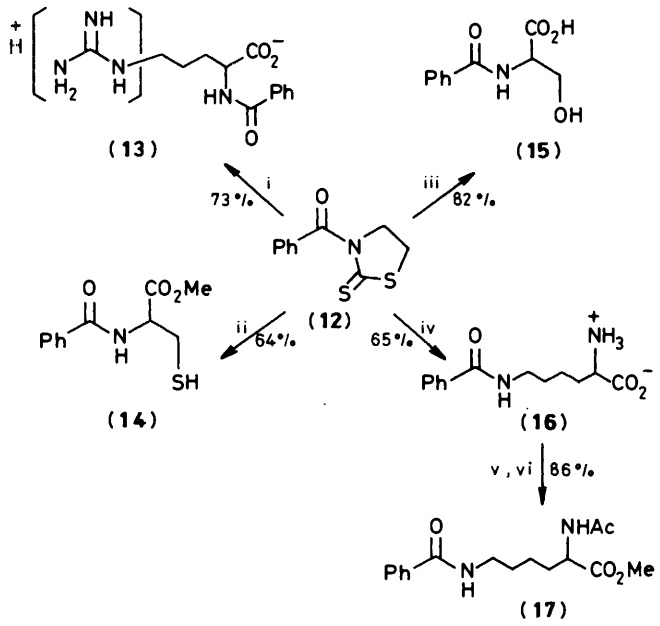
Table 2. Synthesis of Z (or Boc)-peptides (**4**), (**6**), (**7a**), and (**8a**)

Z (or Boc)-Peptide	Reaction time (min)	Yield (%)	M.p. (°C)	$[\alpha]_D^t$	(c; solvent; t/°C)
Z-L-Ala-Gly-OEt (**4a**)	15[a]	94	98—99	−19.7	(1.0; EtOH; 23)
	80	94	99—100	−22.7	(1.1; EtOH; 17)
Z-L-Ala-Gly-OH (**4b**)	1	89	128—129	−15.4	(0.91; EtOH; 23)
Z-L-Ala-L-Ala-OH (**4c**)	90	85	154—156	−32.5	(1.0; MtOH; 21)
Z-L-Ala-L-Ser-OH (**4d**)	30	88	194—196	+21.1	(0.4; DMF; 19)
Z-L-Ala-L-Thr-OH (**4e**)	30	93	139—141	−8.2	(0.94; EtOH; 23)
Z-L-Met-Gly-OEt (**4f**)	20[a]	88	95.5—96.5	−17.0	(0.73; EtOH; 23)
	110	94	96—97	−19.6	(1.1; EtOH; 17)
Z-L-Ala-L-Phe-OH (**4g**)	5[a]	87	124—126	+39.2	(0.5; dioxane; 21)
Z-L-Met-L-Phe-OH (**4h**)	20[a]	95	125—126	+3.3	(1.0; EtOH; 19)
Boc-L-Phe-Gly-OH (**4i**)	3	93	163—164	−5.5	(0.5; dioxane; 21)
Z-L-Lys(Boc)-Gly-OH (**4j**)	5	90	127—128	−12.7	(1.0; MeOH; 22)
Z-L-Lys(Boc)-L-Ala-OH (**4k**)	overnight	88	107—109	−14.8	(1.0; MeOH; 21)
Z-L-Lys(Boc)-L-Leu-OH (**4l**)	overnight	79	131—132	−16.3	(1.0; MeOH; 21)
Z-L-Lys(Boc)-L-Phe-OH (**4m**)	1 h	78	131—132	+6.0	(2.0; EtOH; 21)
Z-L-Ala↓[L-Ala]$_2$-OH[b] (**6a**)	6 h	87	224—227 (decomp.)	−57.9	(1.0; MeOH; 21)
Z-L-Lys(Boc)↓[L-Ala]$_2$-OH[b] (**6b**)	2 d	99	163—164	−34.9	(1.0; MeOH; 21)
Z-L-Lys(Boc)↓[L-Ala]$_3$-OH[b] (**7a**)	2 d	87	205—207 (decomp.)	−45.1	(1.0; MeOH; 21)
Z-L-Lys(Boc)↓[Gly]$_4$-OH[b] (**8a**)	overnight	96	144—152 (decomp.)	−4.4	(1.0; DMF; 21)

[a] 1.5—2.0 Mol equiv. of Et_3N were employed. In other cases, 1.1 mol equiv. of Et_3N were used. [b] The arrow mark indicates the reaction point.



Scheme 2. *Reagents:* i, HBr–AcOH (1:3); ii, PhCOCl, Na_2CO_3, AcOEt–water; iii, Gly-OEt·HCl, Et_3N, THF–water (3:1)



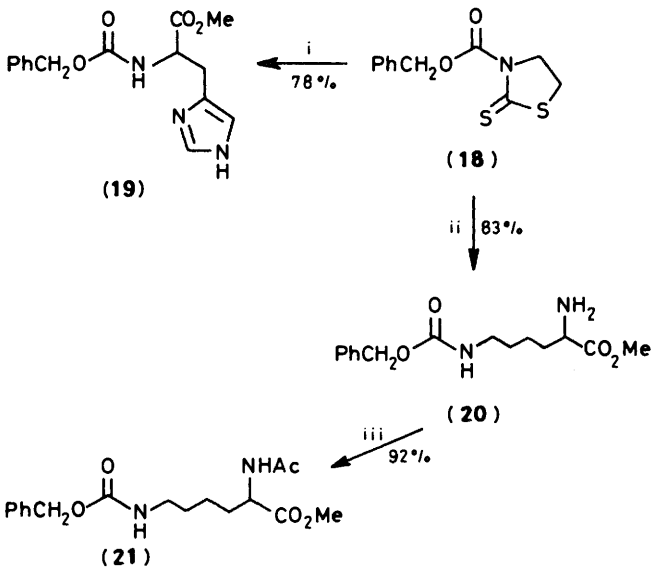
Scheme 3. *Reagents:* i, L-arginine, THF–water (1:1); ii, L-cysteine methyl ester hydrochloride, Et_3N, THF–EtOH (1:4); iii, L-serine, Et_3N, THF–water (1:1); iv, L-lysine, THF–water (3:4), v, $SOCl_2$, MeOH, reflux; vi, Ac_2O, pyridine

method [acetylation of (**16**) or (**20**) to the corresponding diamide (**17**) or (**21**)].

