CHAPTER II

EXPERIMENTAL

2.1 Starting materials and purification of solvents

2.1.1 Starting materials

Chemicals used in this research were obtained from the following sources:

Tetrahydrofuran (THF), triethylamine, benzoylchloride, isobutyl chloroformate, benzyl chloroformate, L-valine, L-tyrosine, L-phenylalanine, absolute methanol, chloroform, diethyl ether, thionyl chloride, petroleum ether, dimethyl sulfoxide, ethyl acetate, hexane, sodium hydroxide, sodium bicarbonate, sodium chloride, were purchased from Fluka Company. All of them were A.R. grade except hexane, chloroform, and ethyl acetate.

Dodecylamine, decylamine, trypsin (EC 3.4.21.4), α-chymotrypsin(EC3.4.21.1), t-Butyloxycarbonyl-L-alanine-*p*-nitro phenylester (BAN), succinyl-L-alanyl-L-prolyl-L-phenylalanine-*p*-nitroanilide (Suc-Ala-Ala-Pro-Phe-*p*NA), N-α-benzoyl-dl arginine-*p*-nitroanilide(BAPNA), the buffer: N-2-hydroxyethyl piperazine-N-2-ethanesulfonic acid (HEPES) were purchased from Sigma Chemical Company, U.S.A.

Human leukocyte elastase was obtained from Professor Dr.Bela Ternai of La Trobe University,Australia.

2.1.2 Purification of solvents

Tetrahydrofuran was purified and dried by refluxing it with sodium metal for 2 hours before it was distilled and stored over molecular sieves (type 4A)[61].

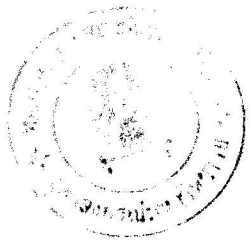
Triethylamine was distilled over potassium hydroxide and stored over molecular sieves (type 4A).

Chloroform, hexane, and ethyl acetate (commercial grade) were distilled before use.

2.2 Syntheses

2.2.1 Preparation of N-benzoyl-L-valine, BZ-V

To a solution of valine (35.1 g,300 mmoles)in 4.0 M sodium hydroxide (75 mL) kept at 0 °C, were added concurrently over a period of 45 minute, benzoyl chloride (30%) in toluene (180 mL) and 4.0 M sodium hydroxide (87 mL). The addition was carried out with vigorous stirring at 0 °C. The resulting solution was acidified with 2.7 M HCl (10%) and the oil that precipitated was extracted into ethyl acetate (3x90 mL). The combined extracts were cooled to 0 °C and washed with ice-cooled 10% sodium bicarbonate solution (3x50 mL).The solution was dried with sodium sulphate and the solvent was evaporated.



,82 °C in petroleum ether), was obtained.

2.2.2 Preparation of carbobenzoxyvaline, Z-V

L-valine (11.7g, 100 mmoles) was dissolved in 25 mL of 1.0 M sodium hydroxide. With cooling in an ice bath, there were added simultaneously with stirring, over a period of forty five minutes, 14 mL(100 mmoles) of carbobenzoxy chloride solution. The solution was acidified with hydrochloric acid, whereupon an oil was deposited. This was then dissolved in 1.0 M sodium hydroxide and precipitated by acidifying this solution with 1.0 M hydrochloric acid. The precipitate was stored in a refrigerator whereupon it slowly crystallized over a period of weeks to white solid (18 g,71.6%), mp. 57-59 °C. ([lit 62],58-61 °C).

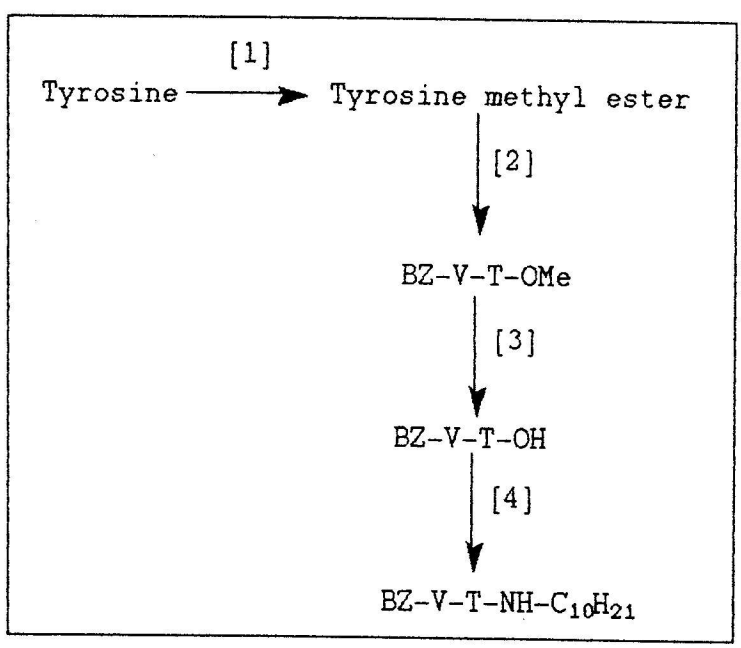
2.2.3 Preparation of N-t-Butyloxycarbonyl-valine, BOC-V

L-valine (11.7 g,100 mmole) was dissolved in dioxane: water 2:1 (300mL) and 1.0 M sodium hydroxide (100 mL).The solution was cooled to -5 °C and di-t-butylcarbonate (24 g, 110 mmole) was added over a period of 10 minute. The solution was then stirred at room temperature for 3 hours. The solution was concentrated, ice-cooled and mixed with ethyl acetate(75 mL). The mixture was acidified to pH 2-3 with 0.5 M potassium hydrogen sulphate.The organic layer was separated and the

hydrogen sulphate.The organic layer was separated and the aqueous layer extracted with ethyl acetate (2x60 mL).The combined organic layers were dried with sodium sulphate and the solvent evaporated,to yield a light coloured oil. The oil was left at 4 °C to crystalize to white solid (17 g,78 %), mp. 75-76 °C.

2.2.4 Preparation of BZ-V-T-NH-$C_{10}H_{21}$ (compound I)

There are four steps of reaction involved in this preparation which are schematically shown below:



Scheme 2.1 Preparation of BZ-V-T-NH-$C_{10}H_{21}$

Step 1: Preparation of tyrosine methyl ester.

L-tyrosine (36.2 g, 200 mmoles) was suspended in absolute methanol (200 mL). Thionyl chloride (15 mL) was

added dropwise and then the solution was refluxed for 2 hours. The solvent was removed with the aid of a rotary evaporator under reduced pressure to yield a very viscous oil and permitted to stand in the refrigerator for a few hours. The hygroscopic solid of the hydrochloride salt of tyrosine methyl ester,white powder (42.0 g, 90.6%), m.p.183-185 °C (lit.[67] m.p. 185-190 °C decompose) was obtained after washing with absolute ether.

Step 2: Preparation of BZ-V-T-OMe.

BZ-V from 2.2.1 (12.5 g,31.3 mmoles) was dissolved in dry THF(90 mL) and triethylamine(4.4 mL,32 mmoles). The solution was cooled at -5 to -10 °C with stirring before the addition of isobutyl chloroformate (4.2 mL,32 mmoles). The stirring was continued further at the same temperature for 15 minutes before the addition of a solution of tyrosine methyl ester HCl from step 1 (7.4 g, 32 mmoles) in THF (30 mL) and triethylamine (4.4 mL, 32 mmoles). The solution was stirred continuously for 10 hours at room temperature. After this time, the solution was placed in a separating funnel and ethyl acetate (60 mL) and water (15 mL) were added. The organic layer was collected and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ (15 mL), water (15 mL), 1 M HCl (15 mL) and water (15 mL) respectively and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and then a transparent glassy crystalline (12.5 g,

31.3 mmoles, 91.6 %), m.p. 150-153°C, was obtained after a few weeks of storage.

Step 3: Preparation of BZ-V-T-OH.

BZ-V-T-OMe (12.5 g, 31.3 mmoles) previously prepared in step 2, was dissolved in methanol (30 mL). With stirring at room temperature, 1 M NaOH (15 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. A gelatinous precipitate was formed and was acidified with 1 M HCl with cooling. The mixture was kept at 4 °C overnight in a refrigerator, and extracted three times with a mixture of ethyl acetate and sodium hydrogen carbonate (5:1). The organic layer was separated and the solvent was removed under reduced pressure to yield an oil (11.5 g, 95.6 %).

Step 4: Preparation of BZ-V-T-$NHC_{10}H_{21}$.

BZ-V-T-OH (3.9 g, 10.1 mmoles), the oil previously prepared in step 3, was dissolved in dry THF (60 mL) and triethylamine (1.1 mL, 10.2 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutylchloroformate (1.4 mL, 10.2 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine (2.1 mL, 10.2 mmoles) in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$ (15 mL), H_2O (15 mL), 1 M HCl (15 mL)

Na_2SO_4, the ethyl acetate was evaporated and the solid was recrystallized from ethyl acetate to form white shiny light crystal (4.74 g, 89.9 %), m.p. 96-100 °C.

TLC: R_f= 0.55 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{31}H_{45}N_3O_4$:

cal'd:C 71.10, H 8.66, N 8.02

found:C 71.05, H 8.68, N 8.05

IR (KBr) υ (cm^{-1}) (Fig. I.1):

3600 (-OH stretching);3320 (-NH stretching); 3100 (-CH aromatic); 2960,2920, 2840 (-CH stretching); 1660, 1620 (HN-C=O stretching); 1520, 1500 (-NH bending).

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.14):

0.83 (t,3H,-CH_3); 0.83,0.60 (br.,6H,CH_3-CH-CH_3); 1.21 (s,16H,CH_2's chain); 2.04 (m,1H,CH_3-CH-CH_3); 2.49 (q,2H,-CH_2-Ar); 2.70 (d,2H,-NH-CH_2); 4.21(t, 1H,NH-CH-C=O); 4.40(q,1H,-NH-CH-CH-CH_3);6.56,6.96 (db.of db.,4H,—⬡—); 7.47 (m,5H,Ar-C=O); 7.70 (t,1H,-OH); 7.86 (d,1H,-NH-CH-CH-CH_3); 8.20 (t, 1H,-NH-CH_2-); 9.01 (s,1H,-NH-CH_2-Ar).

^{13}C NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.27):

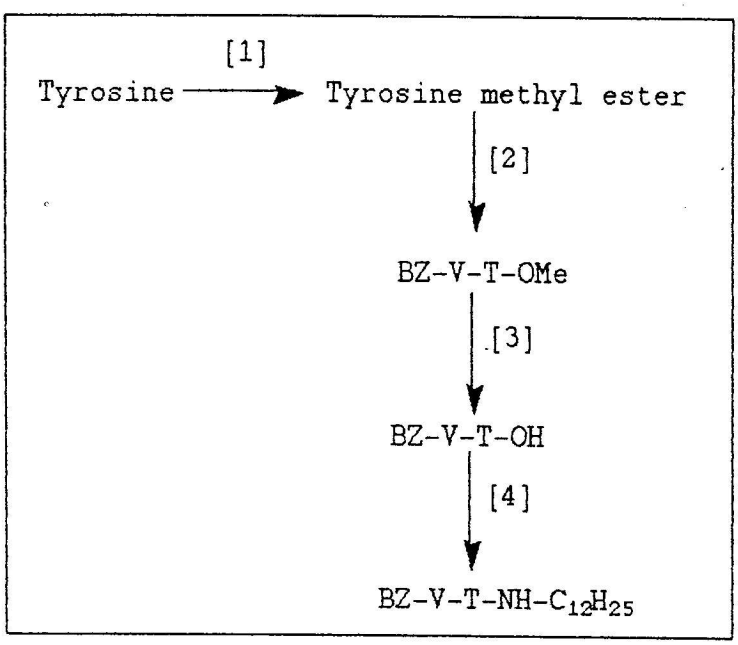
13.6 (-CH_3); 18.5 (-CH[CH_3]$_2$); 18.7 (-CH[CH_3]$_2$); 22.0, 26.5, 28.7,29.2,29.7, 31.2 (-CH_2's chain); 36.0 (-NH-CH_2-), 38.4 (-CH_2-Ar); 55.2 (-NH-CH-CH[CH_3]$_2$); 60.2 (-NH-CH-CH_2-); 114.1, 127.5, 127.9, 129.2, 130.2, 134.5, 155.9 (-CH_2-Ar-OH,Ar-C=O); 167.5 (Ar-C=O); 170.2 (O=C-CH-

OH, **Ar**-C=O); 167.5 (Ar-**C**=O); 170.2 (O=**C**-CH-CH-CH_3); 171.5 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.40) in CH_3OH:CH_3Cl = 1:19

2.2.5 Preparation of BZ-V-T-NH-$C_{12}H_{25}$ (compound II)

There are four steps of reaction involved in this preparation which is schematically shown below:



Scheme 2.2 Preparation of BZ-V-T-NH-$C_{12}H_{25}$

Steps 1,2 and 3 had already been carried out in the synthesis of BZ-V-T-NH-$C_{10}H_{21}$ (I)

Step 4: Preparation of BZ-V-T-NH-$C_{12}H_{25}$.

