Notes

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Amino Acids and Peptides. XI.[1,2] Synthesis of Attractant and Repellent Peptides for *Aedes aegypti* and *Blattella germanica*

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Z–Gly–Val–Ser–Phe–Val–Leu–OMe and related peptides were synthesized by the conventional solution method and their attractant and repellent activities for *Aedes aegypti* (mosquito) and *Blattella germanica* (cockroach) were examined. Z–Val–Leu–OMe exhibited potent repellent activity against not only *Aedes aegypti* but also *Blattella germanica.*

Keywords——peptide; chemical synthesis; attractant activity; repellent activity; *Aedes aegypti* (mosquito); *Blattella germanica* (cockroach)

In 1974, Sholudchrnko *et al.*,[3] synthesized a protected hexapeptide, Z–Gly–Val–Ser–D,L-Phe–Val–Leu–OMe, and reported that the hexapeptide was a repellant and the related peptides, Z–D,L-Phe–Val–Leu–OMe and Z–Gly–Val–Ser–OMe, were attractants for the mosquito (*Aedes aegypti*).

In order to study the relationship between the structure of peptides and attractant and repellent activities against not only *Aedes aegypti* (mosquito) but also *Blattela germanica* (cockroach), we synthesized the protected hexapeptide[3] and related peptides and examined their attractant and repellent activities. As illustrated in Fig. 1 (R=Me), two kinds of stereoisomeric hexapeptides were prepared using L and D-phenylalanine; Sholudchrnko *et al.*, had used D,L-phenylalanine to prepare the peptide. Our synthetic route was different from that described previously.[3] Z–Val–OH and H–Leu–OMe were coupled by the DCC method to give Z–Val–Leu–OMe (**1**).[4] After removal of the Z group by catalytic hydrogenation, Z–Phe–OH or Z–D-Phe–OH[5] was coupled with DCC to afford Z–Phe–Val–Leu–OMe (**3**) or Z–D-Phe–Val–Leu–OMe (**4**), respectively. Z–Val–OH and H–Ser–OMe were coupled by the ONp active ester method to give Z–Val–Ser–OMe (**2**).[6] After removal of the Z group of **2**, Z–Gly–OH was coupled with the corresponding dipeptide ester by the active ester method to give Z–Gly–Val–Ser–OMe (**5**), which was converted to the corresponding hydrazide (**6**). The tripeptide azide prepared from **6** was combined with H–Phe–Val–Leu–OMe or H–D-Phe–Val–Leu–OMe to yield Z–Gly–Val–Ser–Phe–Val–Leu–OMe (**7**) or Z–Gly–Val–Ser–D-Phe–Val–Leu–OMe (**8**), respectively. The activity of the synthetic peptides against *Aedes aegypti* and *Blattella germanica* is summarized in Table I. The dipeptide **1**, which consisted of hydrophobic amino acids (Val and Leu), exhibited fairly potent repellent activity against *Aedes aegypti* and *Blattella germanica* (72.7 and 64%, respectively). Introduction of a Phe or D-Phe residue into the peptide **1** gave a tripeptide, **3** or **4**, and decreased the repellent activity against *B. germanica*. Compound **3** exhibited attractant activity for *A. aegypti*, in accordance