Through these reactions we observed the following. BzTT (**12**) does not react with SH and OH groups less nucleophilic than an NH_2 group. BzTT (**12**) and ZTT (**18**) can differentiate between the more nucleophilic and less hindered ω-amino group from the α-amino group in L-lysine and its methyl ester. These reagents can also differentiate the more nucleophilic α-amino group from the protonated guanidino group in L-arginine and from the imidazole group in L-histidine methyl ester. We achieved highly chemoselective NH_2-acylation of the multifunctional amino acids without special protection of the acylation-sensitive groups. This method has been utilized for the total synthesis of parabactin (**22**), a spermidine-containing siderophore.[12]

Crosslinking Reaction between Two Amino Acids.—Crosslinking (see Figure) in a protein (or enzyme) or between proteins (or enzymes) is interesting from the viewpoint of protein and enzyme technology.[13] We synthesized two types of compound, (**23**) as a homo-bifunctional reagent and (**26**) as a hetero-bifunctional reagent. The former must have a high reactivity towards amino groups, while the latter must have high reactivities towards amino and mercapto groups. Compound (**23**) was prepared by treating succinyl dichloride with 2 mol equiv. of the thallium(I) salt of 1,3-thiazolidine-2-thione (TT) in THF.[2b,11] Compound (**26**) was prepared by dehydrative condensation, in the presence of DCC (dicyclohexylcarbodiimide), between TT and carboxylic acid (**25**) which was derived from 2,4-dinitrophenylsulphenyl chloride and 3-mercaptopropionic acid.[14]

On treatment with L-lysine (2 mol equiv), succinyl TT



Scheme 4. *Reagents:* i, L-histidine methyl ester hydrochloride, Et_3N, CH_3CN, reflux, ii, L-lysine methyl ester dihydrochloride, Et_3N, THF–EtOH (1:4); iii, Ac_2O, pyridine

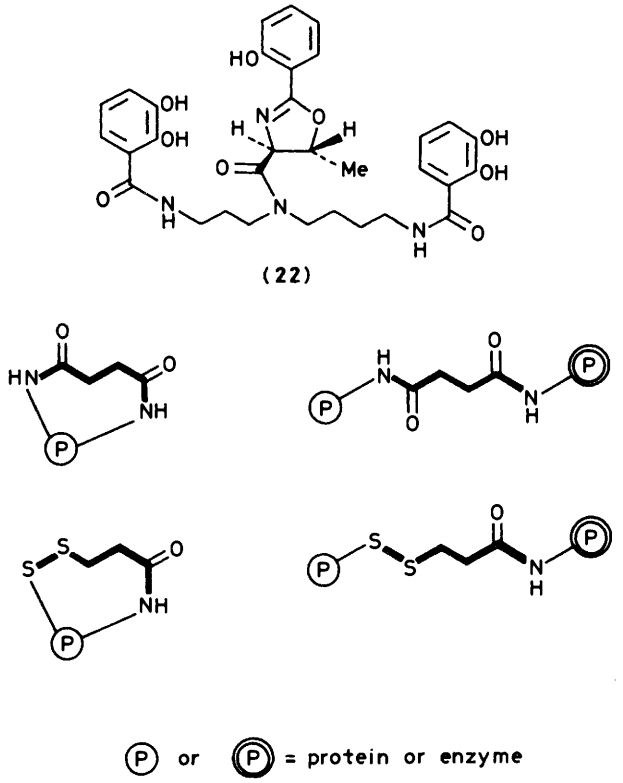
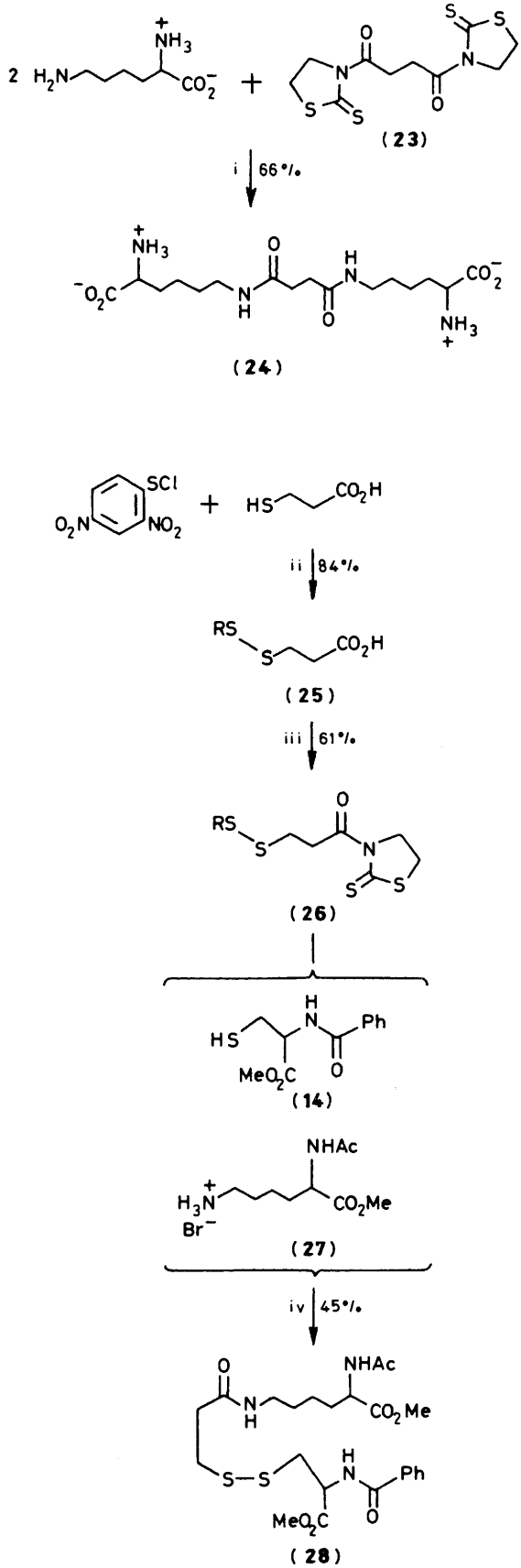


Figure. Crosslinking mode.

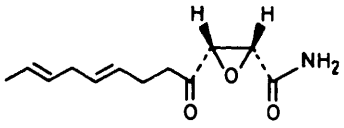
diamide (**23**) afforded the L-lysine diamide (**24**) in 66% yield. The hetero-bifunctional reagent (**26**) was treated with a mixture of an equimolar amount of SH enzyme model compound (**14**) and another model compound (**27**) in the presence of Et_3N to yield the desired product (**28**) in 45% yield (see Scheme 5).

Interestingly, the hetero-bifunctional reagent (**26**) showed inhibition activity towards a fatty acid synthetase in *Brevibacterium ammoniagenes*.[15] Its activity ($I.C._{50}$ 50 μg ml^{-1}) was

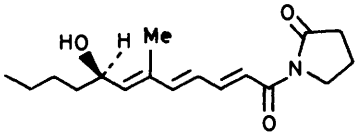


Scheme 5. *Reagents:* i, THF–water (2:1); ii, THF, 0 °C; iii, 1,3-thiazolidine-2-thione, DCC, 0 °C; iv, Et_3N, $CHCl_3$–EtOH–THF. R = 2,4-dinitrophenyl

found to be lower than that (I.C.$_{50}$ 1 μg ml^{-1}) of cerulenin (**29**) but is almost the same as that (I.C.$_{50}$ 20 μg ml^{-1}) of variotin (**30**).[15] We are now investigating in detail the crosslinking reaction of some enzyme using our reagents (**23**) and (**26**).



