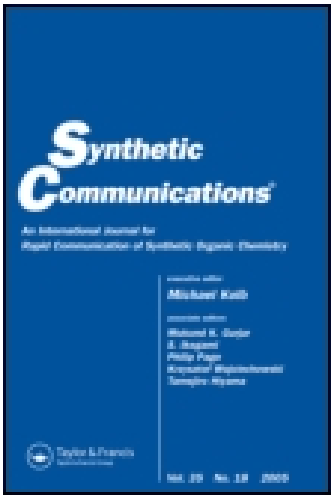

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New Ion-Exchange cum Separation Technique: A Study for the Synthesis of ω-Guanidine containing Peptides using ω-Amino Acid as Surrogate

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New Ion-Exchange cum Separation Technique: A Study for the Synthesis of ω-Guanidine containing Peptides using ω-Amino Acid as Surrogate

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Abstract: This article describes a systematic study for the introduction of ω-guanidine function at a late stage of synthesis using a protected amino group as a surrogate to improve overall yield. This concept was used to design and synthesize pseudo-peptides as GP IIb–IIIa receptor antagonist wherein glycine in endogenous ligand Arg-Gly-Asp (RGD) is replaced by 2-amino-thiazole-4-ylacetic acid (Tha) as a spacer. Further, we describe here a unique salt exchange cum purification technology based on reverse phase (RP-18) medium-pressure liquid chromatography.

Keywords: amino group as surrogate, GP IIb–IIa, RGD, salt exchange cum RP-18 purification, ω-guanidine function

1 INTRODUCTION

Fibrinogen is a major blood glycoprotein. It plays an essential role in haemostasis. Fibrinogen maintains blood viscosity and has a role in developing the haemostatic plug.[1] Higher levels of fibrinogen have found to be associated with increased age, high blood pressure, obesity, smoking, high stress, and diabetes.

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With the discovery of inhibitory effects of Arg-Gly-Asp (RGD) residue against the binding of fibrinogen to GP IIb–IIIa, a plethora of information accumulated in the literature describing synthetic mimics of RGD as GP IIb–IIIa antagonists in both peptide and nonpeptide areas.[2–8] The essential features identified during previous structure–activity studies indicate the presence of a basic functional moiety such as guanidino, amino, or benzamidino and a carboxylic acid moiety appropriately oriented with respect to each other. It is equally important to place them at a certain distance apart on a suitable template.

We have designed our molecule for the synthesis of GP IIb–IIIa antagonist based on the idea of replacing glycine with a small spacer. We had used the 2-amino-thiazole-4-yl-acetyl group as a glycine substitute. The assumption was that the amino group of thiazole would make a pseudo-peptide linkage while the CH_2 group between the thiazole ring and carboxylic group, because of its free rotation, would achieve the right orientation. We also believed that replacement of arginine by other ω-guanidino acids would help us to optimize the critical distance between the basic and the acidic moiety as well as the role of a-amino group of arginine. It is well established now that introduction of a hydrophobic residue around C-terminus and amidation of α-carboxylic acid of Asp residue increases GP IIb–IIIa antagonistic activity.[2,9] We had incorporated these thoughts in our synthetic strategy. Thus we had decided to synthesize nonpeptides of the general structure shown in Fig. 1. Another objective of the present study was to develop a synthetic strategy for the introduction of ω-guadino functionality at a late stage of synthesis.