BZ-V-T-OH (3.84 g,9.98 mmoles, the oil previously prepared in step 3, was dissolved in dry THF(60 mL) and triethylamine(1.4 mL,10 mmoles). The solution was stirred and cooled at -5 to -10 °C.After adding isobutyl chloroformate (1.3 mL,10 mmoles),the mixture was stirred at the same temperature for 10 minutes before a solution of dodecylamine (1.9 g,10 mmoles) in dry THF (30 mL) was added. The solution was then stirred at room temperature for 10 hours. To the filtered white solid was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was separated and washed with 5% $NaHCO_3$ (15 mL),H_2O (15 mL), 1 M HCl (15 mL) and H_2O (15 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The residue had been dried in the air for a few weeks. The white crystalline solid(5.0 g,90.8 %),m.p. 173-175 °C was washed with petroleum ether.

TLC: R_f= 0.56 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{33}H_{49}N_3O_4$:

cal'd:C 71.83, H 8.95, N 7.62

found:C 71.54, H 8.87, N 7.56

IR(KBr) υ (cm^{-1}) (Fig. I.2):

3480 (-OH stretching); 3320 (-NH stretching); 3060, 3020 (-CH aromatic); 2920, 2850 (-CH stretching); 1640, 1620 (HN-C=O stretching); 1530, 1510 (-NH bending)

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.15):

0.86 (t,3H,-CH_3); 0.65, 0.86 (br.,6H,CH_3-CH-CH_3);

1.18 (s,20H,C**H**$_2$'s chain); 1.95 (m,1H,CH_3-C**H**-CH_3); 2.51 (m,2H,-NH-C**H**$_2$-); 2.67 (d,2H,-C**H**$_2$-Ar); 4.03 (t,1H,-NH-C**H**-CH-CH_3); 4.40 (q,1H,-NH-C**H**-CH_2-Ar); 6.56,6.97 (db.of db.,4H,-⟨O⟩-);7.39 (m,5H,**Ar**-C=O); 7.48 (t,1H,-O**H**); 7.82 (d,1H,-N**H**-CH_2-); 8.05(t, 1H, -N**H**-CH-CH-CH_3); 8.62 (s,1H,-N**H**-CH-CH_2-Ar).

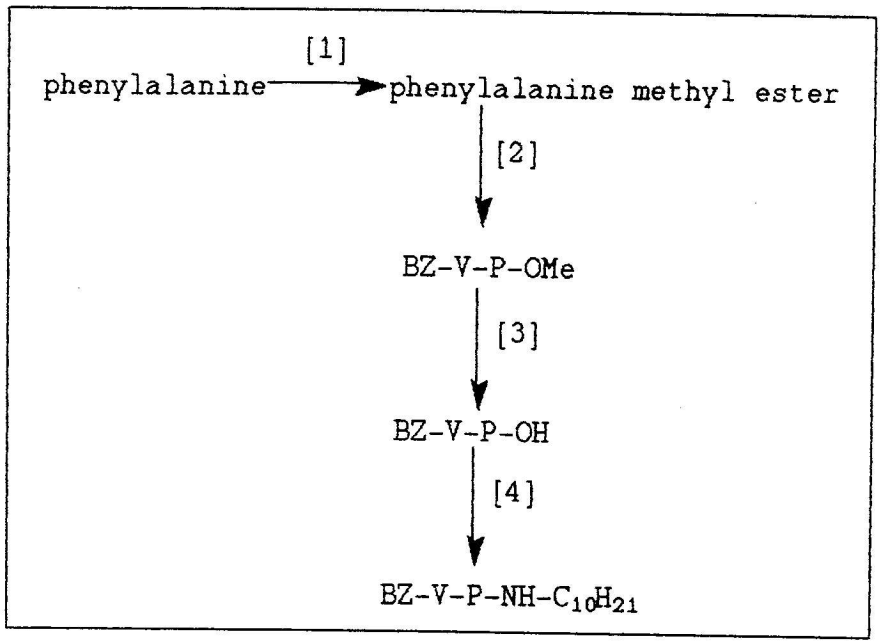
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.28):

13.8 (-C**H**$_3$); 18.9 (-CH[C**H**$_3$]$_2$); 19.0 (-C**H**[CH_3]$_2$); 22.2,26.6,28.9,29.2,29.5,31.4 (-C**H**$_2$'s chain); 36.4 (-NH-C**H**$_2$-); 39.0(-C**H**$_2$-Ar); 54.7 (-NH-C**H**-CH[CH_3]$_2$); 60.6 (-NH-C**H**-CH_2-); 114.8,127.3,127.8,129.8,130.8, 134.0, 155.6 (-CH_2-**Ar**-OH,**Ar**-C=O); 167.1 (Ar-C**=**O); 170.7 (O=C**H**-CH-CH-CH_3); 171.2 (O=C**H**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.41) in CH_3OH:CH_3Cl = 1:19

2.2.6 Preparation of BZ-V-P-NH-$C_{10}H_{21}$ (compound III)

There are four steps of reaction involved in this preparation which is schematically shown below:



Scheme 3.1 Preparation of BZ-V-P-NH-$C_{10}H_{25}$

Step 1: Preparation of phenylalanine methyl ester

Phenylalanine (33.0 g,200 mmoles) was suspended in absolute methanol (200 mL). Thionyl chloride (15 mL) was added dropwise and the solution was then refluxed for 2 hours. The product was obtained as hygroscopic white needle-like crystals of the hydrochloride salt of phenylalanine methyl ester,the white powder(41 g,95.0 %),m.p.156-158 °C (lit.[62], m.p. 158-160 °C), was obtained after washing with absolute ether.

Step 2: Preparation of BZ-V-P-OMe.

BZ-V from 2.2.1 (6.6 g,29.8 mmoles) was dissolved in dry THF (60 mL) and triethylamine (4.2 mL,30 mmoles). The solution was cooled at -5 to -10 °C with stirring before the addition of isobutyl chloroformate (3.9 mL,30 mmoles). The stirring was continued further at the same temperature for 15 minutes before the addition of a solution of phenylalanine

methyl ester HCl from step 1 (6.5 g,30 mmoles) in THF (30 mL), water (15 mL) and triethylamine (4.2 mL,30 mmoles). The solution was stirred continuously for 10 hours at room temperature. After this time, the solution was placed in a separating funnel and ethyl acetate (75 mL) and water (15 mL) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3x30 mL). The combined organic layers were washed with saturated $NaHCO_3$ (15 mL), water (15 mL), 1 M HCl (15 mL) and water (15 mL) respectively and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure, leaving a white crystal(10.2 g,89.4 %) mp.145-148°C.

Step 3: Preparation of BZ-V-P-OH

BZ-V-P-OMe (10.2 g,26.7 mmoles), previously prepared in step 2, was added to methanol (60 mL). With stirring at room temperature, 1 N NaOH (15 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. Then it was acidified with 1 M HCl with cooling and extracted with ethyl acetate (90 mL). The ethyl acetate layer was separated and dried with Na_2SO_4 and then the solvent was removed under reduced pressure. The residue was a white crystalline solid (7.42 g, 75.3%),mp.206-208°C.

Step 4: Preparation of BZ-V-P-NH-$C_{10}H_{21}$.

BZ-V-P-OH (1.84 g,5 mmoles) previously prepared in step 3,was dissolved in dry THF (60 mL)and triethylamine (1.4

mL, 10.1 mmoles). The solution was stirred and cooled at -5 to -10°C. After adding isobutylchloroformate (1.3 mL, 10.1 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine (2 mL, 10.1 mmoles) in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL), H_2O(15 mL) 1M HCl(15 mL) and H_2O(15 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated and the white powder (5.3 g, 73.0 %), mp. 87-90°C, was obtained.

TLC: R_f= 0.71 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{31}H_{45}N_3O_3$:

cal'd: C 73.34, H 8.93, N 8.28

found: C 73.12, H 9.02, N 8.20

IR(KBr) υ (cm^{-1}) (Fig. I.3):

3300(-NH stretching); 3060, 3020(-CH aromatic); 2950, 2920, 2840 (-CH stretching); 1680, 1650, 1625 (HN-C=O stretching); 1530 (-NH bending)

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.16):

0.84 (t, 9H, -C**H**$_3$, C**H**$_3$-CH-C**H**$_3$); 1.24 (s, 16H, C**H**$_2$'s chain); 2.05(m, 1H, CH_3-C**H**-CH_3); 3.04 (m, 2H, -NH-C**H**$_2$-); 3.67 (d, 2H, -C**H**$_2$-Ar); 4.01 (t, 1H, -NH-C**H**-CH-CH_3); 4.26 (q, 1H, -NH-C**H**-CH_2-Ar); 5.94 (d, 1H, -N**H**-CH_2-); 5.52(t, 1H, -N**H**-CH-CH-CH_3); 6.78 (s, 1H, -N**H**-CH-CH_2-Ar) 7.19 (m, 4H, **Ar**-CH_2-); 7.41, 7.78 (m, 5H, **Ar**-C=O).

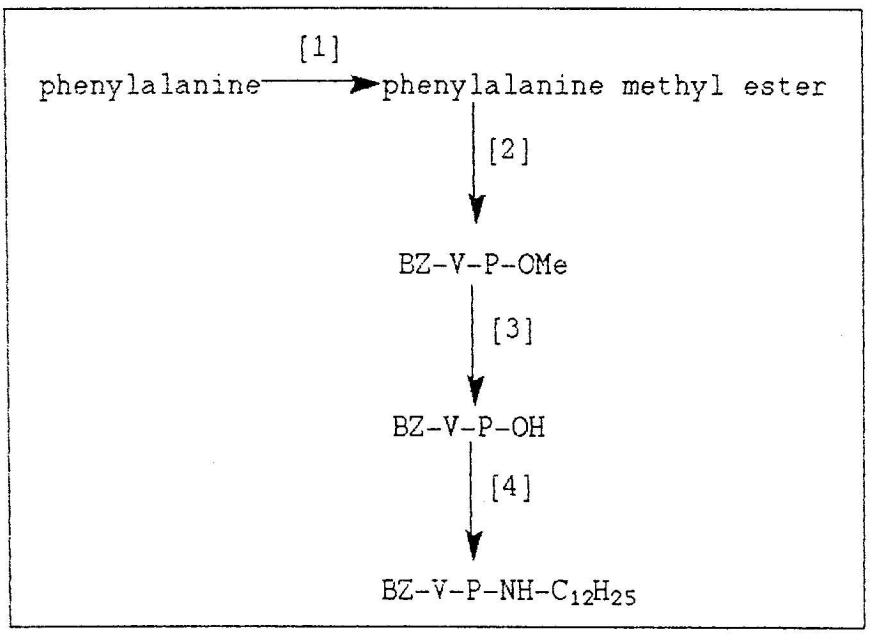
^{13}C NMR ($CDCl_3$) δ(ppm) (Fig. I.29):

14.1 (-**C**H_3); 19.0 (-CH[**C**H_3]$_2$); 19.1 (-**C**H[CH_3]$_2$); 22.6, 26.7, 27.9, 29.5,29.6,31.8(-**C**H_2'schain);39.0 (-NH-**C**H_2-) 39.3 (-**C**H_2-Ar); 56.4 (-NH-**C**H-CH[CH_3]$_2$); 71.1 (-NH-**C**H-CH_2-); 126.5, 127.9, 128.0, 128.4, 129.2,131.4,136.8(**Ar**-CH_2-,**Ar**-C=O);156.2 (Ar-**C**=O); 167.0 (O=**C**-CH-CH-CH_3); 169.8 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.42) in CH_3OH:CH_3Cl = 1:19

2.2.7 Preparation of BZ-V-P-NH-$C_{12}H_{25}$ (compound IV)

There are four steps of reaction involved in this preparation which is schematically described below:



Scheme 2.4 Preparation of BZ-V-P-NH-$C_{12}H_{25}$

Step 1,2 and 3 had already been carried out in the synthesis of BZ-V-P-NH-$C_{10}H_{21}$ (IV).