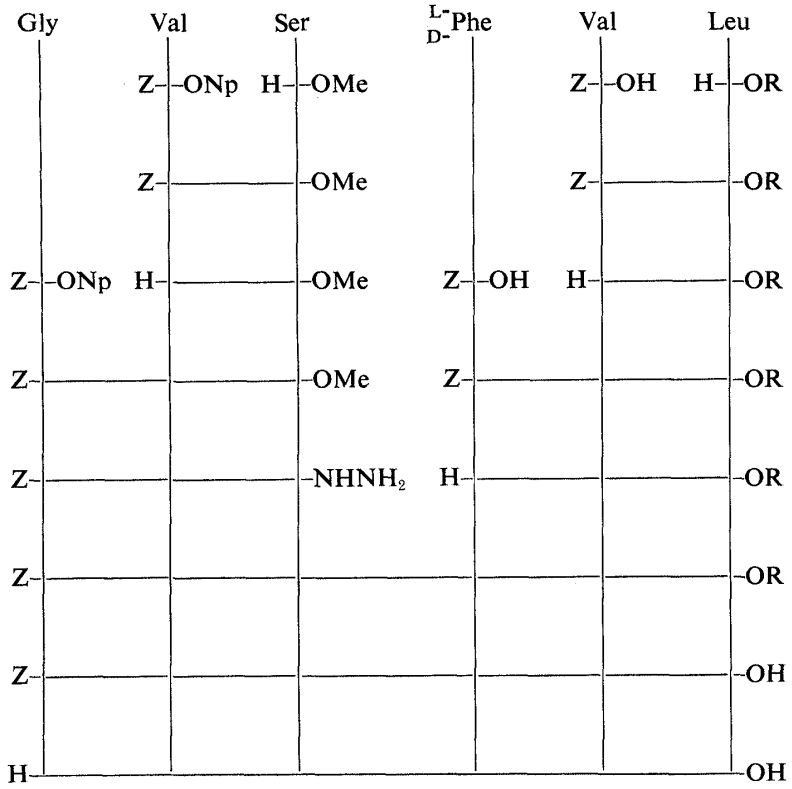

Fig. 1. Synthetic Scheme for Hexapeptides

R = Me; *tert*-Bu.

TABLE I. Activities of Synthetic Peptides towards *Aedes aegypti* and *Blattella germanica*

No.	Compound	*Blattella germanica* Repellent activity (%)	*Aedes aegypti* Attractant activity (%)	*Aedes aegypti* Repellent activity (%)
1	Z–Val–Leu–OMe	64	15	72.7
2	Z–Val–Ser–OMe	6	45	18.2
3	Z–Phe–Val–Leu–OMe	5	65	n.d.[b]
4	Z–D-Phe–Val–Leu–OMe	13.1	n.d.	n.d.
5	Z–Gly–Val–Ser–OMe	0	40	27.3
6	Z–Gly–Val–Ser–$NHNH_2$	−11.1	n.d.	n.d.
7	Z–Gly–Val–Ser–Phe–Val–Leu–OMe	0	25	54.3
8	Z–Gly–Val–Ser–D-Phe–Val–Leu–OMe	24.9	n.d.	n.d.
	DETA[a]	77—85.5	—	100
	Control (EtOH)	—	55	—

a) *N*, *N*-Diethyl-*m*-toluylamide. *b*) Not determined.

with the finding of Sholudchrnko *et al.*[3] Although they reported that Z–Gly–Val–Ser–OMe (**5**), which they obtained as an oily material, exhibited attractant activity for *A. aegypti*, our compound **5** (mp 136—141 °C) did not show any attractant activity. The reason for this discrepancy is unclear. They also reported that Z–Gly–Val–Ser–D,L-Phe–Val–Leu–OMe exhibited repellent activity against *A. aegypti*.[3] Of the two stereoisomers **7** and **8**, Z–Gly–Val–Ser–Phe–Val–Leu–OMe (**7**) exhibited significant repellent activity (54%) against *A. aegypti*

Table II. Activities of Synthetic Peptides towards *Blattella germanica*

No.	Compound	Repellent activity (%)
9	Z–Val–Leu–OBu^t	34
10	Z–Phe–Val–Leu–OBu^t	0
11	Z–D-Phe–Val–Leu–OBu^t	16.2
12	Z–Gly–Val–Ser–Phe–Val–Leu–OBu^t	5.8
13	Z–Gly–Val–Ser–D-Phe–Val–Leu–OBu^t	−5.7
14	Z–Gly–Val–Ser–Phe–Val–Leu–OH	26.0
15	Z–Gly–Val–Ser–D-Phe–Val–Leu–OH	−2.9
16	H–Gly–Val–Ser–Phe–Val–Leu–OH	47.6
	DETA	77—85.5

Table III. Activities of Synthetic Peptides towards *Blattella germanica*

No.	Compound	Repellent activity (%)
21	Z–Val–Leu-ol	10.7
22	Z–Val–Leu–OH	7.3
1	Z–Val–Leu–OMe	64
23	Z–Val–Leu–OEt	47.4
9	Z–Val–Leu–OBu^t	34
24	Z–Val–Leu–OBzl	25.4
25	Z–Val–Leu–NH_2	37.4
26	Z–Val–Leu–$NHCH_3$	18.7
27	Z–Val–Leu–NHC_2H_5	21.0
28	Z–Val–Leu–NHC_2H_4OH	25.9
29	Z–Val–Leu–$NHNH_2$	34.5
30	Z–Val–Ile–OMe	58.6
31	H–Leu–OEt	38.9
32	H–Leu–OBzl·Tos–OH	6.9
17	Z–Leu–NH_2	17.4
18	Z–Leu–$NHCH_3$	22.0
19	Z–Leu–NHC_2H_5	13.5
20	Z–Leu–NHC_2H_4OH	−3.3
	DETA	85.5—98

but it did not show any repellent activity against *B. germanica*. However, Z–Gly–Val–Ser–D-Phe–Val–Leu–OMe (**8**) exhibited slight repellent activity against *B. germanica*. It is clear that the responses of *A. aegypti* and *B. germanica* to various peptides are quite different.