(**29**)



(**30**)

Experimental

M.p.s were determined with a Yanagimoto microapparatus. I.r. spectra were run using KBr plates, unless otherwise stated, on a JASCO A-202 spectrophotometer. Optical rotations were measured on a JASCO DIP-181 polarimeter. E.i. (electron impact) and f.a.b. (fast-atom bombardment) mass spectra were recorded on a JEOL JMS-DX300 mass spectrometer. ^{1}H N.m.r. spectra were recorded on a JEOL JNM-FX100 spectrometer in $CDCl_3$ solutions, unless otherwise stated, with Me_4Si as internal standard. Extracts were dried over Na_2SO_4. Kieselgel 60 (70—230 mesh) (Merck) and Sephadex LH20 (Pharmacia Fine Chemicals) were used for column chromatography. Preparative t.l.c. was performed on precoated plates (Kieselgel 60 F254) (Merck). DMF is dimethylformamide. Light petroleum refers to that fraction boiling over the range 30—60 °C.

Typical Preparation of 1,3-*Thiazolidine*-2-*thione Amides* (**3**) *from Z* (*or Boc*)-*Amino Acid* (**1**).—DCC (4.12 g, 20 mmol) was added to an ice-cooled, stirred solution of Z-L-Ala-OH (**1a**) (4.46 g, 20 mmol) and 1,3-thiazolidine-2-thione (TT) (**2**) (2.38 g, 20 mmol) in CH_2Cl_2 (20 ml). The mixture was stirred at 0 °C for 5 h and a precipitate (dicyclohexylurea) was filtered off. Evaporation of the filtrate under reduced pressure left an oily residue which was crystallized from $CHCl_3$–Et_2O to afford TT-amide (**3a**) (3.94 g, 61% yield) as yellow plates.

Physical Data of TT-amides (**3**).—3-(N-*Benzyloxycarbonyl*-L-*alanyl*)-1,3-*thiazolidine*-2-*thione* (**3a**). Yellow plates, m.p. 163—165 °C (from $CHCl_3$–Et_2O); $[\alpha]_D^{17}$ −120° (*c* 2.0 in $CHCl_3$); $\nu_{max.}$ 3 400, 1 718, and 1 698 cm^{-1}; δ 1.44 (3 H, d, *J* 7 Hz), 3.23 (2 H, t, *J* 8 Hz), 4.50 (2 H, t, *J* 8 Hz), 5.08 (2 H, s), 5.56 (1 H, br d, *J* 8 Hz), 6.14 (1 H, dq, *J* 8 and 7 Hz), and 7.32 (5 H, s) (Found: C, 51.75; H, 5.0; N, 8.8%; M^+, 324. $C_{14}H_{16}N_2O_3S_2$ requires C, 51.85; H, 4.95; N, 8.65%; *M*, 324).

3-(N-*Benzyloxycarbonyl*-L-*methionyl*)-1,3-*thiazolidine*-2-*thione* (**3b**). Yellow needles, m.p. 99—101 °C (from $CHCl_3$–Et_2O); $[\alpha]_D^{17}$ −97.2° (*c* 2.0 in $CHCl_3$): $\nu_{max.}$ 3 350, 1 700, 1 685, and 1 538 cm^{-1}; δ 1.86 (1 H, A part of ABX-type signal, *J* 3 and 8 Hz), 2.04 (3 H, s), 2.28 (1 H, B part of ABX-type signal, *J* 3 and 8 Hz), 2.52 (2 H, t, *J* 8 Hz), 3.22 (2 H, t, *J* 7 Hz), 4.10 (2 H, t, *J* 7 Hz), 5.06 (2 H, s), 5.50 (1 H, br d, *J* 8 Hz), 6.16 (1 H, X part of ABX-type signal *J* 3 and 8 Hz), and 7.28 (5 H, s) (Found: C, 49.95; H, 5.3; N, 7.4%; M^+, 383. $C_{16}H_{20}N_2O_3S_3$ requires C, 50.0; H, 5.25; N, 7.3%; *M*, 383).

3-(N-*Benzyloxycarbonyl*-L-*leucyl*)-1,3-*thiazolidine*-2-*thione* (**3c**). Yellow needles, m.p. 78—79 °C (from Et_2O–light petroleum); $[\alpha]_D^{21}$ −78.4° (*c* 2.0 in $CHCl_3$); $\nu_{max.}$ 3 360, 1 170, 1 690, and 1 530 cm^{-1}; δ 0.91 (6 H, m), 1.15—1.95 (3 H, m), 3.21 (2 H, t, *J* 7 Hz), 4.47 (2 H, t, *J* 7 Hz), 5.04 (2 H, s), 5.21 (1 H, br d, *J* 10 Hz), and 7.27 (5 H, s) (Found: C, 55.7; H, 6.05; N, 7.55%; M^+, 406. $C_{17}H_{22}N_2O_3S_2$ requires C, 55.75; H, 6.05; N, 7.65%; *M*, 406).

3-(N-*t*-*Butoxycarbonyl*-L-*phenylalanyl*)-1,3-*thiazolidine*-2-*thione* (**3d**). Yellow needles, m.p. 168.5—170.5 °C (from $CHCl_3$–hexane); $[\alpha]_D^{19}$ −29.8° (*c* 2.0 in $CHCl_3$); $\nu_{max.}$ 3 400, 1 705, 1 690, and 1 520 cm^{-1}; δ 1.36 (9 H, s), 2.90 (2 H, m), 3.27 (2 H, t, *J* 7.5 Hz), 4.52 (2 H, t, *J* 7.5 Hz), 5.10 (1 H, br s), 6.44 (1 H, s-like), and 7.25 (5 H, s) (Found: C, 55.45; H, 6.05; N, 8.05%; M^+, 366. $C_{17}H_{22}N_2O_3S_2$ requires C, 55.75; H, 6.05; N, 7.65%; *M*, 366).

3-(N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-*lysyl*)-1,3-*thiazolidine*-2-*thione* (**3e**). Yellow fine prisms, m.p. 102—103 °C (from Et_2O–benzene); $[\alpha]_D^{22}$ −66.3° (*c* 1.0 in $CHCl_3$); $\nu_{max.}$ 3 353, 1 690, and 1 530 cm^{-1}; δ 1.05—1.65 (15 H, m), 3.05 (2 H, m), 3.23 (2 H, t, *J* 7 Hz), 4.48 (2 H, t, *J* 7 Hz), 5.04 (2 H, s), 5.55 (1 H, br d, *J* 8 Hz), 6.07 (1 H, m), and 7.26 (5 H, s) (Found: C, 54.75; H, 6.6; N, 8.5%; M^+, 424. $C_{22}H_{31}N_3O_5S_2$ requires C, 54.85; H, 6.5; N, 8.7%; *M*, 424).

Typical Preparation of Z (*or Boc*)-*Dipeptides* (**4**).—A solution of glycine (82.5 mg, 1.1 mmol) in water (5 ml) was added to a yellow solution of compound (**3a**) (324 mg, 1 mmol) in THF (5 ml). After the addition of Et_3N (0.15 ml, 1.1 mmol) the mixture was stirred at room temperature for 1 min (the original yellow colour disappeared). Evaporation of the solvent under reduced pressure gave an oily residue which was dissolved in AcOEt (100 ml). The solution was washed in turn with 5% HCl and brine, dried, and evaporated under reduced pressure to give an oily residue which was purified on a Sephadex LH-20 column with MeOH to give Z-Ala-Gly-OH (**4b**) (249 mg, 89% yield).