Step 4: Preparation of BZ-V-P-NH-$C_{12}H_{25}$.

BZ-V-P-OH (5 g, 9.1 mmoles) from step 3 and dodecylamine (1.7 g,9.2 mmoles) were used. The reaction was carried out under the same reaction conditions described for the synthesis of Bz-V-T-NH-$C_{12}H_{25}$. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated and the residue was recrystallized from ethyl acetate to yield a white powder (5.3 g,73.0%), m.p.103-105°C.

TLC: R_f= 0.73 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{33}H_{49}N_3O_3$:

cal'd:C 73.98, H 9.22, N 7.84

found:C 73.80, H 9.20, N 7.72

IR(KBr) υ (cm^{-1}) (Fig. I.4):

3300 (-NH stretching); 3040 (-CH aromatic); 2960, 2940, 2860 (-CH stretching); 1680, 1650 (HN-C=O stretching); 1540, 1520 (-NH bending).

^1H NMR ($CDCl_3$) δ (ppm) (Fig. I.17):

0.89 (d,9H,-C**H**$_3$, C**H**$_3$-CH-C**H**$_3$); 1.23 (s,20H,C**H**$_2$'s chain);1.92 (m,1H,CH_3-C**H**-CH_3);3.02 (m,4H,-NH-C**H**$_2$-, -C**H**$_2$-Ar); 3.73(m,2H,-NH-C**H**-CH_2-Ar); 3.95(t,1H,-NH-C**H**-CH-CH_3, 4.30(q,1H,-NH-C**H**-CH_2); 5.60(br.,1H,-N**H**-CH-CH-CH_3); 6.14(br.,1H,-N**H**-CH_2); 6.92(br.,1H,-N**H**-CH-CH_2);7.17(m,5H,**Ar**-CH_2-);7.36,7.75(m,5H,**Ar**-C=O).

^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.30):

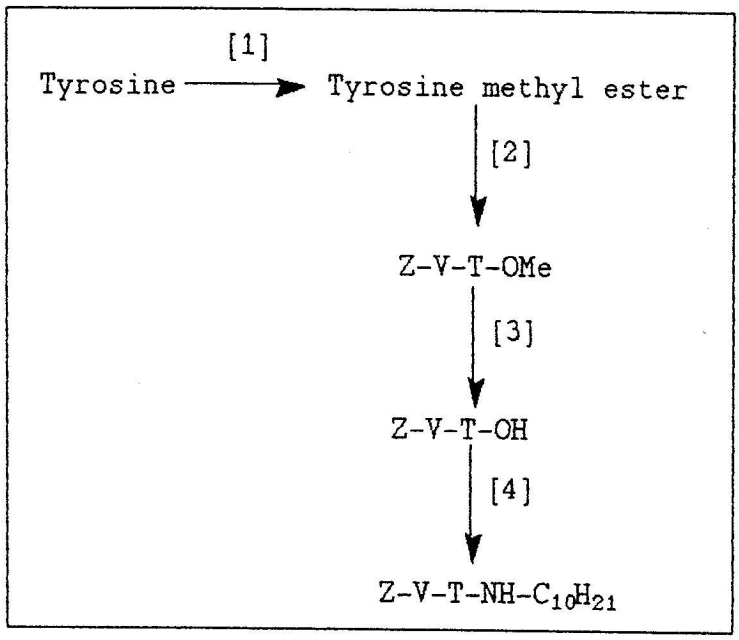
14.1 (-C**H**$_3$); 19.0 (-CH[C**H**$_3$]$_2$, -C**H**[CH_3]$_2$); 22.6, 26.7,27.7,29.3,29.6,31.8 (-C**H**$_2$'s chain);36.4 (-NH-C**H**$_2$-); 39.3 (-C**H**$_2$-Ar); 56.3 (-NH-C**H**-CH[CH_3]$_2$);

71.1 (-NH-**C**H-CH_2-); 126.7,127.2,128.3,128.4,129.2, 131.2, 136.8 (**Ar**-CH_2-,**Ar**-C=O); 156.0 (Ar-**C**=O); 166.8 (O=**C**-CH-CH-CH_3); 170.5 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.43) in CH_3OH:CH_3Cl = 1:19

2.2.8 Preparation of Z-V-T-NH-$C_{10}H_{21}$ (compound V)

There are four steps of reaction involved in this preparation which are schematically shown below:



Scheme 2.5 Preparation of Z-V-T-NH-$C_{10}H_{21}$

Step 1 had already been carried out in the synthesis of BZ-V-T-NH-$C_{10}H_{21}$ (I).

Step 2: Preparation of Z-V-T-OMe.

Z-V from 2.2.2 (7.5 g,30 mmoles) was dissolved in dry THF(90 mL) and triethylamine(4.2 mL,30.5 mmoles). The solution was cooled at -5 to -10 °C with stirring before the addition of isobutyl chloroformate(3.9 mL,30.5 mmoles)The stirring was continued further at the same temperature for 15 minutes before the addition of a solution of tyrosine methyl ester HCl from step 1 (7.1 g, 30.5 mmoles) in THF (30 mL) and triethylamine(4.2 mL, 30.5 mmoles).The solution was stirred continuously for 10 hours at room temperature. After this time, the solution was placed in a separating funnel and ethyl acetate (60 mL) and water (15 mL) were added. The organic layer was collected and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with saturated $NaHCO_3$ (15 mL), water (15 mL), 1 M HCl (15 mL) and water (15 mL) respectively and then dried with anhydrous Na_2SO_4.The solvent was removed under reduced pressure and then a the white powder crystalline solid was obtained (11.0 g, 85.3%), mp.142-145 °C

Step 3: Preparation of Z-V-T-OH.

Z-V-T-OMe (11.0 g,25.6 mmoles) previously prepared in step 2,was dissolved in methanol(30 mL).With stirring at room temperature, 1N NaOH (15 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. A gelatinous precipitate was formed and was acidified with 1 M HCl with cooling.The mixture was kept at 4 °C overnight in a

was separated and the solvent was removed under reduced pressure to the white solid (9.1 g, 85.9 %), mp. 225 °C decompose.

Step 4: Preparation of Z-V-T-$NHC_{10}H_{21}$.

Z-V-T-OH (4.1 g,10 mmoles), previously prepared in step 3, was dissolved in dry THF (60 mL) and triethylamine (1.4 mL,10.1 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutylchloroformate (1.3 mL,10.1 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine (2 mL,10.1 mmoles) in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$ (15 mL), H_2O (15 mL), 1 M HCl (15 mL) and H_2O (15 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated and the waxy white solid was washed with ether many times. The needle crystal (4.3 g, 78.0 %),m.p. 142-143 °C was obtained.

TLC: R_f= 0.55 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{32}H_{47}N_3O_5$:

cal'd:C 69.41, H 8.56, N 7.59

found:C 69.34, H 8.74, N 7.67

IR(KBr) υ (cm^{-1}) (Fig. I.5):

3600 (-OH stretching); 3300 (-NH stretching); 3020

(-CH aromatic);2960,2920,2860(-CHstretching);1750, 1690, 1640 (-NH-C=O stretching); 1540, 1520 (-NH bending).

^{1}H NMR ($CDCl_3$) δ (ppm) (Fig. I.18):

0.82 (t,3H,-**CH_3**); 0.79,0.82 (db. of db.,6H,**CH_3**-CH-**CH_3**); 1.18 (s,16H,**CH_2**'s chain); 1.95 (m,1H,CH_3-**C**H-CH_3); 2.49 (q,1H,-NH-**CH_2**-); 2.88 (d,2H,**CH_2**-Ar); 3.97 (m,1H,-NH-C**H**-CH-CH_3); 4.53 (m,1H,-NH-C**H**-CH_2-Ar); 5.00 (s,2H,Ar-**CH_2**-O-); 8.81 (s,1H,-N**H**-CH-CH_2-Ar); 5.65 (d,1H,-N**H**-CH-CH-CH_3); 6.60, 6.89 (db. of db.,4H,—⟨O⟩—); 6.54 (d,1H,-O**H**);7.26 (s,5H,**Ar**-C=O); 7.72 (t,1H,-N**H**-CH_2-).

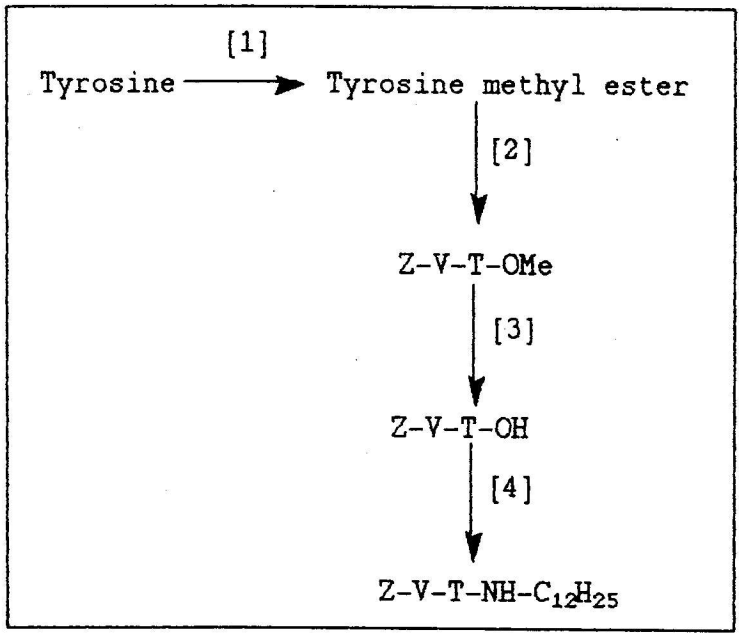
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.31):

14.1 (-**CH_3**); 17.9 (-CH[**CH_3**]$_2$); 19.1 (-**C**H[CH_3]$_2$); 22.6, 26.7, 27.8, 29.4, 29.6, 31.4 (-**CH_2**'s chain); 37.1 (-NH-**CH_2**-); 39.0 (-**CH_2**-Ar); 53.1 (-NH-**C**H-CH[CH_3]$_2$); 59.8 (-NH-**C**H-CH_2-); 67.1 (-O-**CH_2**-Ar); 115.5, 126.5, 127.9, 128.3, 130.2, 136.4 (-CH_2-**Ar**-OH,-O-**CH_2**-Ar);155.8(Ar-CH_2-O-**C**=O);156.8(O=**C**-CH-CH-CH_3); 171.9 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.44) in CH_3OH:CH_3Cl = 1:19

2.2.9 Preparation of Z-V-T-NH-$C_{12}H_{25}$ (compound VI)

There are four steps of reaction involved in this preparation which is schematically described below:



Scheme 2.6 Preparation of Z-V-T-NH-$C_{12}H_{25}$

Step 1,2 and 3 had already been carried out in the synthesis of Z-V-T-NH-$C_{10}H_{21}$ (V).

Step 4: Preparation of Z-V-T-NH-$C_{12}H_{25}$.

Z-V-T-OH (4.1 g, 10 mmoles), previously prepared in step 3, was dissolved in dry THF(60 mL) and triethylamine (1.4 mL, 10.1 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutylchloroformate (1.3 mL, 10.1 mmoles) , the mixture was stirred at the same temperature for 10 minutes before a solution of dodecylamine (2 mL, 10.1 mmoles) in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$ (15 mL), H_2O (15 mL), 1 M HCl (15 mL) and H_2O (15 mL) respectively.

After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The residue had been dried in the air and washed with petroleum ether then the white crystalline solid (4.4 g, 76.0 %), mp. 160-163°C was obtained.

TLC: R_f= 0.57 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{34}H_{51}N_3O_5$:

cal'd:C 70.19, H 8.84, N 7.22

found:C 69.40, H 8.42, N 7.01

IR(KBr) υ (cm^{-1}) (Fig. I.6):

3600 (-OH stretching); 3300(-NH stretching); 3040, 3020(-CH aromatic);2960,2920,2840(-CH stretching); 1690, 1640 (HN-C=O stretching); 1530, 1520 (-NH bending).