In order to examine the repellent and attractant activities of deblocked hexapeptides, we attempted to saponify Z–L- or D-Phe–Val–Leu–OMe (**3** or **4**). However, the saponification rate was very slow. So, according to the scheme shown in Fig. 1 (R=Bu^t), the deblocked hexapeptides were prepared starting with H–Leu–OBu^t.[7] The repellent activity of the obtained peptides against *B. germanica* is summarized in Table II. The deblocked hexapeptide, H–Gly–Val–Ser–Phe–Val–Leu–OH (**16**) exhibited more potent repellent activity (47%) than blocked hexapeptide. It is of interest that the repellent activity of Z–Val–Leu–OBu^t (**9**) decreased to 34% compared with that of Z–Val–Leu–OMe (**1**, 64%). The *tert*-butyl group is bulkier than the methyl group and bulk might affect the repellent activity. In order to study the effect of the bulky moiety at the C-terminal position of **1** on the repellent activity, we synthesized several dipeptide derivatives modified at the C-terminal position and their

repellent activity against *B. germanica* was examined. The results are summarized in Table III. The repellent activity of **1** is the most potent among Z–Val–Leu–OR (R = H, CH_3, C_2H_5, $C(CH_3)_3$ and $CH_2C_6H_5$). The methyl group is more favourable for repellent activity than any other group so far examined. The reduction of **1** with $NaBH_4$ gave the alcohol derivative (**21**), which showed decreased repellent activity. The amide (**25**) or alkyl amide (**26**—**28**) derivatives of Z–Val–Leu–OH are less potent than **1**. Z–Val–Leu–$NHNH_2$ (**29**) exhibited 34.5% repellent activity. Z–Val–Ile–OMe (**30**) showed fairly potent activity (58.6%), indicating that a hydrophobic amino acid at the C-terminal position is suitable for repellent activity. Amino acid derivatives exhibited slight repellent activity except for Z–Leu–$NHCH_2CH_2OH$ (**20**). Among the present compounds, Z–Val–Leu–OMe (**1**) has the most potent repellent activity against *A. aegypti* and *B. germanica* (78 and 64%, respectively), whereas *N*,*N*-diethyl-*m*-toluylamide (DETA), a commercially available repellant, exhibited 100 and 85% repellent activity, respectively. The effect of the synthetic peptides on other insects is under investigation in our laboratory and the results will be described elsewhere.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-180 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates were determined with an amino acid analyzer, K-101 AS (Kyowa Seimitsu Co., Ltd.). Thin-layer chromatography (TLC) was performed on silica gel plates (Kieselgel G, Merck) using the following solvent systems: Rf^1 $CHCl_3$, MeOH and H_2O (8 : 3 : 1, lower phase); Rf^2 $CHCl_3$, MeOH and AcOH (90 : 8 : 2); Rf^3 benzene and AcOEt (1 : 1); Rf^4 *n*-BuOH, pyridine, AcOH and H_2O (4 : 1 : 1 : 2); Rf^5 *n*-BuOH, AcOH and H_2O (4 : 1 : 5, upper phase); Rf^6 $CHCl_3$; Rf^7 *n*-BuOH, pyridine, AcOH and H_2O (30 : 20 : 6 : 24) and Rf^8 $CHCl_3$ and ether (4 : 1).

Z–Phe–Val–Leu–OMe (3)——Z–Phe–OH (4.2 g) and H–Val–Leu–OMe·HCl (prepared from 5.3 g of Z–Val–Leu–OMe[4] by catalytic hydrogenation in 30 ml of MeOH containing 14 ml of 1 N HCl) were dissolved in DMF (50 ml) containing Et_3N (1.96 ml). The solution was cooled with ice-salt. DCC (3.0 g) was added to the solution and the reaction mixture was stirred at room temperature overnight. After removal of the urea derivative and the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Ether and petroleum ether were added to the residue to afford crystals, which were collected by filtration and recrystallized from AcOEt and ether, yield 4.0 g (54.0%), mp 176—179 °C, $[\alpha]_D^{28}$ −42.8° (c = 1.0, MeOH), Rf^3 0.77. *Anal.* Calcd for $C_{29}H_{39}N_3O_6$: C, 66.3; H, 7.48; N, 8.0. Found: C, 66.0; H, 7.38; N, 8.1. Amino acid ratios in an acid hydrolysate: Phe 0.94; Val 1.00; Leu 0.99 (average recovery 87.1%).