Physical Data of Z (*or Boc*)-*Dipeptides* (**4**).—N-*Benzyloxycarbonyl*-L-*alanylglycine Ethyl Ester* (**4a**). Needles, m.p. 99—100 °C (from $CHCl_3$–hexane) (lit.,[16] 97—98 °C); $[\alpha]_D^{17}$ −22.7° (*c* 1.1 in EtOH) {lit.,[16] $[\alpha]_D^{20}$ −24.0 (*c* 1.0 in EtOH)]; $\nu_{max.}$ 3 290, 1 755, 1 690, 1 645, and 1 535 cm^{-1}; δ 1.28 (3 H, t, *J* 7 Hz), 1.42 (3 H, d, *J* 7 Hz), 2.02 (2 H, d, *J* 5 Hz), 2.22 (2 H, q, *J* 7 Hz), 2.28 (1 H, dq, *J* 8 and 7 Hz), 5.12 (2 H, s), 5.38 (1 H, br d, *J* 8 Hz), 6.60 (1 H, br s), and 7.32 (5 H, s) (Found: C, 58.5; H, 6.6; N, 9.05%; M^+, 308. Calc. for $C_{15}H_{20}N_2O_5$: C, 58.45; H, 6.55, N, 9.1; *M*, 308).

N-*Benzyloxycarbonyl*-L-*alanylglycine* (**4b**). Fine prisms, m.p. 128—129 °C (from $CHCl_3$–hexane); $[\alpha]_D^{23}$ −15.4° (*c* 0.91 in EtOH); $\nu_{max.}$ 3 320, 1 725, 1 675, 1 665, 1 635, 1 550, and 1 530 cm^{-1}; δ (CD_3OD) 1.36 (3 H, d, *J* 7 Hz), 3.90 (2 H, s), 4.20 (1 H, q, *J* 7 Hz), 5.07 (2 H, s), and 7.34 (5 H, s) (Found: C, 55.9; H, 5.8; N, 9.95%; M^+, 280. $C_{13}H_{16}N_2O_5$ requires C, 55.7; H, 5.75; N, 10.0%; *M*, 280).

N-*Benzyloxycarbonyl*-L-*alanyl*-L-*alanine* (**4c**). Prisms, m.p. 154—156 °C (from AcOEt–MeOH–hexane); $[\alpha]_D^{21}$ −32.5° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 290, 1 685, 1 642, and 1 535 cm^{-1}; δ (CD_3OD) 1.05—1.53 (6 H, m), 3.93—4.53 (2 H, m), 5.03 (2 H, s), and 7.24 (5 H, s) (Found: C, 57.05; H, 6.25; N, 9.5%; M^+, 294. $C_{14}H_{18}N_2O_5$ requires C, 57.15; H, 6.15; N, 9.5; *M*, 294).

N-*Benzyloxycarbonyl*-L-*alanyl*-L-*serine* (**4d**). Needles, m.p. 194—196 °C (from MeOH–water); $[\alpha]_D^{23}$ +21.1° (*c* 0.4 in DMF); $\nu_{max.}$ 3 475, 3 275, 1 720, 1 630, and 1 542 cm^{-1}; δ [$(CD_3)_2SO$] 1.24 (3 H, d, *J* 7 Hz), 3.42 (1 H, br s), 3.68 (2 H, m), 4.00—4.40 (2 H, m), 5.02 (2 H, s), 7.34 (6 H, br s), and 7.94 (1 H, br d, *J* 8 Hz) (Found: C, 54.3; H, 6.0; N, 9.1%, M^+, 310. $C_{14}H_{18}N_2O_6$ requires C, 54.2; H, 5.85; N, 9.05; *M*, 310).

N-*Benzyloxycarbonyl*-L-*alanyl*-L-*threonine* (**4e**). Fine prisms, m.p. 139—141 °C (from $CHCl_3$–hexane); $[\alpha]_D^{23}$ −8.2° (*c* 0.94 in EtOH); $\nu_{max.}$ 3 400sh, 3 270, 1 710, 1 685, 1 650, and 1 530 cm^{-1}; δ [$(CD_3)_2SO$] 1.06 (3 H, d, *J* 6 Hz), 1.25 (3 H, d, *J* 7 Hz), 3.34 (1 H,

br s), 4.00—4.20 (3 H, m), 5.04 (2 H, s), 7.34 (6 H, s), 7.50 (1 H, br d), and 7.64 (1 H, br d) (Found: C, 55.35; H, 6.3; N, 8.6%; M^+, 324. $C_{15}H_{20}N_2O_6$ requires C, 55.55; H, 6.2; N, 8.65%; M, 324).

N-*Benzyloxycarbonyl*-L-*methionylglycine Ethyl Ester* (**4f**). Needles, m.p. 96—97 °C (from CH_2Cl_2–hexane) (lit.,[17] 94—96 °C); $[\alpha]_D^{17}$ −19.6° (*c* 1.1 in EtOH) {lit.,[17] $[\alpha]_D^{27}$ −19.8° (*c* 4.6 in EtOH)}; $\nu_{max.}$ 3 290, 1 715, 1 690, 1 655, and 1 530 cm^{-1}; δ 1.28 (3 H, t, *J* 7 Hz), 1.80—2.24 (5 H, m), 2.61 (2 H, t, *J* 7 Hz), 4.02 (2 H, m), 4.21 (2 H, q, *J* 7 Hz), 4.40 (1 H, m), 5.11 (2 H, s), 5.50 (1 H, br d, *J* 8 Hz), 6.47 (1 H, br s), and 7.35 (5 H, s) (Found: C, 55.35; H, 6.65; N, 7.65%; M^+, 368. Calc. for $C_{17}H_{24}N_2O_5S$: C, 55.45; H, 6.55; N, 7.6%; M, 368).

N-*Benzyloxycarbonyl*-L-*alanyl*-L-*phenylalanine* (**4g**). Fine prisms, m.p. 124—126 °C (from MeOH–water); $[\alpha]_D^{21}$ +39.2° (*c* 0.5 in dioxane); $\nu_{max.}$ 3 300, 1 715sh, 1 690, 1 650, and 1 530 cm^{-1}; δ 1.21 (3 H, d, *J* 7 Hz), 2.94 (1 H, A part of ABX-type signal, *J* 14 and 7 Hz), 3.17 (1 H, B part of ABX-type signal, *J* 14 and 6 Hz), 4.22 (1 H, m), 4.77 (1 H, X part of ABX-type signal), 5.03 (2 H, s), 5.82 (1 H, br s), 6.92 (1 H, br d), 7.12 (5 H, s), 7.28 (5 H, s), and 8.81 (1 H, br s) (Found: C, 65.05; H, 5.95; N, 7.55%; M^+, 370. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.55%; M, 370).

