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The Reaction of S-Aminomethylthiamine with Acid Anhydride —The Synthesis of O,S-Bis(α-aminoacyl)thiamine—

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It was found that the reaction of S-aminomethylthiamine (II) with acid anhydride afforded the corresponding S-acylthiamine (III) or, O,S-diacylthiamine (IV). Furthermore the reaction of II with anhydrides (VII, IX, XII) of N-benzyloxycarbonyl- or N-*t*-butyloxycarbonyl-L-amino acid in tetrahydrofuran at −5—5° gave successfully the novel thiamine compounds with amino acid residues: O,S-bis(α-N-benzyloxycarbonylaminoacyl)thiamine (VIII) or O,S-bis(α-N-*t*-butyloxycarbonylaminoacyl)thiamine (X). O,S-Bis(α-aminoacyl)thiamine trihydrochloride (XI·3HCl) was obtained in good yield by the treatment of X with hydrogen chloride in ethyl acetate at −10°.

Thiamine activities of VIII·HCl and XI·3HCl in thiamine deficient rats were similar to those of equimolar thiamine chloride hydrochloride (I-Cl·HCl).

In the preceding paper,[2] the synthesis of S-aminomethylthiamine (II) by the reaction of an alkaline solution of thiamine chloride hydrochloride (I-Cl·HCl) with a solution of chloromethyldialkylamine in chloroform has been reported. The compound (II) is soluble in organic solvents such as chloroform, tetrahydrofuran, dioxane, acetone and ethanol. This characteristic property of II will favourably serve to carry out the reaction of II with a mixed anhydride which is unstable in an aqueous solvent.

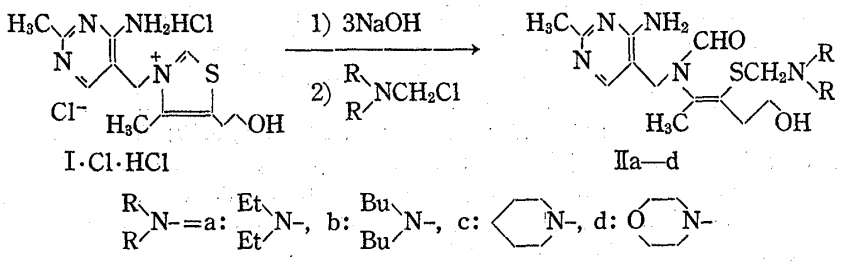

Chart 1

A number of O,S-diacylthiamine derivatives has been reported by several authors,[3] and these compounds are usually synthesized by the treatment of acyl halide or acid anhydride with thiamine (I) in an aqueous alkaline solution. However, there has been no report on the synthesis of O,S-bis(α-aminoacyl)thiamine derivatives in which carbonyl group of α-amino acid couples with both SH and OH groups of thiol type of I. We are interested in the property and the pharmaceutical action of such new thiamine derivatives.

This paper describes the reaction of II with acid anhydride such as benzoic anhydride, butyric anhydride, L-amino acid mixed anhydride and L-amino acid anhydride in tetrahydrofuran (THF).

In order to examine the reactivity of II, the reactions of II with benzoic anhydride and butyric anhydride in THF were preliminarily examined, though Böhme, *et al.*[4] had reported

1) Location: *16-89, Kashima-3-chome, Yodogawa-ku, Osaka, 532, Japan.*
2) H. Yasuo, *Chem. Pharm. Bull.* (Tokyo), **24**, 845 (1976).
3) R. Takata (ed.), "Vitamin-Gakuno-Shinpo," Vol. 1, The Vitamin Society of Japan, 1959, p. 45; R. Takata (ed.), "Shin-Vitamin-Gaku," The Vitamin Society of Japan, 1969, p. 131.
4) H. Böhme and G. Lerche, *Chem. Ber.*, **100**, 2125 (1967).