Z–D-Phe–Val–Leu–OMe (4)——Z–D-Phe–OH[5] (2.7 g) and H–Val–Leu–OMe·HCl (prepared from 4.1 g of Z–Val–Leu–OMe[4] by catalytic hydrogenation as usual) were dissolved in CH_3CN (150 ml) containing Et_3N (1.6 ml) and the solution was cooled to −10 °C. DCC (2.2 g) was added to the solution and the reaction mixture was stirred at room temperature overnight. The desired peptide **4** was isolated in the same manner as described above, yield 2.1 g (36.6%), mp 164—173 °C, $[\alpha]_D^{26}$ −4.4° (c = 1.0, DMF), Rf^2 0.77, Rf^4 0.89. *Anal.* Calcd for $C_{29}H_{39}N_3O_6$: C, 66.3; H, 7.48; N, 8.0. Found: C, 66.4; H, 7.77; N, 8.3. Amino acid ratios in an acid hydrolysate: Phe 0.94; Val 1.00; Leu 0.95 (average recovery 77.4%).

Z–Gly–Val–Ser–OMe (5)——Z–Gly–ONp (5.6 g) and H–Val–Ser–OMe·HCl (prepared from 6.0 g of Z–Val–Ser–OMe[6] by catalytic hydrogenation in 30 ml of MeOH containing 17 ml of 1 N HCl) were dissolved in DMF (60 ml) containing Et_3N (4.8 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% Na_2CO_3, 1 N HCl and water, dried over Na_2SO_4 and evaporated down. Ether was added to the residue to give crystals, yield 5.3 g (76.4%), mp 136—141 °C, $[\alpha]_D^{28}$ −28.3° (c = 1.0, MeOH), Rf^1 0.62, Rf^2 0.40. *Anal.* Calcd for $C_{19}H_{27}N_3O_7$: C, 55.7; H, 6.65; N, 10.3. Found: C, 55.3; H, 6.65; N, 10.1. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 1.07; Ser 0.86 (average recovery 74.0%).

Z–Gly–Val–Ser–$NHNH_2$ (6)——Hydrazine hydrate (80%, 0.5 ml) was added to a solution of Z–Gly–Val–Ser–OMe (2.1 g) in MeOH (30 ml). The reaction mixture was stored at room temperature overnight. Crystals which appeared were collected by filtration, washed with MeOH and dried, yield 1.7 g (83.0%), mp 223—229 °C, $[\alpha]_D^{27}$ +2.1° (c = 1.0, DMF), Rf^1 0.36. *Anal.* Calcd for $C_{18}H_{27}N_5O_6$: C, 52.8; H, 6.65; N, 17.1. Found: C, 52.9; H, 6.64; N, 17.3. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 0.98; Ser 0.93 (average recovery 81.7%).

Z–Gly–Val–Ser–Phe–Val–Leu–OMe (7)——Z–Gly–Val–Ser–N_3 (prepared from 1.3 g of Z–Gly–Val–Ser–$NHNH_2$, 0.88 ml of 7 N HCl/dioxane and 0.43 ml of isoamylnitrite as usual) in DMF (20 ml) was combined with H–Phe–Val–Leu–OMe (prepared from 1.6 g of Z–Phe–Val–Leu–OMe by catalytic hydrogenation) in DMF (20 ml)

containing Et_3N (0.86 ml) under cooling with ice. The reaction mixture was stirred at 4 °C overnight. Removal of DMF followed by addition of EtOH gave white crystals, which were collected by filtration, washed with EtOH and recrystallized from *n*-propanol and water (3 : 1), yield 0.82 g (34.6%), mp 265—268 °C, $[\alpha]_D^{27}$ −10.1° (*c* = 1.0, DMF), Rf^2 0.27. *Anal.* Calcd for $C_{39}H_{56}N_6O_{10}$: C, 60.9; H, 7.34; N, 10.9. Found: C, 60.7; H, 7.43; N, 10.7. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 1.97; Ser 0.89; Phe 0.98; Leu 1.00 (average recovery 77.3%).