N-*Benzyloxylcarbonyl*-L-*methionyl*-L-*phenylalanine* (**4h**). Needles, m.p. 125—126 °C (from $CHCl_3$–hexane); $[\alpha]_D^{19}$ +3.3° (*c* 1.0 in EtOH); $\nu_{max.}$ 3 350, 3 250, 1 705, 1 670, 1 625, and 1 570 cm^{-1}; δ 1.64—2.20 (2 H, m), 2.04 (3 H, s), 2.48 (2 H, t, *J* 7 Hz), 3.00 (1 H, A part of ABX-type signal, *J* 14 and 7 Hz), 3.21 (1 H, B part of ABX-type signal, *J* 14 and 6 Hz), 4.26 (1 H, m), 4.68 (1 H, X part of ABX-type signal) 5.08 (2 H, s) 6.71 (1 H, br d, *J* 9 Hz), 7.21 (5 H, s), 7.33 (5 H, s), and 7.41 (1 H, br d, *J* 8 Hz) (Found: C, 61.3; H, 6.1; N, 6.75%; M^+, 343. $C_{22}H_{26}N_2O_5S$ requires C, 61.4; H, 6.1; N, 6.5%; M, 343).

N-*t*-*Butoxycarbonyl*-L-*phenylalanylglycine* (**4i**). Fine prisms, m.p. 163—164 °C (Et_2O–hexane); $[\alpha]_D^{21}$ −5.5° (*c* 0.5 in dioxane); $\nu_{max.}$ 3 295, 1 720, 1 680, 1 645, and 1 540 cm^{-1}; δ (CD_3OD) 1.34 (9 H, s), 2.79 (1 H, A part of ABX-type signal, *J* 14 and 10 Hz), 3.18 (1 H, B part of ABX-type signal, *J* 14 and 5 Hz), 3.92 (2 H, s), 4.34 (1 H, X part of ABX-type signal, *J* 10 and 5 Hz), and 7.24 (5 H, s) (Found: C, 59.55; H, 7.0; N; 8.6%; M^+, 322. $C_{16}H_{22}N_2O_5$ requires C, 59.6; H, 6.9; N, 8.7%; M, 322).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-L-*lysylglycine* (**4j**). Fine prisms, m.p. 127—128 °C (from AcOEt–CH_2Cl_2); $[\alpha]_D^{22}$ −12.7° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 350, 1 702, 1 678, 1 659, and 1 537 cm^{-1}; δ (CD_3OD) 1.10—2.06 (15 H, m), 3.00 (2 H, t, *J* 6 Hz), 3.87 (2 H, s), 4.10 (1 H, m), 5.05 (2 H, s), and 7.28 (5 H, s) (Found: C, 57.25; H, 7.25; N, 9.45%; M^+, 437. $C_{21}H_{31}N_3O_7$ requires C, 57.65; H, 7.15; N, 9.6%; M, 437).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-L-*lysyl*-L-*alanine* (**4k**). Needles, m.p. 107—109 °C (from Et_2O); $[\alpha]_D^{21}$ −14.8° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 310, 1 690, 1 645, and 1 535 cm^{-1}; δ 0.76—2.08 (18 H, m), 3.02 (2 H, m), 3.98—4.62 (2 H, m), 4.82 (1 H, br s), 5.02 (2 H, s), 5.96 (1 H, br d, *J* 8 Hz), 6.23 (1 H, br s), and 7.00—7.20 (6 H, m) (Found: C, 58.3; H, 7.45; N, 9.25%; M^+, 451. $C_{22}H_{33}N_3O_7$ requires C, 58.5; H, 7.35; N, 9.3%; M, 451).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\alpha}$-*t*-*butoxycarbonyl*-L-*lysyl*-L-*leucine* (**4l**). Fine prisms, m.p. 131—132 °C (from $CHCl_3$–hexane); $[\alpha]_D^{21}$ −16.3° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 340, 1 682, and 1 523 cm^{-1}; δ 0.89 (6 H, d, *J* 5 Hz), 1.00—2.09 (18 H, m), 2.99 (2 H, m), 4.20 (1 H, m), 4.54 (1 H, m), 4.74—4.96 (1 H, br s), 5.05 (2 H, s), 5.99 (1 H, br d, *J* 8 Hz), 7.08 (1 H, br d, *J* 8 Hz), 7.26 (5 H, s), and 8.69 (1 H, br s) (Found: C, 60.5; H, 8.1; N, 8.35%; M^+, 493. $C_{25}H_{39}N_3O_7$ requires C, 60.85; H, 7.95; N, 8.5%; M, 493).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-L-*lysyl*-L-*phenylalanine* (**4m**). Needles, m.p. 131—132 °C (from CH_2Cl_2–hexane); $[\alpha]_D^{21}$ 6.0° (*c* 2.0 in EtOH); $\nu_{max.}$ 3 340, 3 360, 1 741, 1 695, 1 678, 1 611, and 1 538 cm^{-1}; δ 0.99—2.09 (15 H, m), 2.73—3.30 (4 H, m), 4.13 (1 H, m), 4.53 (1 H, m), 5.04 (2 H, s), 5.60—6.13 (2 H, br m), 6.92 (1 H, br d, *J* 8 Hz), 7.11 (5 H, s), 7.26 (5 H, s), and 9.52 (1 H, br s) (Found: C, 63.85; H, 7.2; N, 8.0%; M^+, 527. $C_{28}H_{37}N_3O_7$ requires C, 63.75; H, 7.05; N, 7.95%; M, 527).

Typical Preparation of Z (*or Boc*)-*tripeptides* (**6**).—Z-L-Ala-L-Ala-OH (**4c**) (3.5 g, 12 mmol) and anisole (6 ml) were added to an ice-cooled solution (10 ml) of HBr–AcOH (1:3) and the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure to give an oily residue which was solidified from Et_2O. The solid obtained was repeatedly purified by successive decantations with excess of Et_2O to afford L-Ala-L-Ala-OH·HBr (**5a**) (2.9 g, quantitative) as crystals. Then a solution of compound (**5a**) (1.45 g, 6 mmol) and Et_3N (1.54 ml, 11 mmol) in water (25 ml) was added to a solution of compound (**3a**) (1.62 g, 5 mmol) in THF (25 ml). The mixture was stirred at room temperature under N_2 for 3 days and treated as before to give Z-L-Ala-L-Ala-L-Ala-OH (**6a**) (1.59 g, 87% yield).

Physical Data of Z-Tripeptides (**6**).—N$^{\alpha}$-*Benzyloxycarbonyl*-L-*alanyl*-L-*alanyl*-L-*alanine* (**6a**). Needles, m.p. 224—227 °C (decomp.) (from MeOH); $[\alpha]_D^{21}$ −57.9° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 300, 1 735, 1 682, 1 652, 1 601, and 1 533 cm^{-1}; δ (CD_3OD) 1.05—1.47 (9 H, m), 3.97—4.57 (3 H, m), 5.04 (2 H, s), and 7.25 (5 H, s) (Found: C, 55.8; H, 6.4; N, 11.45%; M^+, 365. $C_{17}H_{23}N_3O_6$ requires C, 55.9; H, 6.35; N, 11.5%; M, 365).

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-L-*lysyl*-L-*alanyl*-L-*alanine* (**6b**). Prisms, m.p. 163—164 °C (from MeOH–hexane–AcOEt); $[\alpha]_D^{21}$ −34.9° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 340, 1 725, 1 680, 1 665, and 1 525 cm^{-1}; δ (CD_3OD) 0.95—1.95 (21 H, m), 3.06 (2 H, t, *J* 7 Hz), 3.95—4.55 (3 H, m), 5.09 (2 H, s), and 7.32 (5 H, s) [Found: C, 57.25; H, 7.4; N, 10.55%; f.a.b.m.s. $(M + Na)^+$, 545. $C_{25}H_{38}N_4O_8$ requires C, 57.45; H, 7.35; N, 10.7%; M, 522].