the reaction of dialkylaminomethylalkyl sulfide with acyl halide. Compound (II) containes two reactive functional groups; hydroxy and amino groups, besides N,S-acetal group. To investigate the reactivities of the active functional groups that react readily with acid anhydride, the reaction of S-diethylaminomethylthiamine (IIa) with an equimolar amount of benzoic anhydride was carried out in THF at 5—10° for 3 hr, although the starting material (IIa) was still remained on thin-layer chromatography (TLC). From the reaction mixture, S-benzoyl thiamine (III: R′=C_6H_5) was obtained in 25.0% yield and O,S-dibenzoylthiamine (IV: R′=C_6H_5) was yielded in only 2.6%. When the reaction time was prolonged to 17 hr, the yield of III increased up to 55.0%. The treatment of 2.2 molar ratio of benzoic anhydride with IIa gave IV in 49.0% yield and a trace amount of III was only detected on TLC. Diethylbenzamide (VI), which was formed by elimination[5] of formalin from diethylaminomethyl benzoate (V), was obtained as a by-product in these reactions. Similar results were obtained by the reaction of IIa with butyric anhydride. When IIc was used instead of IIa, the yield of III was slightly lower. Results were summarized in Table I.

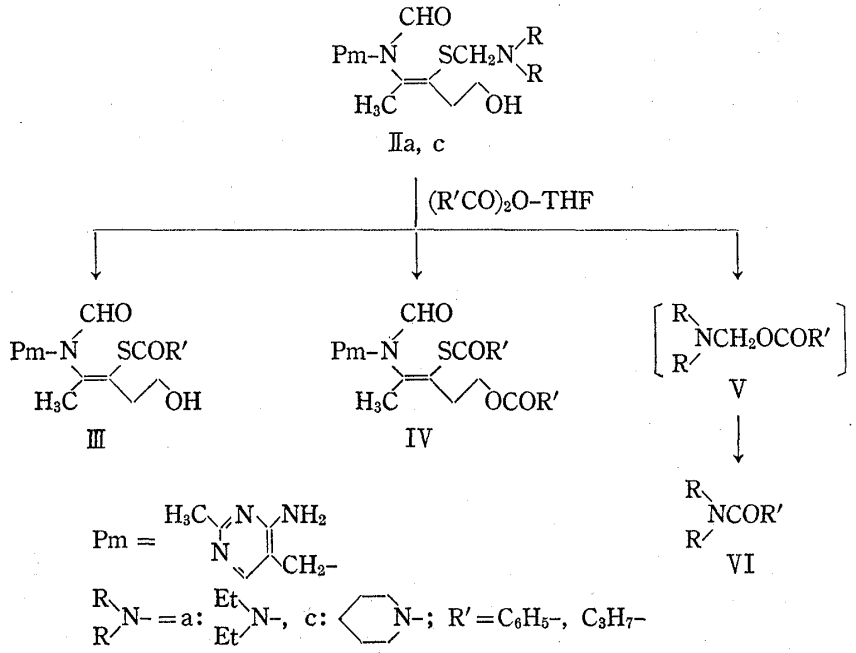

Chart 2

Table I. Reaction of II with $(R'CO)_2O$ in THF at 5—10°

II	R′	Mol. ratio of $(R'CO)_2O$	Reaction Time (hr)	Yield (%) III	IV	VI
a	Ph	1.0	3.0	25.0	2.6	23.0
a	Ph	1.0	17.0	58.0	3.0	28.2
a	Ph	2.2	17.0	trace	49.0	30.0
a	Pr	1.0	17.0	53.0	trace	23.2
c	Pr	1.0	17.0	32.4	trace	40.0

Ph=phenyl, Pr=propyl

From the above results, it was found that the reaction of II with an equimolar amount of acid anhydride in THF afforded S-acylthiamine (III) predominantly, showing that N,S-

5) H. Böhme, J. Bohn, E. Köhler, and J. Roehr, *Ann. Chem.*, **664**, 130 (1963).

acetal group is the most reactive of all the functional groups. O,S-Diacylthiamine (IV) was then obtained by further addition of the anhydride.

In the light of the above findings, the reaction of IIa with L-amino acid anhydrides was investigated to obtain O,S-bis(α-aminoacyl)thiamine. As regards the reaction of I with amino acid and amino acid derivatives of I, several reports has appeared.[6,7]

L-Amino acids used in this report were alanine, valine, proline, phenylalanine and tryptophan with the N-protecting groups such as benzyloxycarbonyl and *t*-butyloxycarbonyl.