Z–Gly–Val–Ser–D-Phe–Val–Leu–OMe (8)——Z–Gly–Val–Ser–N_3 (prepared from 0.78 g of Z–Gly–Val–Ser–$NHNH_2$ as described above) was combined with H–D-Phe–Val–Leu–OMe (prepared from 1.08 g of Z–D-Phe–Val–Leu–OMe by catalytic hydrogenation). The desired compound was obtained in the same way as described above and recrystallized from MeOH, yield 1.2 g (82.0%), mp 224—229 °C, $[\alpha]_D^{27}$ −7.4° (*c* = 1.0, DMF), Rf^2 0.29. *Anal.* Calcd for $C_{39}H_{56}N_6O_{10}$: C, 60.9; H, 7.34; N, 10.9. Found: C, 60.9; H, 7.38; N, 11.2. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 1.97; Ser 0.89; Phe 0.98; Leu 0.92 (average recovery 62.4%).

Z–Val–Leu–OBu^t (9)——a) DCC Method: Z–Val–OH (4.5 g), H–Leu–OBu^t (prepared from 5.79 g of Z–Leu–OBu^t[7] by catalytic hydrogenation Rf^2 0.28) and N-hydroxysuccinimide (2.1 g) were dissolved in CH_3CN (80 ml) and the solution was cooled to −10 °C. DCC (4.5 g) was added to the solution and the reaction mixture was stirred at room temperature for 2 d. After removal of the dicyclohexylurea and the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down to give an oily material. The crude product in $CHCl_3$ (5 ml) was applied to a column of silica gel (3 × 48 cm), which was equilibrated and eluted with $CHCl_3$. The solvent of the eluate (800—1000 ml) was removed by evaporation and petroleum ether was added to the residue to afford crystals, yield 2.4 g (32.2%), mp 65—68 °C, $[\alpha]_D^{28}$ −47.4° (*c* = 1.0, MeOH), Rf^6, 0.36. *Anal.* Calcd for $C_{23}H_{36}N_2O_5$: C, 65.9; H, 8.86; N, 6.7. Found: C, 65.7; H, 8.63; N, 6.7.

b) Active Ester Method: Z–Val–ONp (10.4 g) and H–Leu–OBu^t (prepared from 10.4 g of Z–Leu–OBu^t by catalytic hydrogenation) were dissolved in CH_3CN (75 ml) containing Et_3N (3.9 ml) and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give crystals, yield 10.0 g (85%), mp 64—67 °C, Rf^2 0.87, Rf^6 0.36.

Z–Phe–Val–Leu–OBu^t (10)——Z–Phe–OH (5.9 g) and H–Val–Leu–OBu^t (prepared from 10 g of Z–Val–Leu–OBu^t by catalytic hydrogenation) were dissolved in CH_3CN (200 ml) and cooled with ice-salt. DCC (4.9 g) was added to the solution and the reaction mixture was stirred at room temperature overnight. After removal of the urea derivative and the solvent, the residue was dissolved in AcOEt (200 ml). This solution was washed with 10% citric acid, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume. Ether was added to the residue to give crystals, which were collected by filtration and washed with ether, yield 6.0 g (53.6%), mp 154—157 °C, $[\alpha]_D^{28}$ −47.9° (*c* = 1.0, MeOH), Rf^2 0.67, Rf^3 0.83. *Anal.* Calcd for $C_{32}H_{45}N_3O_6$: C, 67.7; H, 7.99; N, 7.4. Found: C, 67.7; H, 8.25; N, 7.6. Amino acid ratios in an acid hydrolysate: Phe 1.01; Val 1.00; Leu 1.02 (average recovery 51.7%).

Z–D-Phe–Val–Leu–OBu^t (11)——Z–D-Phe–OH (8.68 g) and H–Val–Leu–OBu^t (8.31 g) were dissolved in CH_3CN (300 ml) and cooled with ice-salt. DCC (7.2 g) was added to the solution and the reaction mixture was stirred at room temperature overnight. The title compound was obtained in the same way as described for the synthesis of **10**, yeild 7.9 g (48.2%), mp 148—152 °C, $[\alpha]_D^{25}$ −43.4° (*c* = 0.8, MeOH), Rf^2 0.68. *Anal.* Calcd for $C_{32}H_{45}N_3O_6$: C, 67.7; H, 7.99; N, 7.4. Found: C, 67.6; H, 8.04; N, 7.5. Amino acid ratios in an acid hydrolysate: Phe 1.10; Val 1.00; Leu 1.00 (average recovery 86.9%).