2 RESULTS AND DISCUSSION

Scheme 1 describes our initial strategy to maintain Arg residue at N-terminus, AspNHR as C-terminus, and 2-amino-thiazol-4-yl acetyl (Tha) as spacer. The C-terminal intermediate Asp (OBzl) NHR was prepared in two steps. First, Boc-Asp (OBzl)[10] was converted into the corresponding amide by mixed anhydride procedure using isobutyl chloroformate and NMM.[11] In the next step, the Boc group was removed by formic acid–anisole.[12] The amino

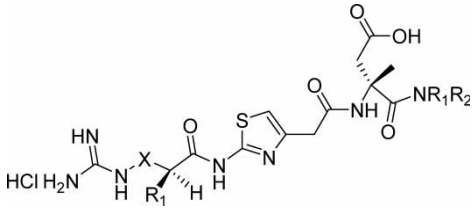
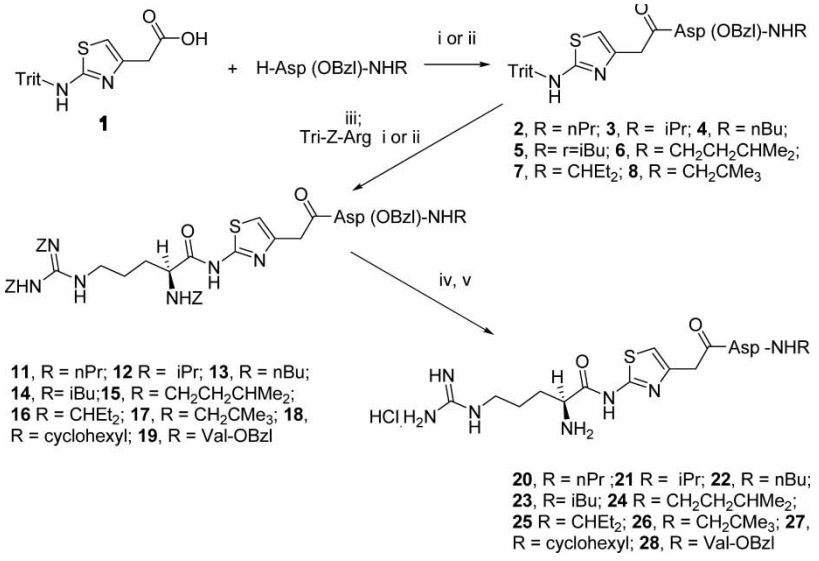


Figure 1. (R1 = NH_2 or H; X = $(CH_2)_n$; n = 0–3).



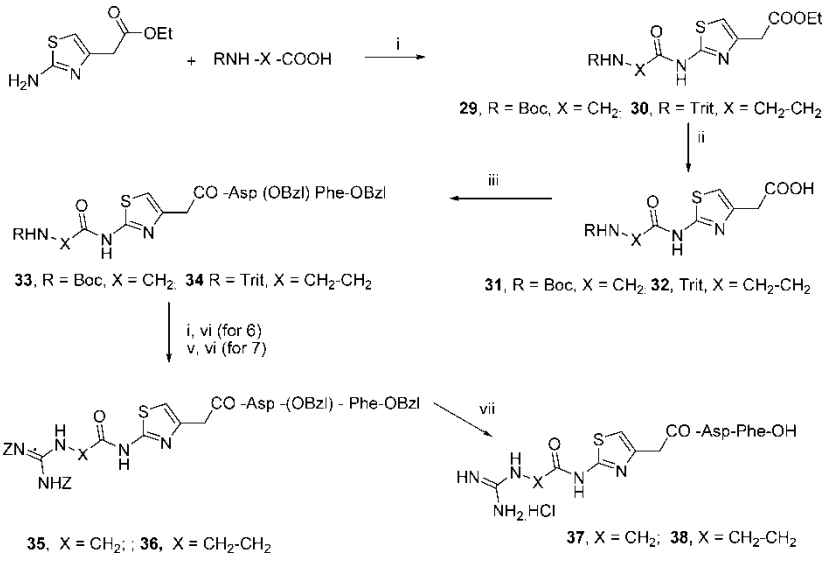
Scheme 1. i) DCC, HOBt, CH_2Cl_2, DMF; ii) 1-methyl morpholine, isobutyl chloroformate, $CHCl_3$, $-15^{\circ}C$; iii) 85% aqueous AcOH, D, 10 min; iv) trifluoromethanesulfonic acid, TFA; v) purification, HCl–ether.