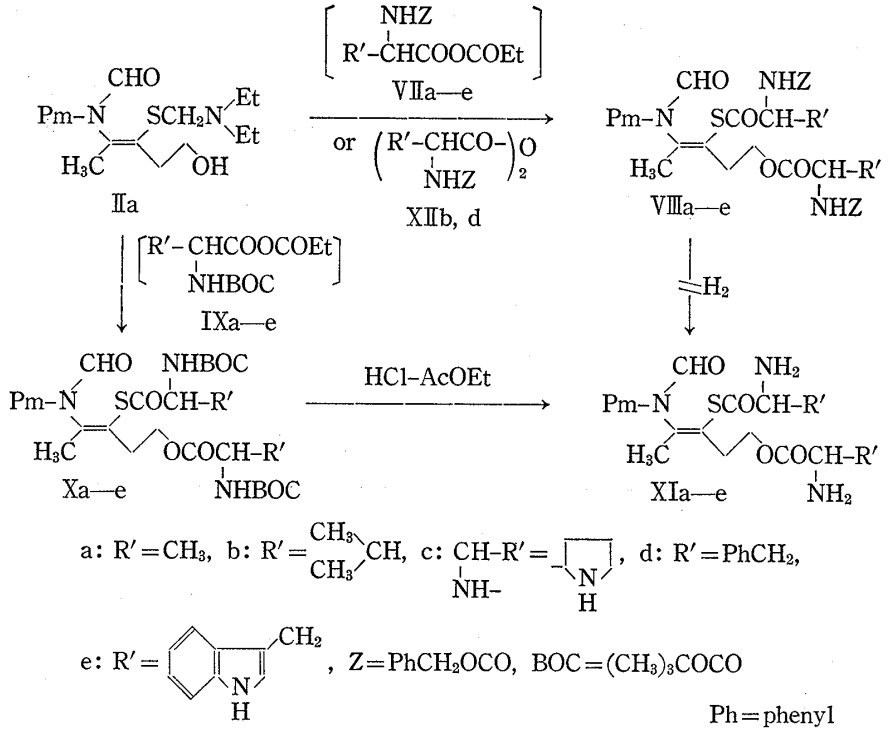

Chart 3

The mixed anhydride (VII) of N-benzyloxycarbonylamino acid was prepared by treatment with ethyl chloroformate in THF in the presence of triethylamine in the usual way.[8] The reaction of IIa with the above mixed anhydride (VII) was carried out in THF at −5—0° overnight and the reaction mixture was washed with cold sodium bicarbonate solution and cold dilute hydrochloric acid to obtain O,S-bis(α-N-benzyloxycarbonylaminoacyl)thiamine (VIII). The corresponding hydrochloride (VIII·HCl) of VIII was prepared by treatment with ethanolic hydrogen chloride and purified by reprecipitation from ethanol–ether as a colourless powder.

Compounds (VIIIb, d) were also obtained by the reactions of IIa with N-benzyloxycarbonylvaline anhydride (XIIb) and N-benzyloxycarbonylphenylalanine anhydride (XIId), which were prepared from the corresponding N-benzyloxycarbonylamino acid and dicyclohexylcarbodiimide.[9] Unfortunately reductive decarbobenzyloxylation of VIII in the presence of Pd-C catalyst did not proceed at all. Yields, physical constants and analytical data of VIII–HCl were shown in Table II.

6) C. Kawasaki, Y. Ito, T. Miyahara, and H. Yokoyama, *Vitamins*(Japan), **35**, 170 (1967); G. Kurata, T. Sakai, T. Miyahara, and H. Yokoyama, *ibid.*, **35**, 136 (1967).
7) M. Nagawa, Y. Baba, and T. Yoshioka, *Takamine Kenkyusho Nempo*, **13**, 31 (1961); V.M. Tursin and T.I. Kanina, *Z. Org. Khim.*, **7**, 2621 (1971); [*Chem. Abstr.*, **76**, 86114*u* (1972)].
8) R.A. Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951).
9) I. Muramatsu, *Nippon Kagaku Zasshi*, **82**, 83 (1961); H. Schüssler and H. Zahn, *Chem. Ber.*, **95**, 1076 (1962).