Z–Gly–Val–Ser–Phe–Val–Leu–OBu^t (12)——Z–Gly–Val–Ser–N_3 (prepared from 1.27 g of Z–Gly–Val–Ser–$NHNH_2$, 0.89 ml of 7 N HCl/dioxane and 0.43 ml of isoamylnitrite as usual) in DMF (20 ml) was combined with a cold DMF solution (20 ml) of H–Phe–Val–Leu–OBu^t (prepared from 1.8 g of Z–Phe–Val–Leu–OBu^t by catalytic hydrogenation). The reaction mixture was stirred at 4 °C overnight. After removal of the solvent, AcOEt and water were added to the residue to give a gelatinous material, which was collected by filtration and recrystallized from MeOH, yield 1.6 g (65.1%), mp 240—246 °C, $[\alpha]_D^{23}$ −13.8° (*c* = 0.9, MeOH), Rf^2 0.90, Rf^3 0.78. *Anal.* Calcd for $C_{42}H_{62}N_6O_{10}$: C, 62.2; H, 7.71; N, 10.4. Found: C, 62.3; H, 8.04; N, 10.9. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 2.04; Ser 0.93; Phe 1.09; Leu 0.97 (average recovery 88.0%).

Z–Gly–Val–Ser–D-Phe–Val–Leu–OBu^t (13)——Z–Gly–Val–Ser–N_3 (prepared from 1.8 g of Z–Gly–Val–Ser–$NHNH_2$, 1.23 ml of 7 N HCl/dioxane and 0.61 ml of isoamylnitrite as usual) was combined with a cold DMF solution (10 ml) of H–D-Phe–Val–Leu–OBu^t (prepared from 2.48 g of Z–D-Phe–Val–Leu–OBu^t by catalytic hydrogenation). The reaction mixture was stirred at 4 °C for 2 d. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 10% citric acid and water, dried over Na_2SO_4 and evaporated down. Petroleum ether was added to the residue to give crude crystalline materials, which were collected and washed with petroleum ether, yield 2.9 g (81.3%). This material in $CHCl_3$ (3 ml) was applied to a column of silica gel (2.1 × 31 cm), which was eluted with $CHCl_3$ (600 ml), and 2% MeOH in $CHCl_3$ (500 ml). The solvent of the latter eluate was removed by evaporation and petroleum ether was added to the residue to afford crystals, yield 2.3 g (64.5%), mp 119 °C (sintering), 218—221 °C (dec.), $[\alpha]_D^{23}$ −48.3° (*c* = 0.9, MeOH), Rf^6 0.62. *Anal.* Calcd for $C_{42}H_{62}N_6O_{10}$: C, 62.2; H, 7.71; N, 10.4. Found: C, 62.2; H, 7.77; N, 10.5. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 2.32; Ser 0.86; Phe 1.01; Leu 0.99 (average recovery 94.4%).

Z–Gly–Val–Ser–Phe–Val–Leu–OH (14)——A solution of Z–Gly–Val–Ser–Phe–Val–Leu–OBu^t (0.8 g) in TFA

(2 ml) containing anisole (0.2 ml) was stored at room temperature for 2.5 h. Ether was added to the solution to yield a precipitate, which was collected by filtration, washed with ether and dried over KOH pellets, yield 0.51 g (71.7%), mp 242—248 °C, $[\alpha]_D^{27}$ −8.4° (c=0.85, DMF), Rf^2 0.18, Rf^4 0.86, Rf^5 0.96. *Anal.* Calcd for $C_{38}H_{54}N_6O_{10}\cdot H_2O$: C, 59.1; H, 7.30; N, 10.9. Found: C, 59.1; H, 7.37; N, 11.1. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 1.85; Ser 0.91; Phe 1.00; Leu 0.93 (average recovery 64.0%).

Z-Gly-Val-Ser-D-Phe-Val-Leu-OH (15)——A solution of Z-Gly-Val-Ser-D-Phe-Val-Leu-OBu^t (0.67 g) in TFA (2 ml) containing anisole (0.2 ml) was stirred at room temperature for 2.5 h. Ether was added to the solution to give a precipitate, which was collected by filtration, washed with ether and AcOEt and dried over KOH pellets, yield 0.49 g (72.2%), mp 148 °C (sintering), 162—172 °C (dec.), $[\alpha]_D^{23}$ −49.0° (c=1.0, MeOH), Rf^1 0.83, Rf^2 0.20. *Anal.* Calcd for $C_{38}H_{54}N_6O_{10}\cdot H_2O$: C, 59.1; H, 7.30; N, 10.9. Found: 58.8; H, 7.13; N, 10.7. Amino acid ratios in an acid hydrolysate; Gly 1.00; Val 2.17; Ser 0.81; Phe 1.01; Leu 0.96 (average recovery 71.0%).