group of Tha was protected by tritylation using trimethylsilyl as transient acid protection as reported earlier[13] to obtain **1**. The reaction of **1** with different Asp (OBzl)–NHR in the presence of DCC–HOBt[14] or the mixed anhydride method resulted in compounds **2**–**10**. The Trit group from **2**–**10** was removed by treatment with 85% AcOH.[15] The resulting amine component was coupled with tri-Z-Arg[16] by the mixed anhydride procedure[11] to yield compounds **11**–**19**. The yield of the coupling of Arg residue was found to be poor in DMF (20–35%), however, when THF–CH_2Cl_2 (or better $CHCl_3$) replaced DMF, the yield improved to 45–55%. The simultaneous removal of both Z and Bzl from **11**–**19** was carried out by treatment with trifluoromethanesulfonic acid in TFA[17] followed by reverse-phase MPLC purification to give **20** to **28** in good yield.

To examine the effect of chain length at N-terminus on activity, the Arg residue was replaced by ω-guanidino acid as shown in Scheme 2 ($n = 0$ or 1; $R_1 = H$, $R_2 =$ Phe in Fig. 1) and Scheme 3 ($n = 5$; $R_1 = H$, $R_2 = n$Bu in Fig. 1).

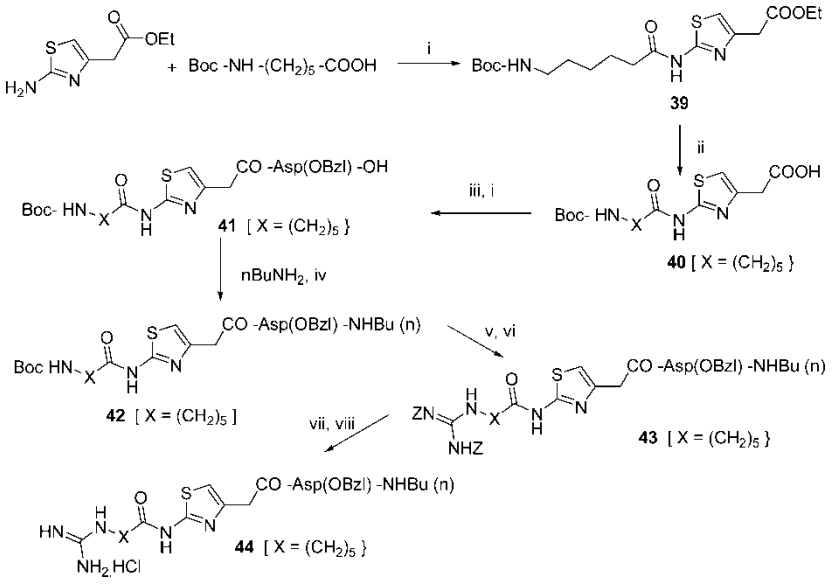
2.1 New Systematic Approach

As described previously, we have observed poor yield while coupling Arg residue with the C-terminal fragment. Thereafter, we developed a novel



Scheme 2. i) DCC, HOBt, EtOAc; ii) 1N NaOH, MeOH; iii) H-Asp (OBzl-Phe-OBzl . HCl, Et_3N, DCC, HOBt; iv) Formic acid, anisole, room temperature; v) 85% aqueous AcOH, 100°C, 10 min; vi) Et_3N, dicartbobenzoxy-S-methyl isothiourea, CH_2C_{12}, reflux; vii) trifluoromethanesulfonic acid, TFA, rt.