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.19):

0.84 (t, 3H, -**CH_3**); 0.75, 0.84(d, 6H, **CH_3**-CH-**CH_3**); 1.20 (s, 20H, **CH_2**'s chain); 1.79(m, 1H, CH_3-**CH**-CH_3); 2.50(q, 2H, -NH-**CH_2**-); 2.70(d, 2H, -**CH_2**-Ar); 3.77 (m, 1H, -NH-**CH**-CH-CH_3); 4.40 (m, 1H, -NH-**CH**-CH_2-Ar); 5.00 (s, 2H, Ar-**CH_2**-O-); 6.58, 6.90 (db.of db., 4H, -  -); 7.28 (s, 5H, **Ar**-CH_2-OH); 7.43 (d, 1H, -O**H**); 7.64(d, 1H, -N**H**-CH_2-); 7.94(d, 1H, -N**H**-CH-CH-CH_3); 8.79(s, 1H, -N**H**-CH-CH_2-Ar).

^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.32):

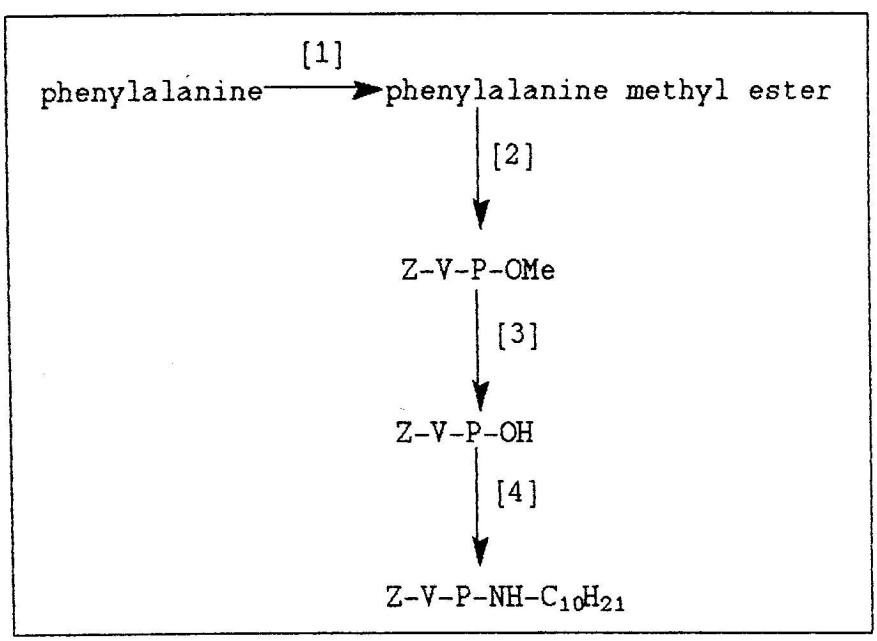
13.8 (-**CH_3**); 18.9 (-CH[**CH_3**]$_2$); 19.0 (-**CH**[CH_3]$_2$); 22.2, 26.5, 28.9, 29.0, 29.2, 31.4 (-**CH_2**'s chain); 36.9 (-NH-**CH_2**-); 38.9 (-**CH_2**-Ar); 54.4 (-NH-**CH**-CH[CH_3]$_2$); 60.7 (-NH-**CH**-CH_2-); 65.7 (-O-**CH_2**-Ar); 114.8, 127.3, 127.6, 128.0, 129.8, 136.5 (-CH_2-**Ar**

-OH, **Ar**-C=O); 155.7 (Ar-CH_2-O-**C**=O); 156.2 (O=**C**-CH-CH-CH_3); 171.6 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.45) in CH_3OH:CH_3Cl = 1:19

2.2.10 Preparation of Z-V-P-NH-$C_{10}H_{21}$ (compound VII)

There are four steps of reaction involved in this preparation which is schematically shown below:



Scheme 2.7 Preparation of Z-V-P-NH-$C_{10}H_{21}$

Step 1 had already been carried out in the synthesis of BZ-V-P-NH-$C_{10}H_{21}$ (III).

Step 2: Preparation of Z-V-P-OMe.

Z-V from 2.2.2 (7.5 g, 30 mmoles) was dissolved in dry THF (90 mL) and triethylamine (4.2 mL, 30 mmoles). The solution was cooled at -5 to -10°C with stirring before the addition of

isobutyl chloroformate (3.9 mL, 30 mmoles). The stirring was continued further at the same temperature for 15 minutes before the addition of a solution of phenylalanine methyl ester HCl from step 1 (6.6 g,30.5 mmoles) in THF (30 mL), water (15 mL) and triethylamine (4.2 mL,30 mmoles). The solution was stirred continuously for 10 hours at room temperature. After this time, the solution was placed in a separating funnel and ethyl acetate (75 mL) and water (15 mL) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3x30 mL). The combined organic layers were washed with saturated $NaHCO_3$ (15 mL), water (15 mL), 1 M HCl (15 mL) and water (15 mL) respectively and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the white powder (9.7 g,79.0%), mp.108-112°C was obtained.

Step 3: Preparation of Z-V-P-OH.

Z-V-P-OMe (9.7 g,23.7 mmoles) previously prepared in step 2 was dissolved in methanol (60 mL). With stirring at room temperature, 1 N NaOH (15 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. Then it was acidified with 1 M HCl.The mixture was extracted three times with a mixture of ethyl acetate and sodium hydrogen carbonate (5:1). The organic layer was separated and dried with Na_2SO_4 and then the solvent was removed under reduced pressure to the shiny white power solid (7.5 g, 79.7%), mp.163-165°C.

Step 4: Preparation of Z-V-P-NH-$C_{10}H_{21}$.

Z-V-P-OH (4.0 g,10 mmoles) previously prepared in step 3,was dissolved in dry THF (60 mL)and triethylamine (1.4 mL,10.1 mmoles). The solution was stirred and cooled at -5 to -10°C. After adding isobutylchloroformate (1.3 mL,10.1 mmoles) , the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine (2 mL,10.1 mmoles) in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL) ,H_2O(15 mL)1M HCl(15 mL) and H_2O(15 mL) respectively. After drying over anhydrous Na_2SO_4,the ethyl acetate was evaporated to give the white powder (3.9 g,73.0 %), mp.137-140°C.

TLC: R_f= 0.75 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{32}H_{47}N_3O_4$:

cal'd:C 71.47, H 8.81, N 7.81

found:C 71.01, H 8.64, N 7.63

IR(KBr) υ (cm^{-1}) (Fig. I.7):

3300(-NH stretching);3060,3020(-CH aromatic);2960, 2920, 2860 (-CH stretching); 1680, 1650 (HN-C=O stretching); 1530 (-NH bending).

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.20):

0.78 (m,9H,-CH_3,CH_3-CH-CH_3); 1.18 (s,20H,CH_2's chain); 1.91 (m,1H,CH_3-CH-CH_3); 2.88 (m,4H,-CH_2-Ar,-NH-CH_2-); 3.96(t,1H,-NH-CH-CH-CH_3);4.58 (q,1H,

-NH-C**H**-CH_2-Ar); 5.00 (s,2H,Ar-C**H_2**-O-); 6.32 (d,1H, -**N**H-CH_2-); 7.11 (s,5H,**Ar**-CH_2-CH-); 7.24 (s,5H,**Ar**-CH_2-O-); 7.78(br.,t,1H,-**N**H-CH-CH-CH_3); 7.90 (d,1H, -**N**H-CH-CH_2-Ar).

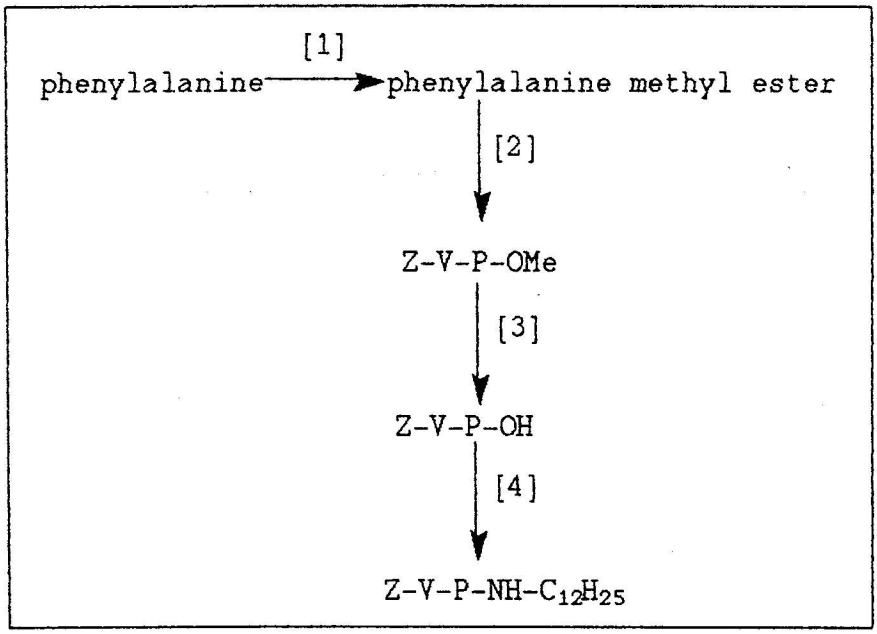
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.33):

14.1 (-**C**H_3);18.0,18.3 (-CH[**C**H_3]$_2$;19.1 (-**C**H[CH_3]$_2$); 22.6,26.9,27.7,29.3,29.7,31.8 (-**C**H_2's chain);38.6 (-NH-**C**H_2-); 39.4(-**C**H_2-Ar); 54.5 (-NH-**C**H-CH[CH_3]$_2$); 60.2(-NH-**C**H-CH_2-); 66.5(Ar-**C**H_2);126.4,127.7,128.1, 129.3, 136.5, 136.8, 136.9 (**Ar**-CH_2-CH-NH-, **Ar**CH_2-O-C=O); 156.2 (O=**C**-O-CH_2-Ar); 170.9 (O=**C**-CH-CH-CH_3); 171.8 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.46) in CH_3OH:CH_3Cl = 1:19

2.2.11 **Preparation of Z-V-P-NH-$C_{12}H_{25}$ (compound VIII)**

There are four steps of reaction involved in this preparation which is schematically described below:



Scheme 2.8 Preparation of Z-V-P-NH-$C_{12}H_{25}$

Step 1,2 and 3 had already been carried out in the synthesis of Z-V-T-NH-$C_{10}H_{21}$ (V).

Step 4: Preparation of Z-V-P-NH-$C_{12}H_{25}$.

Z-V-P-OH (4.0 g, 10 mmoles) previously prepared in step 3, was dissolved in dry THF (60 mL)and triethylamine (1.5 mL, 10.1 mmoles). The solution was stirred and cooled at -5 to -10°C. After adding isobutylchloroformate (1.4 mL, 10.1 mmoles) , the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine (2 mL, 10.1 mmoles) in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL) , H_2O(15 mL)1M HCl(15 mL) and H_2O(15 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The residue had been dried in the air and was

recrystallized with petroleum ether to the white powder (4.4 g, 78.0 %), mp. 146-149 °C.

TLC: R_f= 0.74 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{34}H_{51}N_3O_4$:

cal'd: C 72.18, H 9.09, N 7.43

found: C 72.13, H 9.12, N 7.14

IR(KBr) υ (cm^{-1}) (Fig. I.8):

3300 (-NH stretching); 3080, 3040 (-CH aromatic); 2960, 2920, 2860 (-CH stretching); 1680, 1650 (HN-C=O stretching); 1530 (-NH bending).

^1H NMR ($CDCl_3$) δ (ppm) (Fig. I.21):

0.87 (m, 9H, -**CH_3**, **CH_3**-CH-**CH_3**); 1.25 (s, 20H, **CH_2**'s chain); 2.08 (m, 1H, CH_3-**C**H-CH_3); 3.07 (m, 4H, -**CH_2**-Ar, -NH-**CH_2**-); 4.07 (t, 1H, -NH-**C**H-CH-CH_3); 4.66 (q, 1H, -NH-**C**H-CH_2-Ar); 5.07 (s, 2H, **Ar**-CH_2-O-); 5.57 (d, 1H, -**N**H-CH_2-); 7.21(s, 5H, **Ar**-CH_2-CH-); 7.32(d, 5H, **Ar**-CH_2-O-); 6.15 (d, 1H, -**N**H-CH-CH-CH_3); 7.09 (d, 1H, -**N**H-CH-CH_2-Ar).