H-Gly-Val-Ser-Phe-Val-Leu-OH (16)——Z-Gly-Val-Ser-Phe-Val-Leu-OH (0.4 g) in water and *n*-BuOH (5 ml + 15 ml) was hydrogenated over Pd catalyst for 4.5 h. Crystals formed were dissolved in DMF, and the Pd was removed by filtration. After removal of the solvent, ether was added to the residue to afford crystals, which were collected by filtration and dried, yield 0.31 g (94%), mp 252—260 °C (dec.), $[\alpha]_D^{27}$ −13.4° (c=1.0, DMF), Rf^4 0.64, Rf^7 0.67. *Anal.* Calcd for $C_{30}H_{48}N_6O_8\cdot 3H_2O$: C, 53.4; H, 8.07; N, 12.5. Found: C, 53.0, H, 7.42; N, 12.2. Amino acid ratios in an acid hydrolysate: Gly 1.00; Val 1.87; Ser 0.98; Phe 1.03; Leu 0.95 (average recovery 74.7%).

General Procedure for Synthesis of Z-Leu-NHX (X=CH_3, C_2H_5, C_2H_4OH; 18—20)——Z-Leu-ONp and H-NHX were coupled by the active ester method. The yield, mp, $[\alpha]_D$ value, Rf values and analytical data are summarized in Table IV.

Synthesis of Dipeptide Derivatives (21—29)——Dipeptide derivatives (**21**—**29**) were synthesized according to the scheme shown in Fig. 2, and the yield, mp, $[\alpha]_D$ value, Rf values and analytical data are summarized in Table IV.

Z-Val-Ile-OMe (30)——Z-Val-OH (7.5 g), H-Ile-OMe (prepared from 5.5 g of H-Ile-OMe·HCl and 4.2 ml of Et_3N) and HOBt (4.1 g) were dissolved in DMF (50 ml). The solution was cooled to −10 °C and DCC (7.4 g) was

TABLE IV. Yield, mp, Optical Rotation, Rf Values and Analytical Data

Compound	Yield (%)	mp (°C)	$[\alpha]_D$ (MeOH)	TLC		Formula	Elemental analysis Calcd (Found)		
				Rf^1	Rf^2		C	H	N
Z-Leu-$NHCH_3$ (**18**)	45	130—131	−17.3 (c=1.0)	0.90	0.64	$C_{15}H_{22}N_2O_3$	64.7 (65.0)	7.97 8.09	10.1 10.1)
Z-Leu-NHC_2H_5 (**19**)	68	127—128	−18.1 (c=1.0)	0.93	0.67	$C_{16}H_{24}N_2O_3$	65.7 (65.7	8.27 8.45	9.6 9.5)
Z-Leu-NHC_2H_4OH (**20**)	89	124—125	−18.8 (c=1.0)	0.83	0.51	$C_{16}H_{24}N_2O_4$	62.3 (62.4	7.85 7.79	9.1 9.2)
Z-Val-Leu-ol (**21**)	80	120—123	−35.5 (c=1.0)		0.60	$C_{19}H_{30}N_2O_4$	65.1 (65.2	8.63 8.88	8.0 7.9)
Z-Val-Leu-OH (**22**)	64	128—132	−31.1 (c=1.0)	0.64	0.68	$C_{19}H_{28}N_2O_5$	62.6 (62.5	7.74 7.97	7.7 7.8)
Z-Val-Leu-OEt (**23**)	56	100—102	−42.3 (c=1.0)	0.91	0.81	$C_{21}H_{32}N_2O_5$	64.3 (64.4	8.22 8.84	7.1 7.4)
Z-Val-Leu-OBzl (**24**)	23	115—116	−10.7[b] (c=1.0)		0.71[a]	$C_{26}H_{34}N_2O_5$	68.7 (69.0	7.54 7.61	6.2 6.1)
Z-Val-Leu-NH_2 (**25**)	65	245—246	−10.9[b] (c=1.0)	0.66	0.60	$C_{19}H_{29}N_3O_4$	62.8 (62.5	8.04 8.09	11.6 11.4)
Z-Val-Leu-$NHCH_3$ (**26**)	38	206—208	−12.1[b] (c=0.9)	0.88	0.45	$C_{20}H_{31}N_3O_4$	63.6 (63.7	8.28 8.37	11.1 11.1)
Z-Val-Leu-NHC_2H_5 (**27**)	51	218—220	−16.1[b] (c=1.0)	0.83	0.45	$C_{21}H_{33}N_3O_4$	64.4 (64.3	8.50 8.60	10.7 10.5)
Z-Val-Leu-NHC_2H_4OH (**28**)	63	198—200	−48.8 (c=1.0)	0.61	0.40	$C_{21}H_{33}N_3O_5$	61.9 (61.9	8.16 8.34	10.3 10.4)
Z-Val-Leu-$NHNH_2$ (**29**)	82	124—138	−42.6 (c=1.0)	0.64	0.65	$C_{19}H_{30}N_4O_4$	60.3 (60.7	7.99 8.12	14.8 14.5)