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-L-*lysyl*-L-*alanyl*-L-*alanyl*-L-*alanine* (**7a**).—A solution of L-Ala-L-Ala-L-Ala-OH·HBr (0.72 g, 2.3 mmol), obtained from compound (**6a**) by the usual treatment, and Et_3N (0.64 ml, 4.6 mmol) in water (10 ml) was added to a solution of compound (**3e**) (0.82 g, 1.7 mmol) in THF (10 ml). After being stirred at room temperature for 2 days, the reaction mixture was treated as usual to afford the *Z*-*tetrapeptide* (**7**) (0.91 g, 87%) as fine prisms from MeOH–AcOEt–hexane, m.p. 205—207 °C (decomp.); $[\alpha]_D^{21}$ −45.1° (*c* 1.0 in MeOH); $\nu_{max.}$ 3 300, 1 630, and 1 532 cm^{-1}; δ (CD_3OD) 1.02—1.90 (24 H, m), 3.00 (2 H, m), 3.90—4.54 (4 H, m), 5.09 (2 H, s), and 7.30 (5 H, s) [Found: C, 56.45; H, 7.5; N, 11.4%; f.a.b.m.s. $(M + Na + H)^+$, 617. $C_{28}H_{43}N_5O_9$ requires C, 56.65; H, 7.3; N, 11.8%; M, 593].

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*t*-*butoxycarbonyl*-L-*lysylglycyl*-*glycylglycylglycine* (**8a**).—The usual treatment of commercially available glycylglycylglycylglycine (Tokyo Kasei Co., Tokyo, Japan) (0.74 g, 3 mmol) with compound (**3e**) (0.96 g, 2 mmol) in 1:1 water–THF (30 ml) in the presence of Et_3N (0.42 ml, 3 mmol) afforded Z-L-*Lys*(*Boc*)-[*Gly*]$_4$-*OH* (**8**) (1.17 g, 96%) as fine prisms from water–MeOH–AcOEt. m.p. 144—152 °C (decomp.); $[\alpha]_D^{21}$ −4.4° (*c* 1.0 in DMF); $\nu_{max.}$ 3 302, 1 732, 1 678, 1 650, and 1 530 cm^{-1}; δ (CD_3OD–D_2O) 1.18—1.94 (15 H, m), 3.10 (2 H, t-like, *J* 7 Hz), 3.86—4.12 (8 H, m), 5.00 (2 H, s), and 7.44 (5 H, s) [Found: C, 52.3; H, 6.75; N, 13.5%; f.a.b.m.s. $(M + Na)^+$, 631. $C_{27}H_{40}N_6O_{10}\cdot\frac{1}{2}H_2O$ requires C, 52.5; H, 6.7; N, 13.6%; M, 608].

3-L-*Leucyl*-1,3-*thiazolidine*-2-*thione Hydrobromide* (**9**).—Compound (**3c**) (1.1 g, 3 mmol) was added to an ice-cooled solution (10 ml) of HBr–AcOH (1:3). After being stirred at room temperature under N_2 for 1.5 h, the reaction mixture was treated with Et_2O (50 ml) to give a precipitate which was filtered

off. The precipitate was repeatedly washed with excess of Et_2O and crystallized from MeOH–Et_2O to afford *compound* (**9**) (718 mg, 76%) as yellow needles, m.p. 158—162 °C (decomp.); $\nu_{max.}$ 2 950 and 1 674 cm^{-1}; δ [$(CD_3)_2SO$] 0.90 (6 H, d, *J* 6 Hz), 1.20—2.00 (3 H, m), 3.20—3.72 (2 H, m), 4.20—4.88 (2 H, m), 5.72 (1 H, br s), and 8.32 (3 H, br s) [Found: C, 34.35; H, 5.55; N, 9.0% $(M - HBr)^+$, 232. $C_9H_{17}BrN_2OS_2$ requires C, 34.5; H, 5.45; N, 8.95%; $(M - HBr)$, 232].

3-(N-*Benzoyl*-L-*leucyl*)-1,3-*thiazolidine*-2-*thione* (**10**).—A solution of Na_2CO_3 (504 mg, 3.8 mmol) in water (10 ml) was added to a suspension of compound (**9**) (595 mg, 1.9 mmol) and benzoyl chloride (0.27 ml, 2.3 mmol) in AcOEt (20 ml). After being stirred at room temperature for 1 h the mixture was separated, the organic layer was washed with brine, dried, and evaporated under reduced pressure to give an oily residue which was crystallized from AcOEt–hexane to afford the *benzoyl derivative* (**10**) (498 mg, 78%) as yellow needles, m.p. 132—134 °C; $[\alpha]_D^{16}$ −47.4° (*c* 2 in $CHCl_3$); $\nu_{max.}$ 3 320, 1 695, 1 630, and 1 530 cm^{-1}; δ 1.00 (6 H, d, *J* 6 Hz), 1.40—2.20 (3 H, m), 3.30 (2 H, t, *J* 7 Hz), 4.52 (2 H, t, *J* 7 Hz), 6.32—6.84 (2 H, m), and 7.26—8.04 (5 H, m) (Found: C, 57.2; H, 6.0; N, 8.3. $C_{16}H_{20}N_2O_2S_2$ requires C, 57.15; H, 6.0; N, 8.35%).

N-*Benzoyl*-L-*leucylglycine Ethyl Ester* (**11**).—A solution of glycine ethyl ester hydrochloride (154 mg, 1.1 mmol) in water (3 ml) was added to a solution of 3-(*N*-benzoyl-L-leucyl)-1,3-thiazolidine-2-thione (**10**) (336 mg, 1 mmol) in THF (10 ml). After the addition of Et_3N (0.15 ml, 1.1 mmol), the mixture was stirred at room temperature for 3 h and submitted to the usual work-up to give compound (**11**) (272 mg, 85%) as needles from AcOEt–hexane, m.p. 156—157 °C (lit.,[7] 153—155 °C); $[\alpha]_D^{22}$ −32.4° (*c* 2.0 in EtOH) {lit.,[7] $[\alpha]_D^{20}$ −34.0° (*c* 3.1 in EtOH)}; $\nu_{max.}$ 3 300, 1 750, 1 655, 1 625, and 1 540 cm^{-1}; δ 0.96 (6 H, d, *J* 6 Hz), 1.22 (3 H, t, *J* 6 Hz), 1.44—1.96 (3 H, m), 3.94 (2 H, m), 4.12 (2 H, q), 4.80 (1 H, m), and 6.84—7.84 (7 H, m) (Found: C, 63.85; H, 7.65; N, 8.8%; M^+, 320. Calc. for $C_{17}H_{24}N_2O_4$: C, 63.75; H, 7.55; N, 8.75%; *M*, 320).