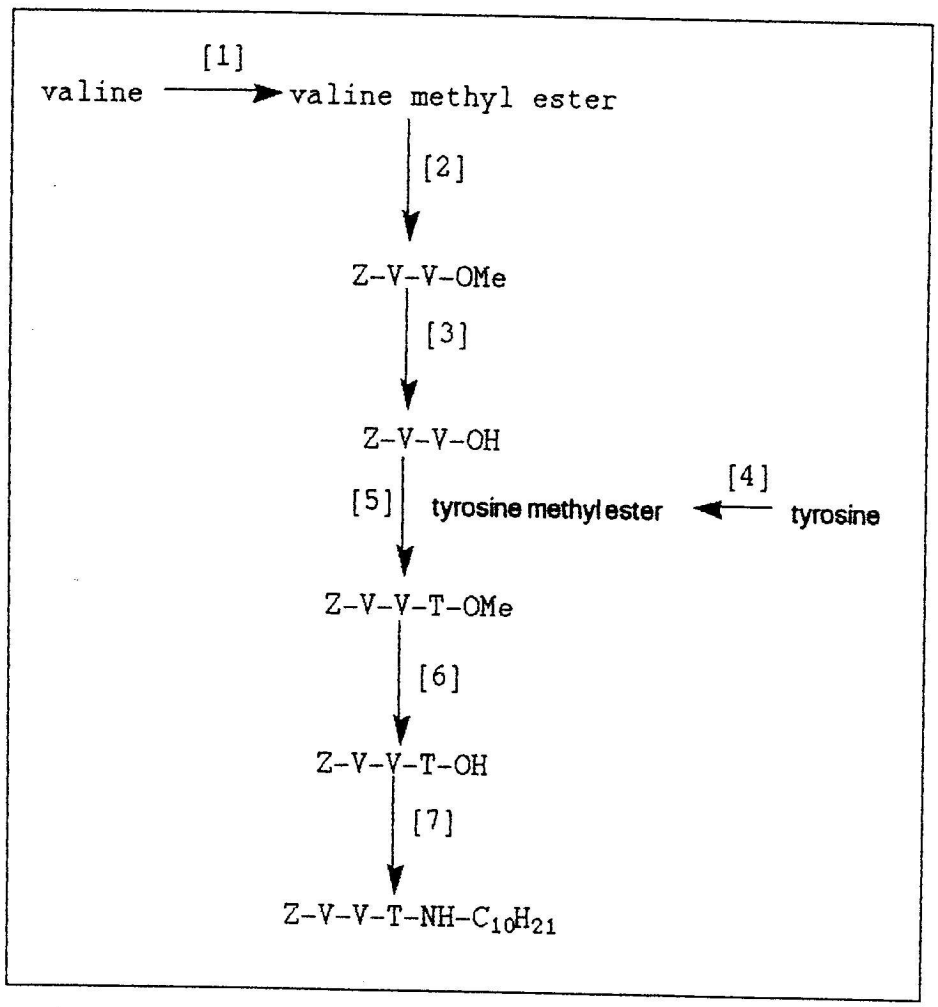
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.34):

14.1 (-**CH_3**); 18.3 (-CH[**CH_3**]$_2$; 19.0(-**C**H[CH_3]$_2$); 22.6, 26.9, 29.3, 29.4, 29.6, 29.7, 31.8 (-**CH_2**'s chain); 38.5 (-NH-**CH_2**-); 39.3 (-**CH_2**-Ar); 54.6 (-NH-**C**H-CH[CH_3]$_2$); 60.2 (-NH-**C**H-CH_2-); 66.5 (Ar-**CH_2**); 126.4, 127.7, 127.8, 128.2, 129.3, 136.7 (**Ar**-CH_2-CH-NH-, **Ar**-CH_2-O-C=O); 156.2(O=**C**-O-CH_2-Ar); 170.6 (O=**C**-CH-CH-CH_3); 171.5 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.47) in CH_3OH:CH_3Cl = 1:19

2.2.12 Preparation of Z-V-V-T-NH-$C_{10}H_{21}$ (compound IX)

There are seven steps of reaction involved in this preparation which is schematically described below:



Scheme 2.9 Preparation of Z-V-V-T-NH-$C_{10}H_{21}$

Step 1: Preparation of valine methyl ester.

L-Valine (23.4 g, 200 mmoles) was suspended in absolute methanol (200 mL). Thionyl chloride (15 mL) was added dropwise

and then refluxed the solution for 2 hours. The solvent was removed under rotatory evaporator reduced pressure to yield a very viscous oil which was permitted to stand in the refrigirator for a few hours. The hygroscopic solid of the hydrochloride salt of valine methyl ester, the needle white crystal (30.8 g, 91.8 %), m.p. 169-171 °C (lit. [62], m.p. 174 °C), was obtained after washing with absolute ether.

Step 2: Preparation of Z-V-V-OMe.

Z-V from 2.2.2 (25.1 g,100 mmoles) was dissolved in dry THF (250 mL) and triethylamine (14 mL,101 mmoles). The solution was cooled at -5 to -10 °C with stirring before the addition of isobutyl chloroformate (14 mL,101 mmoles). The stirring was continued further at the same temperature for 15 minutes before the addition of a solution of phenylalanine methyl ester HCl from step 1 (17 g,101 mmoles) in THF (100 mL) and triethylamine (14 mL,101 mmoles). The solution was stirred continuously for 10 hours at room temperature. After this time, the solution was placed in a separating funnel and ethyl acetate (100 mL) and water (15 mL) were added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (30 mL) many times. The combined organic layers were washed with saturated $NaHCO_3$ (25 mL), water (25 mL),1 M HCl (25 mL) and water (25 mL) respectively and then dried with anhydrous Na_2SO_4. The solvent was removed under reduced pressure and then the pale yellow oil was left. After few weeks, the white needle crystal (25.8 g,70.5%),mp. 84-87 °C was

obtained.

Step 3: Preparation of Z-V-V-OH

Z-V-V-OMe from step 2 was dissolved in methanol (100 mL) .With stirring at room temperature, 1N NaOH (15 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. The gelatinous precipitate was formed and was acidified with 1 M HCl under cooling condition.The mixture was kept at 4 °C overnight in refrigerator, and extracted three times with a mixture of ethyl acetate and sodium hydrogen carbonate (5:1). The organic layer was separated and then the solvent was removed under reduced pressure, the glassy needle crystal in oil (17.4 g,70.6%) was obtained after a few weeks of storage.

Step 4: Preparation of tyrosine methyl ester.

This step had already been carried out in the synthesis of BZ-V-T-NH-$C_{10}H_{21}$; Step 1.

Step 5: Preparation of Z-V-V-T-OMe.

Z-V-V-OH(7 g,20 mmoles) from step 3 dissolved in dry THF(60 mL) and triethylamine (2.8 mL,20 mmoles). The solution was stirred and cooled at -5 to -10 °C.After adding isobutyl chloroformate (2.6 mL,20 mmoles),the mixture was stirred at the same temperature for 10 minutes before a solution of tyrosine methyl ester HCl (5 g,21 mmoles) in dry THF (30 mL) was added. The solution was then stirred at room temperature

was added. The solution was then stirred at room temperature for 10 hours. To the filtered white solid was added ethyl acetate (25 mL) and water (5 mL) in a separating funnel. The organic layer was separated and washed with 5% $NaHCO_3$ (5 mL) ,H_2O (5 mL), 1 M HCl (5 mL) and H_2O (5 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The residue oil had been dried in the air for a few weeks.The white solid(8g,76.0%),mp. 197-200 °C was obtained.

Step 6: Preparation of Z-V-V-T-OH.

Z-V-V-T-OMe (8 g, 15.2 mmoles) from step 5 was dissolved in methanol(30 mL).With stirring at room temperature, 1N NaOH (5 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. The gelatinous precipitate was formed and was acidified with 1 M HCl under cooling condition. The mixture was kept at 4 °C overnight in refrigerator, and extracted three times with a mixture of ethyl acetate and sodium hydrogen carbonate (5:1). The organic layer was separated and then the solvent was removed under reduced pressure to yield an oil(6 g,76.3%).

Step 7: Preparation of Z-V-V-T-NH-$C_{10}H_{21}$.

Z-V-V-T-OH(2.6 g,5 mmoles)from step 6 was dissolved in dry THF and triethyamine (0.7 mL,5 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutyl chloroformate (0.7 mL,5 mmoles),the mixture was stirred at the

solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL), H_2O(15 mL) 1M HCl(15 mL) and H_2O(15 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated and the residue was washed many times with petroleum ether to yield a white powder (2.3 g, 70.0 %), m.p. 99-102 °C

TLC: R_f= 0.59 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{37}H_{56}N_4O_6$:

cal'd:C 68.07, H 8.65, N 8.58

found:C 68.85, H 8.95, N 8.66

IR(KBr) υ (cm^{-1}) (Fig. I.9):

3400 (-OH stretching); 3300 (-NH stretching); 3080, 3040(-CHaromatic); 2960, 2920 (-CH stretching); 1730, 1690, 1640 (HN-C=O stretching); 1530 (-NH bending).

^1H NMR ($CDCl_3$) δ (ppm) (Fig. I.22):

0.90 (m, 15H, -**CH_3**, 2[**CH_3**-CH-**CH_3**]); 1.24 (s, 16H, **CH_2**'s chain); 2.12 (br., 2H, 2[CH_3-**CH**-CH_3]); 3.08 (q, 2H, NH-**CH_2**-); 3.70(d, 4H, **CH_2**-Ar); 4.10(t, 1H, -NH-**CH**-CH_2-Ar); 4.50(m, 2H, 2[-NH-**CH**-CH-CH_3]); 5.07(s, 2H, Ar-**CH_2**-O-); 6.05 (br., 1H, -OH); 6.47 (t, 2H, -**NH**-CH_2-); 6.65, 6.93 (db. of db., 4H, -⬡-); 6.56, 6.78 (d, 1H, -**NH**-CH-CH-CH_3); 7.09 (d, 1H, -**NH**-CH-CH_2-Ar); 7.28 (s, 9H, **Ar**-CH_2-

CH-, **Ar**-CH_2-O-).

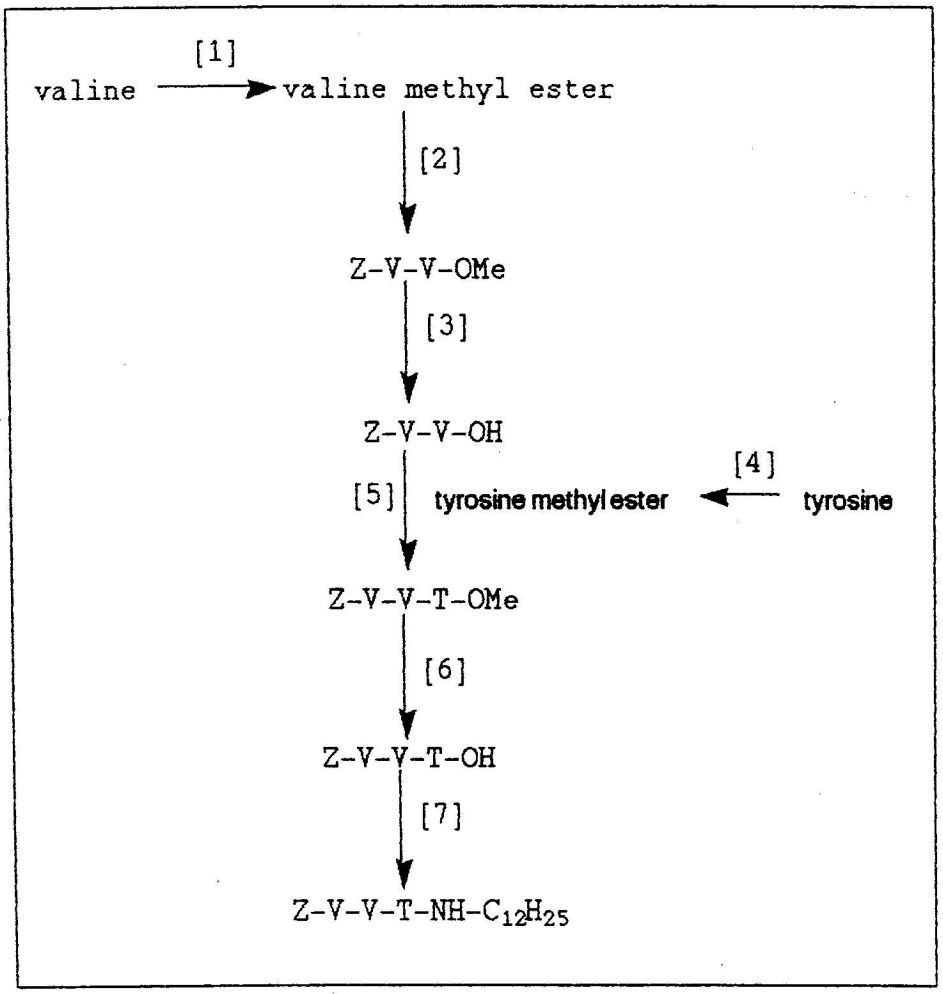
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.35):

17.7 (-**C**H_3); 18.1(-CH[**C**H_3]$_2$);18.9,19.1(-**C**H[CH_3]$_2$); 26.5,27.2,29.4,29.8(**C**H_2's chain); 31.1 (-NH-**C**H_2-); 51.8(-**C**H_2-Ar); 56.8 (-NH-**C**H-CH[CH_3]$_2$); 60.2 (-NH-**C**H-CH_2-); 66.7 (Ar-**C**H_2-O-); 120.5, 121.4, 127.8, 128.2, 130.2, 136.3 (**Ar**-CH_2-CH-NH-, **Ar**-CH_2-O-C=O); 156.0 (O=**C**-O-CH_2-Ar); 170.9 (O=**C**-CH-CH-CH_3); 171.9 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.48) in CH_3OH:CH_3Cl = 1:19

2.2.13 Preparation of Z-V-V-T-NH-$C_{12}H_{25}$ (compound X)

There are seven steps of reaction involved in this preparation which is schematically described below:



Scheme 2.10 Preparation of Z-V-V-T-NH-$C_{12}H_{25}$

Step 1-6 had already been carried out in the synthesis of Z-V-V-T-NH-$C_{10}H_{21}$(IX).