synthetic strategy in which the guanidino functionality was introduced at the later stage once the coupling of all the fragments was over. The highly efficient method for introduction of protected guanidino group to amino acid was published by us[16b] and others.[16c] The protected amino group was used as a precursor for guanidine. Thus Boc-Gly[18] or Trit-β-Ala[19] was coupled with Tha-OEt[20] in the presence of DCC-HOBt to yield compounds **29** and **30** in high yield as shown in Scheme 2. The ethyl esters **29** and **30** were hydrolyzed with 1 N NaOH and MeOH to the corresponding acid **31** and **32** respectively. The free acids **31** and **32** were coupled with Asp (OBzl) Phe-OBzl [obtained by removing amino protection from Boc-Asp (OBzl)-Phe-OBzl by treatment with formic acid–anisole] by DCC–HOBt to get **33** and **34** in high yield. At this stage, the amino protecting groups from **33** and **34** were removed by treatment with formic acid–anisole and 85% AcOH respectively. The resulting amine salt (after neutralizing with Et_3N) was refluxed with dicarbobenzoxy-S-methyl isothiourea[16b,21] to get the Z-protected guanidino compounds **35** and **36** in good yield. Finally, all the protecting groups were removed by treating **35** and **36** with trifluoromethansulfonic acid in TFA, which after purification as described next yielded final compounds **37** and **38**. The synthesis of compound **44**, in which ε-gunidino haxanoyl moiety replaced the Arg residue, was carried out according to Scheme 3 using ε-amino acid as precursor. Thus Boc-ε-Ahx[22] was



Scheme 3. i) DCC, HOBt, EtOAc; ii) 1N NaOH, MeOH; iii) H-Asp (OBzl)-OH, Et_3N, Tms-Cl; iv) N-methyl morpholine, isobutyl chloroformate, DMF; v) Formic acid, anisole, rt; vi) Et_3N, di-cartbobenzoxy-S-methyl isothiourea, CH_2C_{12}, CH_3CN, reflux; vii) Trifluoromethanesulfonic acid, TFA, rt, Purification and salt exchange; viii) HCl-ether.

coupled with Tha-OEt in the presence of DCC–HOBt to obtain **39**. It was hydrolyzed with 1N NaOH in MeOH, giving free acid **40**. The α-carboxylic group of Asp (OBzl) was protected in situ as trimethyl silyl ester using Tms-Cl. The resulting ester hydrochloride was neutralized with triethyl amine and then coupled with **40** in the presence of DCC–HOBt.

The temporary protection was removed by treatment with MeOH and usual workup yielded Boc-ε-Ahx-Tha-Asp (OBzl)-OH (**41**) in very good yield (84%). The activation of **41** through the mixed anhydride method followed by treatment with nBuNH$_2$ afforded **42**, which was converted to the final compound **44** through the intermediate **43** by the same method as described for the synthesis of compound **37**.

The two protons attached to the β-carbon of Asp moiety in most cases showed well separated jeminal coupling followed by vicinal coupling with C-α-proton, resulting in two sets of doublets. Considering the general structure in Fig. 1, the proton trans to C-α-H appeared upfield (δ 2.6–2.8) with higher vicinal coupling constant value (6–8 Hz) in ^{1}H NMR spectrum and is assigned Hβ. On the other hand, the proton cis to C-α-H appears downfield (δ 2.8–3.05) with lower vicinal coupling constant value (4–5 Hz) and is assigned H_α.

2.2 Simple New Method Developed for Salt Exchange and Purification

Initially we had encountered difficulty in exchanging trifluoromethanesulfonate salt of the final compounds into any other biologically acceptable salt(s). Direct exchange with HCl or use of a different type of ion exchanger failed to give desired results. Finally, we developed a unique reverse-phase purification protocol. Thus the crude material was dissolved in cold water basified the solution by adding cold dilute NaOH to make the guanidino moiety free basified the solution. Because guanidine is a strong base, use of organic bases or a weak inorganic base could not be used. The solution was immediately loaded on RP-18 MPLC column. After initial washing with water (to remove sodium trifluoromethanesulfonate), further purification was carried out using the required eluting solvent that always contained acetic acid (see Experimental). The pure product was obtained as acetate salt. Thus we call this technique as "salt exchange cum purification." The acetate salt thus obtained was converted into hydrochloride salt by the usual procedure. The same technique was utilized in all the cases where trifluoromethanesulfonic acid salt had to be exchanged. Thus compounds **20**–**28, 37, 38**, and **44** could be obtained in reasonably good yields.