Benzoylation of L-*Arginine*.—A solution of L-arginine (191 mg, 1.1 mmol) in water (3 ml) was added to a yellow solution of 3-benzoyl-1,3-thiazolidine-2-thione (**12**) (223 mg 1 mmol) in THF (3 ml). The mixture was stirred at room temperature until the yellow colour disappeared (4 h) and the solvent was evaporated under reduced pressure to leave an oily residue which was purified on a Sephadex LH-20 column with MeOH to give N^{α}-benzoyl-L-arginine (**13**) (202 mg, 73%) as prisms from AcOEt–hexane, m.p. 298—300 °C (decomp.) [lit.,[10] 298 °C (decomp.)]; $\nu_{max.}$ ($CHCl_3$) 3 350 and 1 630 cm^{-1}; δ (D_2O) 1.40—2.12 (4 H, m), 3.23 (2 H, t, *J* 7 Hz), 4.42 (1 H, t, *J* 7 Hz), and 7.36—7.94 (5 H, m) (Found: C, 55.6; H, 6.55; N, 20.15. Calc. for $C_{13}H_{18}N_4O_3$: C, 56.1; H, 6.5; N, 20.15%).

Benzoylation of L-*Cysteine Methyl Ester*.—A solution of L-cysteine methyl ester hydrochloride monohydrate (773 mg, 4.4 mmol) in EtOH (20 ml) was added to a solution of 3-benzoyl-1,3-thiazolidine-2-thione (**12**) (872 mg, 4 mmol) in THF (5 ml). After the addition of Et_3N (0.62 ml, 4.4 mmol), the mixture was stirred at room temperature under N_2 for 10 h and subjected to the usual work-up to give N-*benzoyl*-L-*cysteine methyl ester* (**14**) (610 mg, 64%) as prisms from $CHCl_3$–hexane, m.p. 63—65 °C; $\nu_{max.}$ 3 400sh, 3 300, 1 730, 1 630, and 1 520 cm^{-1}; δ 1.40 (1 H, br t-like, *J* 9 Hz), 3.12 (2 H, dd, *J* 9 and 4 Hz), 3.80 (3 H, s), 5.06 (1 H, dt, *J* 8 and 3 Hz), 7.06 (1 H, br t, *J* 8 Hz), and 7.24—8.00 (5 H, m) (Found: C, 55.6; H, 5.55; N, 5.85%; M^+, 239. $C_{11}H_{13}NO_3S$ requires C, 55.25; H, 5.5; N, 5.85%; *M*, 239).

Benzoylation of L-*Serine*.—A solution of L-serine (116 mg, 1.1 mmol) in water (5 ml) was added to a yellow solution of compound (**12**) (223 mg, 1 mmol) and Et_3N (0.2 ml, 1.5 mmol) in THF (5 ml). After being stirred at room temperature under N_2 for 18 h, the reaction mixture was treated as usual to afford *N*-benzoyl-L-serine (**15**) (181 mg, 82% yield) as needles from MeOH, m.p. 145—147 °C (lit.,[9] 147—149 °C); $\nu_{max.}$ 3 520, 3 340, 2 950, 1 715, 1 625, and 1 540 cm^{-1}; δ [$(CD_3)_2SO$] 3.74 (2 H, d, *J* 5 Hz), 4.44 (1 H, dt, *J* 8 and 5 Hz), 7.00—7.96 (5 H, m), and 8.32 (1 H, br d, *J* 8 Hz) (Found: C, 57.2; H, 5.25; N, 6.6%; M^+, 209. Calc. for $C_{10}H_{11}NO_4$: C, 57.4; H, 5.3; N, 6.7%; *M*, 209).

Benzoylation of L-*Lysine*.—A solution of L-lysine (160 mg, 1.1 mmol) in water (4 ml) was added to a yellow solution of compound (**12**) (223 mg, 1 mmol) in THF (3 ml). After being stirred at room temperature for 3 min, a precipitate was filtered off and repeatedly washed with water and Et_2O to give N^{ε}-benzoyl-L-lysine (**16**) (163 mg, 65% yield) as crystals, m.p. 240—243 °C (decomp.) [lit.,[10] 240 °C (decomp.)]; $\nu_{max.}$ 3 320, 1 645, and 1 580 cm^{-1}; (Found: C, 62.25; H, 7.3; N, 11.0. Calc. for $C_{13}H_{18}N_2O_3$: C, 62.4; H, 7.25; N, 11.2%). The n.m.r. spectrum of compound (**16**) could not be determined because of its low solubility. Therefore, compound (**16**) was converted into a more soluble derivative (**17**). Thionyl chloride (0.11 ml) was added to a suspension of compound (**16**) (120 mg, 0.48 mmol) in MeOH (1 ml) and the mixture was refluxed and stirred under N_2 for 30 min. The solvent was evaporated under reduced pressure to give an oily residue which was crystallized from MeOH–Et_2O to afford N^{ε}-benzoyl-L-lysine methyl ester hydrochloride (143 mg, 99%). Acetylation of this compound (95 mg) with acetic anhydride (1 ml) and pyridine (3 ml) gave the acetamide (**17**) (84 mg, 87%) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 440, 1 735, 1 660, and 1 520 cm^{-1}; δ 1.08—2.00 (6 H, m), 1.98 (3 H, s), 3.44 (2 H, dt, *J* 8 and 6 Hz), 3.72 (3 H, s), 4.56 (1 H, dt, *J* 6 and 8 Hz), 6.40 (2 H, br m), and 7.28—7.88 (5 H, m) (Found: C, 62.45; H, 7.45; N, 9.1%; M^+, 306. $C_{16}H_{22}N_2O_4$ requires C, 62.7; H, 7.25; N, 9.15%; *M*, 306).

Benzyloxycarbonylation of L-*Histidine Methyl Ester*.—Et_3N (1.2 ml, 8.8 mmol) was added to a suspension of 3-benzyloxycarbonyl-1,3-thiazolidine-2-thione (**18**) (1.01 g, 4 mmol) and L-histidine methyl ester hydrochloride (1.064 g, 4.4 mmol). After being refluxed under N_2 for 8 h, the reaction mixture was treated as usual to afford N^{α}-*benzyloxycarbonyl*-L-*histidine methyl ester* (**19**) (944 mg, 78%) as a pale yellow oil, $\nu_{max.}$ ($CHCl_3$) 3 350, 3 200, 1 720sh, and 1 700 cm^{-1}; δ ($CDCl_3$) 3.06 (2 H, d, *J* 6 Hz), 3.60 (3 H, s), 4.14 (1 H, dt, *J* 8 and 6 Hz), 5.03 (2 H, s), 6.25 (1 H, br s), 6.70 (1 H, s), 7.24 (5 H, s), 7.42 (1 H, s), and 9.77 (1 H, br s) (Found: C, 59.1; H, 5.6; N, 14.0%; M^+, 303. $C_{15}H_{17}N_3O_4$ requires C, 59.4; H, 5.65; N, 13.85%; *M*, 303).