Step 7: Preparation of Z-V-V-T-NH-$C_{12}H_{25}$.

Z-V-V-T-OH (2.6 g, 5 mmoles) from step 6 was dissolved in dry THF and triethyamine (0.7 mL,5 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutyl chloroformate (0.7 mL, 5 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of dodecylamine (1 g,5 mmoles) in dry THF(30 mL) was added. The solution was then stirred at room temperature for 10 hours. To

the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL), H_2O (15 mL), 1M HCl (15 mL) and H_2O (15 mL) respectively.After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The white crystalline solid (2 g, 60.0 %), m.p. 236-238°C was obtained.

TLC: R_f= 0.57 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{39}H_{60}N_4O_6$:

calc'd:C 68.79, H 8.88, N 8.23

found :C 68.64, H 8.74, N 8.43

IR(KBr) υ (cm^{-1}) (Fig. I.10):

3400 (-OH stretching); 3300(-NH stretching); 3060, 3020 (-CH aromatic); 2950 (-CH stretching); 1680, 1630 (HN-C=O stretching);1540, 1520 (-NH bending).

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.23):

0.68, 0.82 (m,15H,-CH_3,2[CH_3-CH-CH_3]);1.20 (s,16H, CH_2's chain);1.93(br.,2H,2[CH_3-CH-CH_3]);2.83(q,2H, -NH-CH_2-); 3.56(d,4H,CH_2-Ar);3.98 (t,1H,-NH-CH-CH_2 -Ar); 4.23,4.42(m,2H,2[-NH-CH-CH-CH_3]); 5.00(s,2H, Ar-CH_2-O-);6.59,6.91(db.of db.,4H,-⟨o⟩-);7.29(s,5H, Ar-CH_2-O);7.59(d,1H,-NH-CH-CH_2-Ar);7.77(t,2H,-NH-CH_2-);8.15 (d,2H,-NH-CH-CH-CH_3);9.05 (br.,1H,-OH).

^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.36):

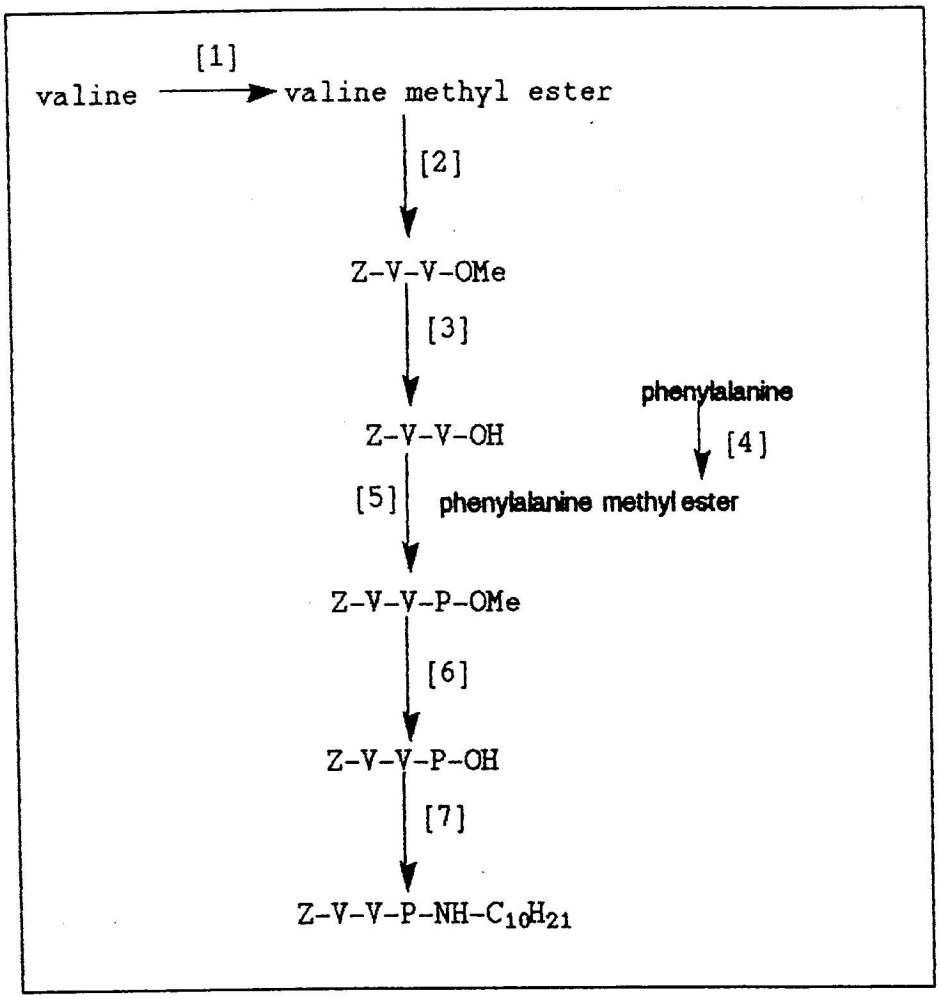
15.6 (-CH_3); 16.5 (-CH[CH_3]$_2$); 17.6 (-CH[CH_3]$_2$); 26.8, 29.1, 29.3 (CH_2's chain); 35.1 (-NH-CH_2-); 50.3 (-CH_2-Ar); 53.1, 56.8 (-NH-CH-CH[CH_3]$_2$);

59.9 (-NH-**C**H-CH_2-); 65.0 (Ar-**C**H_2-O-); 113.9, 126.5, 126.6, 127.1, 128.9 (**Ar**-CH_2-CH-NH-, **Ar**-CH_2-O-C=O); 155.2 (O=**C**-O-CH_2-Ar); 170.4, 170.9 (O=**C**-CH-CH-CH_3); 171.8 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.49) in CH_3OH:CH_3Cl = 1:19

2.2.14 Preparation of Z-V-V-P-NH-$C_{10}H_{21}$ (compound XI)

There are seven steps of reaction involved in this preparation which is schematically described below:



Scheme 2.11 Preparation of Z-V-V-P-NH-$C_{10}H_{21}$

Step 1,2 and 3 had already been carried out in the synthesis of Z-V-V-T-NH-$C_{10}H_{21}$(IX).

Step 4 had already carried out in the synthesis of BZ-V-P-NH-$C_{10}H_{21}$(III).

Step 5: Preparation of Z-V-V-P-OMe.

Z-V-V-OH (7.0 g,20 mmoles) from step 3 was dissolved in dry THF(30 mL) and triethylamine(2.9 mL, 20.5 mmoles). The solution was stirred and cooled at -5 to -10 °C.After adding isobutyl chloroformate (2.9 mL, 20.5 mmoles), the mixture was stirred at the same temperature for 10 minutes before a

solution of phenylalanine methyl ester HCl(4.4 g, 20.5 mmoles) from step 4 in dry THF (10 mL) was added. The solution was then stirred at room temperature for 10 hours. To the filtered white solid was added ethyl acetate (20 mL) and water (5 mL) in a separating funnel. The organic layer was separated and washed with 5% $NaHCO_3$(5 mL),H_2O(5 mL),1 M HCl (5 mL) and H_2O (5 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The residue oil had been dried in the air for a few weeks. The white powder(7.9 g,77.5%),mp.192-194 °C was obtained.

Step 6: Preparation of Z-V-V-P-OH.

Z-V-V-P-OMe(8 g,16 mmoles)from step 5 was dissolved in methanol (30 mL).With stirring at room temperature, 1 N NaOH (5 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. The gelatinous precipitate was formed and acidified with 1 M HCl under cooling condition. The mixture was kept at 4 °C overnight in refrigerator, and extracted three times with a mixture of ethyl acetate and sodium hydrogen carbonate (5:1). The organic layer was separated and then the solvent was removed under reduced pressure to a waxy pale yellow gel(5.6 g, 72.3 %)

Step 7: Preparation of Z-V-V-P-NH-$C_{10}H_{21}$.

Z-V-V-P-OH (2.5 g,5 mmoles) from step 6 as dissolved in dry THF and triethyamine (0.7 mL,5 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutyl

chloroformate (0.8 mL, 5.5 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine (1.1 mL,5.5 mmoles) in dry THF(30 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL), H_2O (15 mL), 1M HCl (15 mL) and H_2O (15 mL) respectively.After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated.The white powder (1.8 g,58.0 %), mp.151-153 °C was obtained.

TLC: R_f= 0.70 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{37}H_{56}N_4O_5$:

calc'd: C 69.78, H 8.86, N 8.80

found : C 69.82, H 8.90, N 8.72

IR(KBr) υ (cm^{-1}) (Fig. I.11):

3300 (-NH stretching); 3040 (-CH aromatic); 2940, 2860 (-CH stretching);1680,1640 (HN-C=Ostretching) ; 1530 (-NH bending).

^1H NMR ($CDCl_3$) δ (ppm) (Fig. I.24):

0.87(m,15H,-**CH_3**,2[**CH_3**-CH-**CH_3**]); 1.24 (s,16H,**CH_2**'s chain); 2.10 (m,2H,2[CH_3-**CH**-CH_3]); 3.10 (q,2H,-NH-**CH_2**-);3.70(s,4H,-**CH_2**-Ar,);4.00(t,1H,-NH-**CH**-CH_2-Ar) 4.22,4.85 (t,2H,2[-NH-**CH**-CH-CH_3]); 5.10 (s,4H,Ar-**CH_2**-O-); 5.32, 6.44 (d,2H,2[-**NH**-CH-CH-CH_3]); 6.25 (d,1H,**NH**-CH_2); 7.08 (br.,1H,-**NH**-CH-CH_2-Ar); 7.25 (s,5H,**Ar**-CH_2-O-); 7.33 (s,5H,**Ar**-CH_2-CH-)

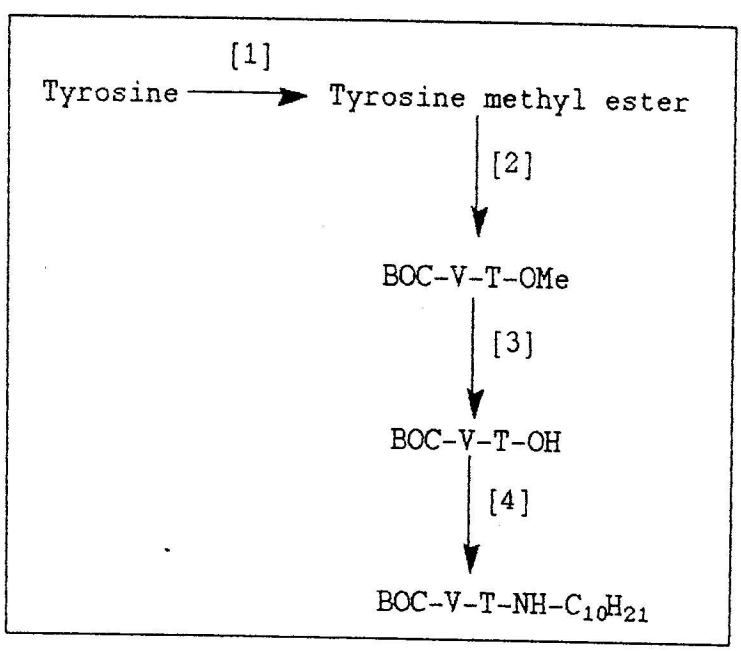
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.37):

16.4 (-**C**H_3); 16.6 (-CH[**C**H_3]$_2$);18.3,18.5(-CH[**C**H_3]$_2$); 26.0,27.1,28.4,28.6 (**C**H_2's chain); 30.2(-NH-**C**H_2-); 51.5 (-**C**H_2-Ar); 56.4 (-NH-**C**H-CH[CH_3]$_2$); 60.4 (-NH-**C**H-CH_2-);67.5 (Ar-**C**H_2-O-);116.1, 126.8, 127.4, 127.6, 127.9, 128.2, 136.5 (**Ar**-CH_2-CH-NH-,**Ar**-CH_2-O-C=O); 154.4 (O=**C**-O-CH_2-Ar); 170.1 (O=**C**-CH-CH-CH_3); 171.7 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.50) in CH_3OH:CH_3Cl = 1:19

2.2.15 Preparation of BOC-V-T-NH-$C_{10}H_{21}$ (compound XII)

There are seven steps of reaction involved in this preparation which is schematically described below:



Scheme 2.12 Preparation of BOC-V-T-NH-$C_{10}H_{21}$

Step 1 had already been carried out in the synthesis of BZ-V-NH-$C_{10}H_{21}$(I).