The compounds synthesized in the present series were tested for GP IIb–IIIa receptor antagonism; however, they did not show any significant activity. The introduction of Tha in place of Gly possibly has either altered the orientation of N-terminus guanidine and C-terminus carboxylic groups or the required length was not achieved for the RGD type of binding.

3 CONCLUSION

We have successfully synthesized the newly designed analogues of RGD, wherein Tha, a heterocyclic to give metabolic stability, replaced the central glycine part. However, this did not yield any positive result in terms of FRA activity.

While executing the present study, we have developed a systematic approach to synthesizing ω-guanidino acid containing peptides/pseudo peptides by carrying the surrogate amino acid untill the late stage of peptide assembly. This approach enabled us to overcome the low yield associated with the coupling of Arg or other guanidino acid. As far as we know, this is the first systematic study wherein a protected ω-guanidino group was introduced at a late stage of the synthesis. There is another example in the literature where a Boc-protected guanidino group was introduced at the final stage of synthesis for Daptomycin analogues. However, they used {[*tert*-butoxycarbonylimino]-pyrazol-1-yl-methyl}-carbamic acid *tert*-butyl ester as guanidinylating agent.[23]

During the present investigation, we developed a unique reverse-phase (RP-18) purification protocol whereby in one step, exchange of unwanted

trifluorosulfonate salt followed by purification of the desired compound could be achieved. In principle, this can be applied to any salt exchange. We believe this method may find wide application in purification technology.

4 EXPERIMENTAL

4.1 General Procedures

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 157 spectrophotometer as KBr film unless otherwise mentioned. ^{1}H NMR spectra were recorded in $CDCl_3$ unless otherwise mentioned on a Jeol FT-90 spectrometer or Bruker ACP 300 spectrometer with TMS as internal standard, and coupling constant values are expressed in Hertz. Light petroleum refers to the fraction of bp 60–80°C. For flash Column chromatography, silica gel (finer than 0.08-mm particle size) was used. For reverse-phase MPLC purification, RP-18 (30-μ particle size) was used. Precoated (silica-gel 60 F_{254}) TLC plates were used for checking purity of compounds. UV light at 366 nm and 254 nm as well as spray reagents such as 0.2% ninhydrin in acetone, Draggendroff, and I_2 vapor was used to detect the spots on TLC. All compounds were homogeneous on TLC and gave proper spectral characteristics. The yield of the reactions and physicochemical properties were described in Table 1 for most of the compounds.

4.2 Boc-Asp (OBzl)-Val-OBzl

A solution of Boc-Asp (OBzl)-OH (4.8 g; 15 mmol) in DMF (30 ml) was chilled to −30°C. 1-Methyl morpholine (1.65 ml; 15 mmol) and isobutylchloroformate (1.95 ml; 15 mmol) were added to the solution in quick succession. In a separate round-bottomed flask, Val-OBzl–PTSA[24] (1.25 g; 16.5 mmol) was dissolved in DMF (20 ml) and chilled to −30°C and neutralized with Et_3N (2.31 ml; 16.5 mmol). The resulting free amine was added to the mixed anhydride after 2 min. The reaction mixture was stirred at −20°C for 1 h. It was stored in a freezer overnight. Solvent was removed under reduced pressure, and the residue was stirred with EtOAc (30 ml) and 1N $NaHCO_3$ (10 ml) and transferred into a separating funnel. The organic layer was separated and washed with water, 1N citric acid, and brine. It was dried over anhydrous Na_2SO_4. Solvent was removed, and the residue was purified by crystallization from EtOAc–light petroleum to yield 7.49 g (96.4%) of the title compound. IR (KBr): 3360–3300 (br), 2950, 1730 (br), 1685, 1670 cm^{-1}; ^{1}H NMR (60 MHz): 0.84, 0.91 [2 × d, 6H, $J = 7.6$, CH (CH_3)$_2$], 1.43 [s, 9H, C (CH_3)$_3$], 2.85 [m, 2H, CH (CH_2COO-)-], 4.4–4.7 (2 × m, 2 × C$^{\alpha}H$ of Asp and Val), 5.12 (s, 4H, 2 × PhCH_2O), 7.32 (br, 10H, PhH).