Benzyloxycarbonylation of L-*Lysine Methyl Ester*.—A solution of L-lysine methyl ester dihydrochloride (1.03 g, 4.4 mmol) and Et_3N (0.93 ml, 6.6 mmol) in EtOH (20 ml) was added to a solution of ZTT (**18**)[11] (986 mg, 3.9 mmol) in THF (5 ml). After being stirred at room temperature for 33 h, the reaction mixture was treated as usual to give N^{ε}-*benzyloxycarbonyl*-L-*lysine methyl ester* (**20**) (947 mg, 83%) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 450, 1 715, and 1 515 cm^{-1}; δ 1.16—2.00 (8 H, m), 3.20 (2 H, m), 3.42 (1 H, m), 3.72 (3 H, s), 4.86 (1 H, br s), 5.10 (2 H, s), and 7.32 (5 H, s) (Found: C, 61.5; H, 7.75; N, 9.75%; M^+, 294. $C_{15}H_{22}N_2O$ requires C, 61.2; H, 7.55; N, 9.5%; *M*, 294). Compound (**20**) (935 mg, 3.18 mmol) was subjected to acetylation in the usual manner with acetic anhydride (5 ml) and pyridine (5 ml) to afford N^{α}-*acetyl*-N^{ε}-*benzyloxycarbonyl*-L-*lysine methyl ester* (**21**) (984 mg, 92%) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 420, 1 720, 1 670, and 1 518 cm^{-1}; δ 1.04—1.90 (6 H, m), 2.00 (3 H, s), 3.18 (2 H, m), 3.72 (3 H, s), 4.58 (1 H, dt, *J* 8 and 6 Hz), 4.88 (1 H, br s), 5.10 (2 H, s), 6.20 (1 H, br d, *J* 8 Hz), and 7.32 (5 H, s) (Found: C, 60.35;

H, 7.25; N, 8.35%; M^+, 336. $C_{17}H_{24}N_2O_5$ requires C, 60.7; H, 7.2; N, 8.35%; *M*, 336).

Treatment of L-*Lysine with Homo-bifunctional Reagent* (**23**).—A solution of L-lysine (161 mg, 1.1 mmol) in water (5 ml) was added to a solution of compound (**23**) (160 mg, 0.5 mmol) in THF (5 ml). After being stirred at room temperature for 8 min, the mixture was treated in the usual manner go give *diamide* (**24**) (123 mg, 66%) as fine prisms, m.p. 225 °C (decomp.) $\nu_{max.}$ 3 440, 3 330, 1 635, 1 580, and 1 545 cm^{-1}; δ (D_2O) 1.04—2.08 (12 H, m), 2.54 (4 H, s), 3.23 (4 H, t, *J* 8 Hz), and 3.77 (2 H, t, *J* 6 Hz) (Found: C, 50.25; H, 8.2; N, 14.6. $C_{16}H_{30}N_4O_6 \cdot \frac{1}{2}H_2O$ requires C, 50.1; H, 8.15; N, 14.6%).

Disulphide (**25**).—A solution of 3-mercaptopropionic acid (6.36 g, 60 mmol) in THF (50 ml) was added to a stirred suspension of 2,4-dinitrophenylsulphenyl chloride (11.7 g, 50 mmol) in THF (200 ml) at 0 °C under N_2 during 30 min. The mixture was then stirred at 0 °C for 3.5 h and the solvent was evaporated under reduced pressure to give a yellow oily residue which was kept in a refrigerator overnight to afford crude yellow needles. They were recrystallized from MeOH–$CHCl_3$ to afford pure *compound* (**25**) (12.84 g, 84%) as yellow plates, m.p. 127—129 °C; $\nu_{max.}$ 3 400 and 1 705 cm^{-1}; δ 2.84 (2 H, t-like, *J* 6 Hz), 3.08 (2 H, t-like, *J* 6 Hz), 3.10—4.10 (1 H, br m), 8.56 (2 H, s), and 9.82 (1 H, br s) (Found: C, 35.45; H, 2.65; N, 9.05%; M^+, 304. $C_9H_8N_2O_6S_2$ requires C, 35.55; H, 2.65; N, 9.2%; *M*, 304).

Hetero-bifunctional Reagent (**26**).—DCC (227 mg, 1.1 mmol) was added to an ice-cooled solution of carboxylic acid (**25**) (304 mg, 1 mmol) and 1,3-thiazolidine-2-thione (130 mg, 1.1 mmol) in THF (10 ml) and the mixture was stirred at room temperature overnight. The precipitate (dicyclohexyl urea) was filtered off and the filtrate was evaporated under reduced pressure to give a yellow oily residue which was purified by preparative t.l.c. with $CHCl_3$–acetone (9:1) to afford *compound* (**26**) (248 mg, 61%) as a yellow oil, $\nu_{max.}$ ($CHCl_3$) 1 695, 1 595, and 1 530 cm^{-1}; δ 3.08 (2 H, t, *J* 6 Hz), 3.28 (2 H, t, *J* 8 Hz), 3.62 (2 H, t, *J* 6 Hz), 4.52 (2 H, t, *J* 8 Hz), 8.44 (2 H, br s), and 9.04 (1 H, br s) (Found: C, 36.05; H, 2.8; N, 10.2. $C_{12}H_{11}N_3O_5S_4$ requires C, 35.55; H, 2.75; N, 10.35%).

Treatment of L-*Cysteine Derivative* (**14**) *and* L-*Lysine Derivative* (**27**) *with Hetero-bifunctional Reagent* (**26**).—A solution of N^{α}-acetyl-L-lysine methyl ester hydrobromide (**27**) (170 mg, 0.6 mmol) in EtOH (2 ml) and a solution of Et_3N (0.15 ml, 1 mmol) in $CHCl_3$ (2 ml) were added to a solution of N^{α}-benzoyl-L-cysteine methyl ester (**14**) (143 mg, 0.6 mmol) in $CHCl_3$ (5 ml). After the addition of a solution of compound (**26**) (203 mg, 0.5 mmol) in THF (5 ml), the mixture was stirred at room temperature under N_2 for 10 min. Then the solvent was evaporated under reduced pressure to give an oily residue which was dissolved in excess of AcOEt. This organic solution was washed in turn with 5% HCl and brine, dried, and evaporated under reduced pressure to give an oily residue which was purified by Sephadex LH20 column chromatography with MeOH and then by preparative t.l.c. with $CHCl_3$–MeOH (95:5) to afford *compound* (**28**) (118 mg, 45%) as an oil, $\nu_{max.}$ ($CHCl_3$) 3 430, 1 735, 1 660, and 1 520 cm^{-1}; δ 1.04—1.92 (6 H, m), 1.98 (3 H, s), 2.56 (2 H, t, *J* 7 Hz), 2.80—3.40 (6 H, m), 3.67 (3 H, s), 3.75 (3 H, s), 4.52 (1 H, q, *J* 7 Hz), 5.06 (1 H, q, *J* 7 Hz), 6.60 (2 H, br d, *J* 7 Hz), and 7.24—7.96 (6 H, m) (Found: C, 44.9; H, 5.45; N, 6.55. $C_{23}H_{33}N_3O_7S_2 \cdot CHCl_3$ requires C, 44.55; H, 5.3; N, 6.5%).

Acknowledgements

We thank Mr. M. Yagi (for some technical assistance), Mrs. K. Ohmine (for n.m.r. spectra), Mrs. T. Terada (for mass spectra), and Miss T. Kishimoto (for elemental analysis) of our Institute. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 56430035) from the Ministry of Education, Science and Culture in Japan.

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Received 19*th March* 1984; *Paper* 4/429