Table II. Yields, Physical Constants and Analytical Data of VIII·HCl

VIII HCl	Yield (%) Method A[a]	Yield (%) Method B[b]	mp (°C) (decomp.)	$[\alpha]_D^{25}$[c]	Formula	Analysis (%) Calcd. (Found) C	H	N	S
a	55.8	—	95—97	−30.9	$C_{34}H_{41}O_8N_6SCl\cdot 1/2H_2O$	55.31 (55.34)	5.73 (5.79)	11.38 (11.27)	4.34 (4.31)
b	66.7	27.0	92—94	−25.0	$C_{38}H_{49}O_8N_6Cl\cdot H_2O$	56.81 (56.67)	6.27 (6.25)	10.46 (10.52)	3.99 (3.61)
c	54.0	—	94—96	−3.7	$C_{38}H_{45}O_8N_6SCl\cdot 2H_2O$	55.83 (56.17)	6.04 (6.03)	10.28 (10.76)	3.92 (3.46)
d	85.0	45.0	90—92	−32.5	$C_{46}H_{49}O_8N_6SCl\cdot H_2O$	61.42 (61.42)	5.60 (5.65)	9.34 (9.53)	3.56 (3.66)
e	62.0	—	125—127	−21.0	$C_{50}H_{51}O_8N_8SCl\cdot H_2O$	61.43 (60.98)	5.46 (5.51)	11.46 (11.73)	3.28 (3.37)

a) mixed anhydride method
b) anhydride method
c) c=1, EtOH

The reaction of IIa with N-*t*-butyloxycarbonylamino acid mixed anhydride (IX), in which the protecting group can be easily removed by treatment with acid, was carried out similarly. The mixed anhydrides (IX) freshly prepared in the usual way[8] were allowed to react with IIa in THF at −5—0°. After standing overnight, the reaction mixture was washed with cold sodium bicarbonate solution and cold dilute acetic acid to obtain O,S-bis(α-N-*t*-butyloxycarbonylaminoacyl)thiamine (X) as an amorphous powder. By treatment with hydrogen chloride in ethyl acetate[10] at about −10°, the protecting group was removed. A precipitated O,S-bis(α-aminoacyl)thiamine trihydrochloride (XI·3HCl) was purified by reprecipitation from ethanol–ether as a colourless powder. Yields, physical constants and analytical data of XI·3HCl were summarized in Table III.

Table III. Yields, Physical Constants and Analytical Data of XI·3HCl

XI 3HCl	Yield (%)	mp (°C) (decomp.)	$[\alpha]_D^{25}$[a]	PPC *Rf*	Formula	Analysis (%) Calcd. (Found) C	H	N	S
a	45.0	153—155	−49.4	0.23	$C_{18}H_{31}O_4N_6SCl_3\cdot H_2O$	39.17 (39.47)	6.03 (5.92)	15.23 (14.83)	5.81 (5.63)
b	41.4	150—153	−33.0	0.48	$C_{22}H_{39}O_4N_6SCl_3\cdot H_2O$	43.45 (43.33)	6.79 (6.66)	13.82 (13.66)	5.27 (5.20)
c	69.4	138—140	−18.8	0.24	$C_{22}H_{35}O_4N_6SCl_3\cdot H_2O$	43.74 (43.35)	5.67 (5.87)	12.91 (12.73)	5.30 (4.88)
d	64.5	164—166	+58.0	0.47	$C_{30}H_{39}O_4N_6SCl_3\cdot 2H_2O$	49.89 (49.81)	5.58 (5.80)	11.63 (12.02)	4.44 (4.72)
e	71.3	188—190	+64.7	0.44	$C_{34}H_{41}O_4N_8SCl_3\cdot H_2O$	52.20 (52.58)	5.28 (4.93)	14.32 (13.97)	4.09 (4.13)

a) c=1, EtOH

The thiamine activities of these new thiamine derivatives, VIII·HCl and XI·3HCl, were examined on the recovery from thiamine depletion in rats. Male Wistar strain rats, 5 weeks old, were fed *ad libitum* on a thiamine-free diet for approximately 2 weeks, until they

10) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **43**, 1910 (1960).

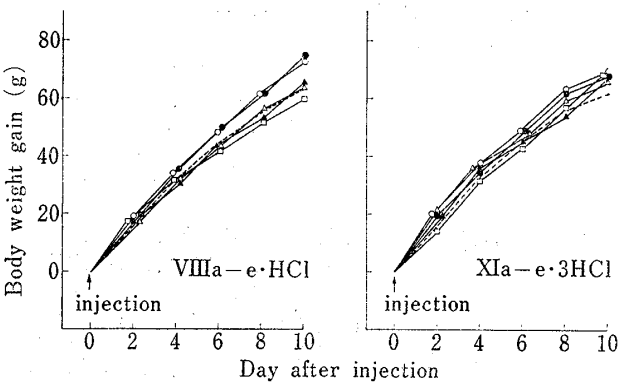

Fig. 1. Thiamine Activities of O,S-Bis(α-N-benzyloxycarbonylaminoacyl)thiamine Hydrochloride (VIII·HCl) and O,S-Bis(α-aminoacyl)-thiamine Trihydrochloride (XI·3HCl) in Thiamine-Deficient Rats