Table 1. Yield and physicochemical properties for identification of compounds

Sub. no.	Yield (%)	Mp (°C)	IR (KBr) (cm^{-1})	^{1}H NMR	Analysis MF; calc. (found) (%)
3	62.6	162–64	3270 (br), 1730 (br), 1635 (br)	2.14, 2.21 (2 × d, 6H, $J = 7.1$), 2.63 (dd, 1H, $J_{gem} = 17.2$, $J = 6.0$), 3.03 (dd, 1H, $J_{gem} = 17.2$, $J = 5.06$), 3.49 (s, 2H), 4.0 (heptate, 1H), 4.64–4.86 (m, 1H), 5.0 (s, 2H), 6.10 (s, 1H), 6.29 (m, 1H), 6.86 (br, 1H), 7.29–7.40 (m, 20H), 8.37 (d, 1H)	$C_{38}H_{38}N_4O_4S$: C, 70.56; H, 5.92; N, 8.66; S, 4.96 (C, 70.43; H, 5.94; N, 8.56; S, 4.83)
4	57.08	117–19	3260 (br), 2930 (br), 1740 (br), 1645 (br)	0.84 (t, 3H, $J = 6.1$), 1.11–1.50 (m, 4H), 2.64 (dd, 1H, $J_{gem} = 17.2$, $J = 6.08$), 3.05 (dd, 1H, $J_{gem} = 17.2$, $J = 4.56$), 3.17 (m, 2H), 3.46 (s, 2H), 4.80 (m, 1H), 4.97 (s, 2H), 6.07 (s, 1H), 6.81 (br, 1H), 7.24–7.35 (m, 20H) 8.43 (d, 1H)	$C_{39}H_{40}N_4O_4S$: C, 70.88; H, 6.10; N, 8.48; S, 4.85(C, 70.65; H, 6.18; N, 8.42; S, 4.71)
5	58.9	127–29	3280 (br), 2940 (br), 1735 (br), 1640 (br)	0.84, 0.91 (2 × s, 6H), 1.56–1.97 (m, 1H), 2.64 (dd, 1H, $J_{gem} = 17.2$, $J = 7.1$), 2.94–3.17 (m, 3H), 3.50 (s, 2H), 4.81 (m, 1H), 5.01 (s, 2H), 6.09 (s, 1H), 6.66 (br, 1H), 6.86 (br, 1H), 7.23–7.40 (m, 20H) 8.51 (d, 1H)	$C_{39}H_{40}N_4O_4S$: C, 70.88; H, 6.10; N, 8.48; S, 4.85 (C, 70.83; H, 6.14; N, 8.41; S, 4.96)
7	97.83	117–18	3280 (br), 2970, 2940, 1740 (br), 1640 (br)	0.76, 0.84 (2 × t, 6H), 1.10–1.57 (m, 4H), 2.60 (dd, 1H, $J_{gem} = 17.2$, $J = 6.08$), 3.05 (dd, 1H, $J_{gem} = 17.2$, $J = 4.05$), 3.49 (s, 2H), 3.57–3.80 (m, 1H), 4.71–4.91 (m, 1H), 5.01 (s, 2H), 6.09 (s, 1H), 6.86 (br, 1H), 7.23–7.40 (m, 20H), 8.53 (d, 1H)	$C_{40}H_{42}N_4O_4S$: C, 71.19; H, 6.27; N, 8.30; S, 4.75 (C, 70.91; H, 6.31; N, 8.25; S, 4.66)