Step 2: Preparation of BOC-V-T-OMe.

BOC-V(6.5,30 mmoles) from 2.2.3 was dissolved in dry THF (90 mL) and triethylamine(4.3 mL,30.5 mmoles). The solution was stirred and cooled at -5 to -10 °C.After adding isobutyl chloroformate (4.0 mL, 30.5 mmoles),the mixture was stirred at the same temperature for 15 minutes before a solution of tyrosine methyl ester (7.1 g,30.5 mmoles) from step 1 in dry THF (30 mL) was added. The solution was then stirred at room temperature for 10 hours. To the filtered white solid was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was separated and washed with 5% $NaHCO_3$ (5 mL),H_2O (5 mL), 1 M HCl (5 mL) and H_2O (5 mL) respectively. After drying over anhydrous Na_2SO_4, the ethyl acetate was evaporated. The residue had been dried in the air for a few weeks. The white powder (8.8 g,22.3 mmoles),mp.161-163 °C was obtained after having been washed with petroleum ether.

Step 3: Preparation of BOC-V-T-OH.

BOC-V-T-OMe (8.8 g, 22.3 mmoles) from step 5 was dissolved in methanol (30 mL).With stirring at room temperature, 1 N NaOH (15 mL) was added dropwise. The solution was stirred for 5 hours at room temperature. The gelatinous precipitate was formed and acidified with 1 M HCl under cooling condition. The mixture was kept at 4 °C overnight in

refrigerator, and extracted three times with a mixture of ethyl acetate and sodium hydrogen carbonate (5:1). The organic layer was separated and then the solvent was removed under reduced pressure to yield an oil(6 g,70.8%)

Step 4: Preparation of BOC-V-T-NH-$C_{10}H_{21}$.

BOC-V-T-OH (3 g,7.9 mmoles) previously prepared in step 3, was dissolved in dry THF and triethyamine(0.7 mL,5 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutyl chloroformate(1 mL,8 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of decylamine(1.1 mL,5.5 mmoles) in dry THF(30 mL) was added. The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL), H_2O (15 mL), 1M HCl (15 mL) and H_2O (15 mL) respectively.After drying over anhydrous Na_2SO_4,the ethyl acetate was evaporated.Yield an oil was left in a dessicator for a few weeks,the white solid (2.7 g, 65.8 %), mp.169-172 °C was obtained after being washed with ether.

TLC: R_f= 0.69 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{29}H_{49}N_3O_5$:

calc'd: C 67.02, H 9.50, N 8.09

found : C 67.19, H 9.73, N 8.09

IR(KBr) υ (cm^{-1}) (Fig. I.12):

3600 (-OH stretching); 3340 (-NH stretching);

3020 (-CH aromatic); 2980, 2940, 2860 (-CH stretching); 1680, 1650 (HN-C=O stretching); 1520 (-NH bending).

^{1}H NMR ($CDCl_3$) δ (ppm) (Fig. I.25):

0.87 (m,9H,-C**H$_3$**, C**H$_3$**-CH-C**H$_3$**); 1.24 (s,16H,C**H$_2$**'s chain);1.45 (s,9H,-C[**CH$_3$**]$_3$);2.16(m,1H,CH_3-C**H**-CH_3); 2.93 (m,4H,-C**H$_2$**-Ar,-NH-C**H$_2$**-); 3.17 (t,1H,-NH-C**H**-CH_2-Ar); 3.88 (m,1H,-NH-C**H**-CH-CH_3);4.57 (q,1H,-N**H**-CH_2-); 4.91 (d,1H,-N**H**-CH-CH_2-Ar);6.14 (br.,1H,-OH) ;6.56 (d,1H,-N**H**-CH-CH-CH_3); 6.76, 7.06 (db. of db. ,4H,-⟨⬡⟩-);6.94(br.,1H,-OH).

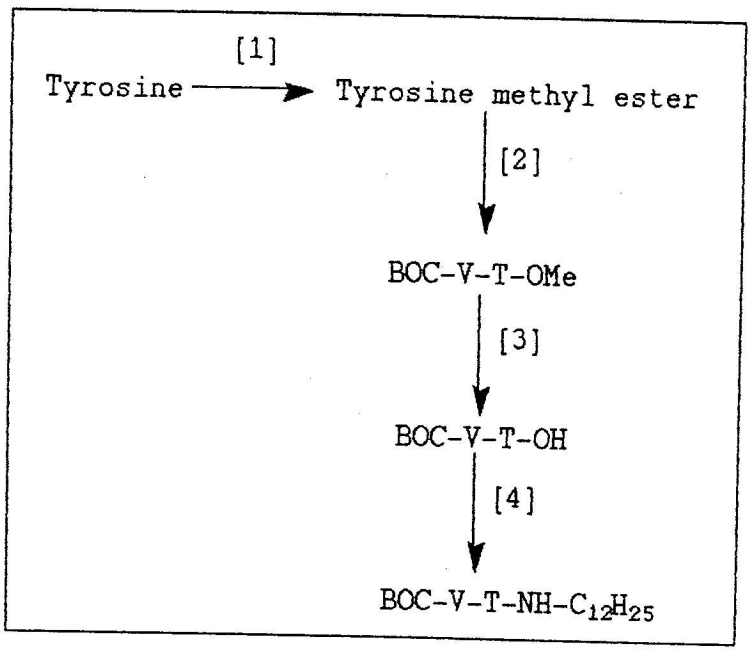
^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.38):

13.8(-**C**H_3); 17.7 (-CH[**C**H_3]$_2$); 18.9 (-CH[**C**H_3]$_2$); 22.2, 26.5, 28.0, 28.9, 29.1, 30.5,31.4 (**C**H_2's chain); 37.2 (-NH-**C**H_2-); 38.9 (-**C**H_2-Ar); 53.9 (-NH-**C**H-CH[CH_3]$_2$); 59.9 (-NH-**C**H-CH_2-); 95.7 (-**C**[CH_3]$_3$); 114.6, 127.1, 129.8 (HO-**Ar**-CH_2); 155.7 (O=**C**-O-C[CH_3]); 170.4 (O=**C**-CH-CH-CH_3); 170.9 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.51) in CH_3OH:CH_3Cl = 1:19

2.2.16 Preparation of BOC-V-T-NH-$C_{12}H_{25}$ (compound XIII)

There are four steps of reaction involved in this preparation which is schematically described below:



Scheme 2.13 Preparation of BOC-V-T-NH-$C_{12}H_{25}$

Step 1,2 and 3 had already been carried out in the synthesis of BOC-V-T-NH-$C_{10}H_{21}$ (XII).

Step 4: Preparation of BOC-V-T-NH-$C_{12}H_{25}$.

BOC-V-T-OH (3.0 g,7.9 mmoles) previously prepared in step 3,was dissolved in dry THF (60 mL). and triethylamine (1.1 mL,8 mmoles). The solution was stirred and cooled at -5 to -10 °C. After adding isobutyl chloroformate (1.4 mL, 10.1 mmoles), the mixture was stirred at the same temperature for 10 minutes before a solution of dodecylamine (1.5 g, 8 mmoles) in dry THF (10 mL) was added.The solution was then stirred at room temperature for 10 hours. To the residue was added ethyl acetate (60 mL) and water (15 mL) in a separating funnel. The organic layer was collected and washed with 5% $NaHCO_3$(15 mL) ,H_2O (15 mL), 1M HCl (15 mL) and H_2O (15 mL) respectively. After drying over anhydrous Na_2SO_4,the ethyl acetate was

evaporated.The residue had been dried in the air and was recrystallized with petroleum ether to the white powder (3.5 g,81.0 %), mp.168-170 °C

TLC: R_f= 0.57 ($CHCl_3$:MeOH:AcOH = 90:5:5)

Elemental Analysis for $C_{31}H_{53}N_3O_5$:

cal'd:C 67.97, H 9.75, N 7.67

found:C 67.41, H 9.34, N 7.46

IR(KBr) υ (cm^{-1}) (Fig. I.13):

3600 (-OH stretching); 3300 (-NH stretching); 3080, 3040 (-CH aromatic); 2920, 2860 (-CH stretching); 1690, 1640 (HN-C=O); 1540 (-NH bending).

^1H NMR ($CDCl_3$+DMSO) δ (ppm) (Fig. I.26):

0.81 (m,9H,-C**H**$_3$,C**H**$_3$-CH-C**H**$_3$); 1.18 (s,20H,C**H**$_2$'s chain); 1.35 (s,9H,-C[C**H**$_3$]$_3$); 1.90 (m,1H,CH_3-C**H**-CH_3); 2.82 (d,2H, -C**H**$_2$-Ar); 3.00 (m, 2H, -NH-C**H**$_2$-); 3.75 (t, 1H, -NH-C**H**-CH-CH_3); 4.45 (q,1H, -NH-C**H**-CH_2-Ar); 5.92 (d,1H, -N**H**-CH_2-); 6.56, 6.92 (db. of db.,4H,-⟨⊙⟩-); 7.15 (d, 1H -N**H**-CH-CH_2-Ar); 7.41(d,1H,-N**H**-CH-CH-CH_3);8.71(br., 1H,-OH).

^{13}C NMR ($CDCl_3$) δ (ppm) (Fig. I.39):

13.8 (-**C**H_3); 17.6 (-CH[**C**H_3]$_2$); 18.9 (-CH[**C**H_3]$_2$); 22.2, 26.5, 28.0, 28.9,29.1,29.2, 30.4, 31.4(**C**H_2's chain); 37.1 (-NH-**C**H_2-); 39.1 (-**C**H_2-Ar); 54.2 (-NH-**C**H-CH[CH_3]$_2$); 60.1 (-NH-**C**H-CH_2-); 95.7 (-**C**[CH_3]$_3$); 114.9, 127.2, 129.8 (HO-**Ar**-CH_2);

155.7 (O=**C**-O-C[CH_3]); 170.4 (O=**C**-CH-CH-CH_3); 171.0 (O=**C**-CH-CH_2-Ar).

HPLC chromatogram (Fig. I.52) in CH_3OH:CH_3Cl = 1:19

2.3 Enzyme kinetic assays

2.3.1 Preparation of solution

Trypsin (0.0507 g) was dissolved in 1 mM HCl (10 mL) and kept in an ice bath or a refrigeator; trypsin 5070 ppm.

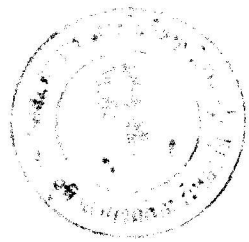
Chymotrypsin (0.0115 g) was dissolved in 1 mM HCl (10 mL) and kept in an ice bath or a refrigerator; chymotrypsin 1150 ppm. Chymotrypsin was diluted to 11.50 ppm because of its high activity.

BAPNA (substrate for trypsin ; 0.0217 g) was dissolved in DMSO (25 mL); BAPNA 2 mM.

Suc-Ala-Ala-Pro-Phe-pNA (substrate for chymotrypsin 0.0312 g) was dissolved in DMSO (25 mL); Suc. 2 mM.

HEPES buffer solution was prepared by mixing 0.1 M HEPES (5.9581 g) and 0.05 M NaCl (0.7305 g) in distilled water (250 mL each), then adjusted to pH 7.0 by adding 10 M NaOH dropwise.