--------: I-Cl·HCl, —●—: a, —○—: b, —▲—: c, —□—: d, —△—: e

were sufficiently depleted of thiamine as recognized by the cessation of growth and the decrease of body weight. The effects of single injection of I-Cl·HCl (3 mg/rat sc) and an equimolar amount of thiamine derivatives on body weight gain were observed for 10 days. From this experiment, it was suggested that all of them exerted the thiamine activity to be approximately equivalent to I-Cl·HCl, as shown in Fig. 1.

Experimental[11]

General Procedure for the Reaction of S-Aminomethylthiamine (IIa,c) with Acid Anhydride——To a stirred solution of anhydride (0.01 mole) in THF (40 ml) was added a solution of S-diethylamino compound (IIa) or S-piperidinomethyl compound (IIc) (0.01 mole) in THF (60 ml) at 5—10°. After stirring was continued for 3 hr or 17 hr[12] at the same temperature, the reaction mixture was evaporated under reduced pressure. The resulting oil was washed with petr. ether to remove the amide (VI). The residue was dissolved in AcOEt and washed with cold $NaHCO_3$ solution, H_2O and then dried over anhyd. Na_2SO_4. Evaporation of the solvent afforded S-acylthiamine (III) and/or O,S-diacylthiamine (IV), which were identical with authentic samples.

General Procedure for the Preparation of O,S-Bis(α-N-benzyloxycarbonylaminoacyl)thiamine (VIIIa—e) from IIa and Mixed Anhydrides (VIIa—e) of N-Benzyloxycarbonyl-L-amino Acid: Method A——To a stirred solution of N-benzyloxycarbonyl-L-amino acid (0.027 mole) and Et_3N (0.03 mole) in THF (70 ml) was added $ClCOOC_2H_5$ (0.025 mole) in THF (20 ml) at −15——10° during 15 min and stirring was continued further 15 min at the same temperature. A solution of IIa (0.01 mole) in THF (100 ml) was added to the suspension of above mixed anhydrides (VIIa—e) at −5—0° and the reaction mixture was stirred overnight at the same temperature. The precipitated Et_3N·HCl was filtered off, and the filtrate was evaporated *in vacuo*. The resulting oil was dissolved in AcOEt and washed with cold $NaHCO_3$ solution, 3% HCl and then dried over anhyd. Na_2SO_4. The solvent was removed under reduced pressure and the residual oil was washed with petr. ether to give an amorphous powder of VIIIa—e. The corresponding hydrochlorides of VIIIa—e were prepared by the treatment with 40% ethanolic hydrogen chloride at −10——5° and purified by reprecipitation from EtOH–ether. Yields, physical constants and analytical data of VIII·HCl were shown in Table II. The IR and NMR spectral data were listed in Table IV.

General Procedure for the Reaction of IIa with N-Benzyloxycarbonyl-L-amino Acid Anhydrides (XIIb,d): Method B——To a stirred solution of XIIb,d (0.013 mole) in THF (70 ml) was added IIa (0.006 mole) in THF (70 ml) at 0—5°. The reaction mixture was stirred overnight at the same temperature and evaporated. The resulting oil was dissolved in AcOEt and washed with cold $NaHCO_3$ solution, 3% HCl, dried over anhyd. Na_2SO_4 and evaporated. The residual oil was washed with petr. ether to give an amorphous powder of VIIIb, d. The corresponding hydrochlorides of VIIIb,d were prepared by the treatment of ethanolic hydrogen chloride at −10——5° in EtOH. The results were shown in Table II.