a) Rf^8. *b*) DMF.

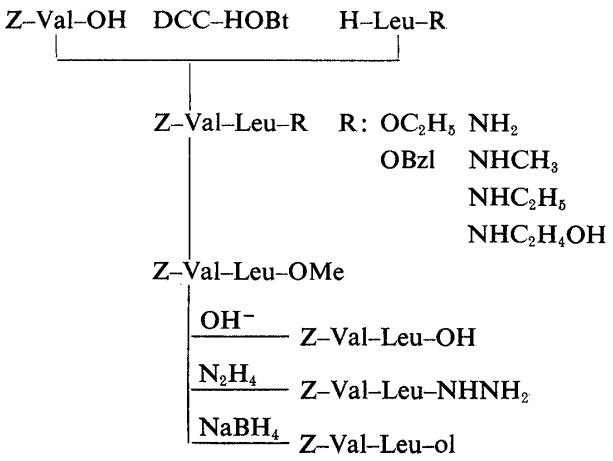

Fig. 2. Synthetic Scheme for Dipeptide Derivatives

added. The reaction mixture was stirred at 4 °C overnight. After removal of the urea derivative and the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and concentrated to a small volume. Petroleum ether was added to the residue to give crystals, yield 8.5 g (74.9%), mp 115—118 °C, $[\alpha]_D^{23}$ −24.0° (c=1.0, MeOH), Rf^2 0.86. *Anal.* Calcd for $C_{20}H_{30}N_2O_5$: C, 63.5; H, 7.99; N, 7.4. Found: C, 63.8; H, 7.96; N, 7.9.

Testing Procedure for Mosquito Repellency——A mouse was covered with 60-mesh nylon netting, on which vinyl tape was fixed to leave a 3×3 cm square at the center of the back of the mouse where mosquitoes could bite. Next, 0.5 ml of 0.5% (w/v) ethanolic solution of a test compound was applied to the site described above. The mouse was put in a cage containing 20 five-day-old female yellow-fever mosquitoes, *Aedes aegypti*. After 30 min, all the mosquitoes were crushed on filter paper and the number of mosquitoes that had bitten was counted. Another mouse, treated with 0.5 ml of ethanol, was used as a control. The repellent efficacy was evaluated on the bases of the ratio calculated as follows:

$$\text{Repellency (\%)} = \frac{A-B}{A} \times 100$$

A: The number of biting mosquitoes in a control experiment.

B: The number of biting mosquitoes in a test.

Testing Procedure for Cockroach Repellency——Thirty adult cockroaches, *Blattella germanica*, were placed in a box containing two pieces of 11×11 cm square filter paper with a cube of sugar (6 g) at the center of each. One of the two filter papers was treated with 0.5 g/m^2 concentration of the test compound and the other was untreated. The weight of sugar taken by the cockroaches during 2 d was measured. The degree of repellency was determined by calculating the percentage as follows:

$$\text{Repellency (\%)} = \frac{A-B}{A} \times 100$$

A: The weight (mg) of sugar taken from the untreated filter paper.

B: The weight (mg) of sugar taken from the treated filter paper.

Testing Procedure for Mosquito Attractant Activity——In a control experiment (see "Testing Procedure for Mosquito Repellency" above), the number of biting mosquitoes was 55% as shown in Table I. In an experiment, the test peptide was regarded as an attractant if the number was more than 55%.

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References and Notes

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