The synthetic inhibitors (10 mM) were dissolved in DMSO then diluted to 0.1 mM.



2.3.2 Determination of the optimum condition for the enzyme kinetics

It is necessary that the optimum temperature of trypsin and chymotrypsin was 37 °C which is equal to the temperature of a human's body. JASCO UVIDEC-650 Double beam spectrophotometer equipped with a 10 mm matched semi-micro precision optical cell and EYELA Digital UNI ACE UA-100 temperature controller was used for studying the enzyme kinetics. The enzyme was preincubated with HEPES buffer (pH 7.0), DMSO, 1 mM HCl for 5 minutes at 37 °C before adding substrate. To a cuvette was added the substrate to make a final volume of 1000 μL, then the mixture was shaken very fast. The initial rate of the production of *p*-nitroaniline was followed by measurement of the increase in absorbance at 400 nm. The absorbance was recorded every 5 second, approximately 25 minutes incubation time till it was not changed. The sample cell contained enzyme, while the reference cell had no enzyme present.

The determination of the appropriate amount of trypsin and chymotrypsin was carried out by preparing the solution as in Table 2.1.

The determination of appropriate amount of substrate has already been done in the determination of appropriate amount of enzyme, Table 2.1, but it can be shown in varying the substrate concentration aspect as in Table 2.2.

Table 2.1 The prepared solution as a function of varying enzyme concentration.

substrate(μL)	enzyme (μL)	HEPES (μL)	HCL (μL)	DMSO (μL)
25	25	500	175	175
	50	500	150	175
	75	500	125	175
	100	500	100	175
	125	500	75	175
	150	500	50	175
	200	500	-	175
50	25	500	175	150
	50	500	150	150
	75	500	125	150
	100	500	100	150
	125	500	75	150
	150	500	50	150
	200	500	-	150
75	25	500	175	125
	50	500	150	125
	75	500	125	125
	100	500	100	125
	125	500	75	125
	150	500	50	125
	200	500	-	125
100	25	500	175	100
	50	500	150	100
	75	500	125	100
	100	500	100	100
	125	500	75	100
	150	500	50	100
	200	500	-	100
125	25	500	175	75
	50	500	150	75
	75	500	125	75
	100	500	100	75
	125	500	75	75
	150	500	50	75
	200	500	-	75
150	25	500	175	50
	50	500	150	50
	75	500	125	50
	100	500	100	50
	125	500	75	50
	150	500	50	50
	200	500	-	50
200	25	500	175	-
	50	500	150	-
	75	500	125	-
	100	500	100	-
	125	500	75	-
	150	500	50	-
	200	500	-	-

Table 2.2 The prepared solution as a function of varying substrate concentration.

enzyme (uL)	substrate (uL)	HEPES (uL)	DMSO (uL)	HCL (uL)
25	25	500	175	175
	50	500	150	175
	75	500	125	175
	100	500	100	175
	125	500	75	175
	150	500	50	175
	200	500	-	175
50	25	500	175	150
	50	500	150	150
	75	500	125	150
	100	500	100	150
	125	500	75	150
	150	500	50	150
	200	500	-	150
75	25	500	175	125
	50	500	150	125
	75	500	125	125
	100	500	100	125
	125	500	75	125
	150	500	50	125
	200	500	-	125
100	25	500	175	100
	50	500	150	100
	75	500	125	100
	100	500	100	100
	125	500	75	100
	150	500	50	100
	200	500	-	100
125	25	500	175	75
	50	500	150	75
	75	500	125	75
	100	500	100	75
	125	500	75	75
	150	500	50	75
	200	500	-	75
150	25	500	175	50
	50	500	150	50
	75	500	125	50
	100	500	100	50
	125	500	75	50
	150	500	50	50
	200	500	-	50
200	25	500	175	-
	50	500	150	-
	75	500	125	-
	100	500	100	-
	125	500	75	-
	150	500	50	-
	200	500	-	-

The results of initial velocities as a function of varying the trypsin, chymotrypsin, BAPNA and Suc-Ala-Ala-Pro-Phe-pNA concentration are shown in Table 2.3,2.4,2.5 and 2.6 respectively.

Table 2.3 Initial velocities as a function of varying trypsin concentration.

BAPNA (μL)	Trypsin (μL)	Initial Velocities (Abs/min)
25	25	0.0040
	50	0.0051
	75	0.0062
	100	0.0166
	125	0.0189
	150	0.0199
	200	0.0211
50	25	0.0057
	50	0.0138
	75	0.0167
	100	0.0254
	125	0.0257
	150	0.0253
	200	0.0258
75	25	0.0098
	50	0.0150
	75	0.0204
	100	0.0258
	125	0.0368
	150	0.0382
	200	0.0393
100	25	0.0165
	50	0.0180
	75	0.0228
	100	0.0289
	125	0.0353
	150	0.0351
	200	0.0355
125	25	0.0169
	50	0.0200
	75	0.0265
	100	0.0354
	125	0.0355
	150	0.0352
	200	0.0368
150	25	0.0560
	50	0.0631
	75	0.0817
	100	0.0929
	125	0.1322
	150	0.1308
	200	0.1320
200	25	0.0550
	50	0.0645
	75	0.0836
	100	0.1300
	125	0.1286
	150	0.1326
	200	0.1374

Table 2.4 Initial velocities as a function of varying chymotrypsin concentration.

Suc-Ala-Ala-Pro-Phe-PNA(μL)	Chymotrypsin (μL)	Initial Velocities (Abs / min)
25	25	0.0021
	50	0.0031
	75	0.0049
	100	0.0053
	125	0.0063
	150	0.0109
	200	0.0117
50	25	0.0043
	50	0.0081
	75	0.0083
	100	0.0089
	125	0.0159
	150	0.0165
	200	0.0173
75	25	0.0048
	50	0.0088
	75	0.0103
	100	0.0130
	125	0.0185
	150	0.0191
	200	0.0199
100	25	0.0074
	50	0.0090
	75	0.0174
	100	0.0183
	125	0.0267
	150	0.0274
	200	0.0309
150	25	0.0081
	50	0.0104
	75	0.0200
	100	0.0236
	125	0.0294
	150	0.0312
	200	0.0318
200	25	0.0080
	50	0.0111
	75	0.0219
	100	0.0251
	125	0.0344
	150	0.0455
	200	0.0520

Table 2.5 Initial velocities as a function of varying BAPNA concentration.

Trypsin (uL)	BAPNA (uL)	Initial Velocities (Abs/min)
25	25	0.0040
	50	0.0057
	75	0.0098
	100	0.0165
	125	0.0189
	150	0.0560
	200	0.0550
50	25	0.0051
	50	0.0158
	75	0.0150
	100	0.0180
	125	0.0200
	150	0.0631
	200	0.0645
75	25	0.0062
	50	0.0167
	75	0.0204
	100	0.0228
	125	0.0265
	150	0.0817
	200	0.0836
100	25	0.0166
	50	0.0254
	75	0.0258
	100	0.0289
	125	0.0354
	150	0.0929
	200	0.1300
125	25	0.0169
	50	0.0257
	75	0.0368
	100	0.0353
	125	0.0355
	150	0.1322
	200	0.1286
150	25	0.0199
	50	0.0253
	75	0.0382
	100	0.0351
	125	0.0352
	150	0.1308
	200	0.1326
200	25	0.0211
	50	0.0258
	75	0.0393
	100	0.0355
	125	0.0368
	150	0.1320
	200	0.1374

Table 2.6 Initial velocities as a function of varying Suc-Ala-Ala-Pro-Phe-*p*NA concentration.

Chymotrypsin (*u*L)	Suc-Ala-Ala-Pro-Phe-PNA (*u*L)	Initial Velocities (Abs/min)
25	25	0.0021
	50	0.0043
	75	0.0048
	100	0.0074
	150	0.0081
	200	0.0080
50	25	0.0031
	50	0.0081
	75	0.0088
	100	0.0090
	150	0.0104
	200	0.0111
75	25	0.0049
	50	0.0083
	75	0.0103
	100	0.0174
	150	0.0200
	200	0.0219
100	25	0.0053
	50	0.0089
	75	0.0130
	100	0.0183
	150	0.0236
	200	0.0251
125	25	0.0063
	50	0.0159
	75	0.0185
	100	0.0267
	150	0.0294
	200	0.0334
150	25	0.0109
	50	0.0165
	75	0.0191
	100	0.0274
	150	0.0312
	200	0.0455
200	25	0.0117
	50	0.0173
	75	0.0199
	100	0.0309
	150	0.0318
	200	0.0520

2.3.3 Determination of the percentage inhibition of synthetic inhibitors.

The optimum conditions obtained in the previous section were used for the determination of percentage inhibition. Each of synthetic inhibitors was varied in different concentrations. The assay was the same as the determination of optimum condition except having the inhibitor in concern. That is the enzyme was preincubated with the inhibitor or without the inhibitor for 5 minutes at 37 °C before the addition of substrate. The sample cell had enzyme plus inhibitor , while the reference cell had only inhibitor without enzyme.

The prepared solutions for determination of the percentage inhibition of synthetic inhibitors are schematically shown below.

Control Run :	Sample cell (μL)	Reference cell (μL)
HEPES	500	500
Enzyme: trypsin	100	-
or chymotrypsin	200	-
1 mM HCl (trypsin)	100	200
(chymotrypsin)	-	200
Inhibitor	-	-
DMSO	100	100
Substrate	200	200

Inhibitor Run :	Sample cell (μL)	Reference cell (μL)
HEPES	500	500
Enzyme: trypsin	100	-
or chymotrypsin	200	-
1 mM HCl (trypsin)	100	200
(chymotrypsin)	-	200
Inhibitor	1, 10, 25, 50, 75, 100	1, 10, 25, 50, 75, 100
DMSO	99, 90, 75, 50, 25, -	99, 90, 75, 50, 25, -
Substrate	200	200

Percentage inhibition was calculated as follows :

$$\% \text{ inhibition} = \frac{a - b}{a} \times 100$$

where a = rate of the release of absorbing species in the absence of inhibitor.

b = rate of the release of absorbing species in the presence of inhibitor.

The results of determination of the percentage of inhibition are shown in Table 2.7.

Table 2.7 Percentage inhibition of synthetic inhibitors.

No.	Inhibitor	Enzyme	% Inhibition					
			10.0uM	7.5uM	5.0uM	2.5uM	1.0uM	0.1uM
I	BVT-10	trypsin	41.76	38.90	28.13	9.45	-	-
		chymotrypsin	73.27	58.46	53.08	43.65	38.85	0
II	BVT-12	trypsin	53.85	51.43	50.99	41.76	23.30	-
		chymotrypsin	53.08	48.27	32.12	1.92	-	-
III	BVP-10	trypsin	38.02	31.43	29.23	23.31	5.49	-
		chymotrypsin	66.73	63.27	44.42	37.50	-	-
IV	BVP-12	trypsin	36.84	22.56	10.90	0	-	-
		chymotrypsin	55.64	39.47	10.90	-	-	-
V	ZVT-10	trypsin	54.95	51.21	46.37	35.60	19.34	0
		chymotrypsin	62.50	60.00	54.23	39.42	-	-
VI	ZVT-12	trypsin	51.65	47.69	42.86	36.26	10.11	-
		chymotrypsin	59.04	58.27	57.88	41.15	31.73	-
VII	ZVP-10	trypsin	48.79	44.40	37.36	11.43	5.49	-
		chymotrypsin	67.69	60.38	54.42	49.04	27.12	0
VIII	ZVP-12	trypsin	44.62	35.16	34.07	24.18	-	-
		chymotrypsin	77.31	69.04	54.42	-	-	-
IX	ZVVT-10	trypsin	60.22	55.82	41.76	40.44	38.68	0
		chymotrypsin	61.15	55.38	53.08	51.15	36.92	-
X	ZVVT-12	trypsin	38.46	37.36	30.33	25.05	3.96	-
		chymotrypsin	57.88	33.27	30.58	27.50	19.55	-
XI	ZVVP-10	trypsin	46.15	41.76	37.58	32.09	3.30	-
		chymotrypsin	56.35	49.04	34.81	30.38	9.40	-
XII	BOC-V-T-10	trypsin	52.31	50.99	32.75	30.11	-	-
		chymotrypsin	71.95	53.65	45.19	23.27	-	-
XIII	BOC-V-T-12	trypsin	51.43	37.14	33.63	16.04	-	-
		chymotrypsin	43.65	42.50	38.85	31.43	14.29	-