General Procedure for the Preparation of O,S-Bis(α-N-*t*-butyloxycarbonylaminoacyl)thiamine (Xa—e) and O,S-Bis(α-aminoacyl)thiamine (XIa—e) from IIa and Mixed Anhydrides (IXa—e) of N-*t*-Butyloxycarbonyl-

11) All melting points were determined in capillaries and uncorrected. Nuclear magnetic resonance (NMR) spectra were taken on a Hitachi Perkin–Elmer R-20A spectrometer in $CDCl_3$ solution with tetramethylsilane (TMS) and in D_2O solution with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. Chemical shifts are given in δ (ppm) values. Abbreviations used are s=singlet, d=doublet, t=triplet, m=multiplet, and b=broad. Infrared (IR) spectra were taken in nujol mull on a Shimadzu IR-27G spectrometer. Optical rotations were measured on a Perkin–Elmer Model 141 automatic polarimeter in 1 dm tubes. Ultraviolet (UV) spectra were taken on a Hitachi EPS-3T spectrophotometer in EtOH. Paper chromatography was performed by the ascending techniques on Toyo filter paper No. 51A in the following solvent system, *n*-BuOH–AcOH–pyridine–H_2O (4: 1: 1: 2). Thin-layer chromatography was carried out on silica gel (Kieselgel G nach Stahl, Merck) in the following solvent systems, $CHCl_3$–EtOH (10: 1) and $CHCl_3$–AcOH–MeOH (85: 3: 15).

12) The end point of the reaction was detected on TLC.

Table IV. Spectral Data of VIII

VIII	IR ν_{max}^{Nujol} cm^{-1}	NMR ($CDCl_3$) ppm
a	1710 1650	1.26, 1.38 (6H, d×2, $CH\underline{CH_3}$×2), 2.05 (3H, s, C=C-CH_3), 2.40 (3H, s, Pm[a]-C_2-CH_3), 5.12 (4H, b, s, $O\underline{CH_2}Ph$[b]×2), 6.03 (2H, b, t, NHZ[c]×2), 6.78 (2H, b, Pm-C_4-NH_2), 7.78 (1H, s, Pm-C_6-H), 7.88 (1H, s, NCHO)
b	1720 1660	0.8—1.0 [12H, m, $CH(\underline{CH_3})_2$×2], 2.0 (3H, s, C=C-CH_3), 2.42 (3H, s, Pm-C_2-CH_3), 4.0—4.4 [6H, m, $\underline{CH}CH(CH_3)_2$×2, Pm-C_5-CH_2, $CH_2\underline{CH_2}OCO$], 5.09, 5.18 (4H, s×2, OCH_2Ph), 5.50 (2H, b, $N\underline{H}Z$×2), 6.20 (2H, b, Pm-C_4-NH_2), 7.80 (1H, s, Pm-C_6-H), 7.82 (1H, s, NCHO)
c	1720 1660	1.8—2.2 (11H, m, Pyrro[c]-C_3,C_4-CH_2×2, C=C-CH_3), 2.45 (3H, s, Pm-C_2-CH_3), 3.3—3.7 (4H, m, Pyrro-C_5-CH_2×2), 6.10 (2H, b, Pm-C_4-NH_2), 5.10, 5.15 (4H, s×2, $O\underline{CH_2}Ph$), 7.30 (s, Ph×2), 7.78 (1H, s, Pm-C_6-H), 7.85 (1H, s, NCHO)
d	1720 1660	1.98 (3H, s, C=C-CH_3), 2.42 (3H, s, Pm-C_2-CH_3), 3.05 (4H, b, d, $CH\underline{CH_2}Ph$×2), 5.05, 5.09 (4H, s×2, OCH_2 Ph×2), 5.60 (2H, b, t, $N\underline{H}Z$×2), 5.90 (2H, b, Pm-C_4-NH_2), 7.1—7.3 (20H, m, Ph×4), 7.78 (1H, s, Pm-C_6-H), 7.82 (1H, s, NCHO)
e	1710 1650	1.80 (3H, s, C=C-CH_3), 2.40 (3H, s, Pm-C_2-CH_3), 2.2—2.7 (2H, m, $\underline{CH_2}CH_2OCO$), 3.25 (4H, b, Ind[e]-C_3-CH_2), 4.4—4.8 (4H, m, $CO\underline{CH}NHZ$×2, Pm-C_5-$\underline{CH_2}$), 5.08, 5.10 (4H, s×2, OCH_2Ph×2), 5.75 (2H, b, $N\underline{H}Z$×2), 6.08 (2H, b, Pm-C_4-NH_2), 6.8—7.5 (10H, m, aromatic.), 7.65 (1H, s, Pm-C_6-H), 7.73 (1H, s, NCHO), 8.85 (2H, b, Ind-NH×2)

a) Pm=pyrimidine ring
b) Ph=phenyl
c) Z=benzyloxycarbonyl
d) Pyrro=pyrrolidine ring
e) Ind=indole ring

Table V. Spectral Data of XI·3HCl

XI 3HCl	UV λ_{max}^{EtOH} nm (log ε)	IR ν_{max}^{Nujol} cm^{-1}	NMR (D_2O) ppm
a	246(4.08)	1740 1660	1.58 (6H, d, $CH\underline{CH_3}$×2), 2.28 (3H, s, C=C-CH_3), 2.61 (3H, s, Pm[a]-C_2-CH_3), 2.9 (2H, t, $\underline{CH_2}CH_2OCO$), 4.2—4.5 (6H, m, $CH_2\underline{CH_2}OCO$, $\underline{CH}CH_3$×2, Pm-C_5-CH_2), 7.96 (1H, s, Pm-C_6-H), 8.10 (1H, s, NCHO)
b	246(4.10)	1740 1660	0.95, 1.05 [12H, d×2, $CH(\underline{CH_3})_2$×2], 2.29 (3H, s, C=C-CH_3), 2.61 (3H, s, Pm-C_2-CH_3), 2.2—2.4 [2H, m, $CH(CH_3)_2$], 2.92 (2H, t, $C\underline{H_2}CH_2OCO$), 4.0—4.5 [6H, m, $\underline{CH}CH(CH_3)_2$×2, $CH_2\underline{CH_2}OCO$, Pm-C_5-CH_2], 8.0 (1H, s, Pm-C_6-H), 8.13 (1H, NCHO)
c	246(4.09)	1740 1660	1.9—2.3 (8H, m, Pyrro[b]-C_3,C_4-CH_2×2), 2.28 (3H, s, C=C-CH_3), 2.60 (3H, s, Pm-C_2-CH_3), 2.90 (2H, t, $\underline{CH_2}CH_2OCO$), 2.3—2.6 (4H, m, Pyrro-C_5-CH_2×2), 4.38 (2H, t, $CH_2\underline{CH_2}OCO$), 7.98 (1H, s, Pm-C_6-H), 8.10 (1H, s, NCHO)
d	246(4.17)	1750 1660	2.18 (3H, s, C=C-CH_3), 2.48 (3H, s, Pm-C_2-CH_3), 3.24 (4H, d, $CH\underline{CH_2}Ph$[c]×2), 4.1—4.6 (6H, m, $CH_2\underline{CH_2}OCO$, $\underline{CH}CH_2Ph$×2, Pm-C_5-CH_2), 7.40 (10H, s, Ph×2), 7.90 (1H, s, Pm-C_6-H), 8.02 (1H, s, NCHO)
e	245(4.43)	1740 1655	2.18 (3H, s, C=C-CH_3), 2.45 (3H, s, Pm-C_2-CH_3), 3.3—3.5 (4H, m, Ind[d]-C_3-CH_2×2), 4.0—4.5 (6H, m, $CH_2\underline{CH}$×2, $CH_2\underline{CH_2}OCO$, Pm-C_5-CH_2), 6.9—7.7 (10H, m, aromatic.), 7.85 (2H, Pm-C_6-H, NCHO)

a) Pm=pyrimidine ring
b) Pyrro=pyrrolidine ring
c) Ph=phenyl
d) Ind=indole ring

L-amino Acid——The mixed anhydrides (IXa—e) were prepared from N-*t*-butyloxycarbonyl-L-amino acid (0.027 mole), $ClCOOC_2H_5$ (0.025 mole) and Et_3N (0.03 mole) in THF (90 ml) as described above. A solution of IIa (0.01 mole) in THF (100 ml) was added to the above mixed anhydrides (IXa—e) at −5°—0° and the reaction mixture was stirred overnight at the same temperature. The precipitated Et_3N·HCl was filtered off and the filtrate was evaporated *in vacuo*. The resulting oil was dissolved in AcOEt and washed with cold $NaHCO_3$ solution, 3% AcOH and then dried over anhyd. Na_2SO_4. The solvent was removed under reduced pressure and the residual oil was washed with petr. ether to give an amorphous powder of Xa—e.

O,S-Bis(α-aminoacyl)thiamine·3HCl (XIa—e·3HCl) were obtained from Xa—e almost quantitatively by the treatment of HCl–AcOEt at −15——10° for 4 hr, and purified by reprecipitation from EtOH–ether or MeOH–ether. Yields, physical constants, analytical data and spectral data of XIa—e·3HCl were listed in Table III and V.

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