8	78.2	Oily	3350–3330, 2970, 1745 (br), 1735 (br), 1660 (br)	0.83–0.84 (m, 9H), 2.61 (dd, 1H, $J_{gem} = 17.2$, $J = 6.08$), 2.81–3.13 (m, 3H), 3.49 (s, 2H), 4.73–4.96 (m, 1H), 5.0 (s, 2H), 6.09 (s, 1H), 6.89 (br, 1H), 7.20–7.43 (m, 20H), 8.63 (m, 1H)	$C_{40}H_{42}N_4O_4S$: C, 71.19; H, 6.27; N, 8.30; S, 4.75 (C, 71.01; H, 6.30; N, 8.13; S, 4.73)
9	53.3	113–15	3300–3260 (br), 2930, 2850, 1740, 1645 (br)	0.85–1.77 (3 × m, 10H), 2.64 (dd, 1H, $J_{gem} = 17.2$, $J = 6.08$), 3.04 (dd, 1H, $J_{gem} = 17.2$, $J = 4.05$, 3.49 (s, 2H), 4.77 (m, 1H), 5.01 (s, 2H), 6.09 (s, 1H), 6.37, 6.83 (2 × br, 2H), 7.23–7.40 (m, 20H), 8.34 (m, 1H)	$C_{40}H_{40}N_4O_4S$: C, 71.69; H, 6.16; N, 8.16; S, 4.67 (C, 71.82; H, 6.34; N, 7.97; S, 4.58)
10	67.07	Oily	3300 (br), 2980 (br), 1740 (br), 1685 (br) 1655 (br)	0.80–0.97 (m, 6H), 1.86–2.31 (br, 1H), 2.68 (dd, 1H, $J_{gem} = 17.2$, $J = 6.08$), 3.05 (2 × quartet, 1H, $J_{gem} = 17.2$, $J = 4.05$, 2.0), 3.49 (s, 2H), 4.49 (dd, 1H), 4.74–5.17 (m, 5H), 6.06 (s, 1H), 6.97 (2 × br, 2H), 7.23–7.37 (m, 20H), 8.57 (m, 1H)	$C_{47}H_{46}N_6O_4S$: C, 71.01; H, 5.83; N, 7.05; S, 4.03 (C, 71.14; H, 5.81; N, 6.98; S, 4.10)
11	34.6	107–09, EtOAc–light petroleum	3405, 3320 (br), 2980 (br), 1750 (br), 1660 (br)	(300 MHz): 0.73 (t, 3H), 1.30 (m, 2H), 1.62–1.82 (m, 4H), 2.64 (dd, 1H, $J_{gem} = 17.85$, $J = 7.1$), 2.87 (dd, 1H, $J_{gem} = 17.85$, $J = 4.5$), 3.0 (m, 2H), 3.49 (s, 2H), 3.88–4.0 (m, 2H), 4.50, 4.72, 4.90 (3 × m, 3H), 4.95–5.15 (m, 8H), 6.15, 6.46 (2 × m, 2H), 6.61 (s, 1H), 7.12–7.35 (m, 20H), 7.70, 9.28, 9.35 (3 × m, 3H)	$C_{49}H_{54}N_8O_{11}S$: C, 61.11; H, 5.65; N, 11.64; S, 3.33 (C, 60.93; H, 5.61; N, 11.49; S, 3.25)

(*continued*)

Table 1. Continued

Sub. no.	Yield (%)	Mp (°C)	IR (KBr) (cm^{-1})	^1H NMR	Analysis MF; calc. (found) (%)
12	52.07	170–72, EtOAc–light petroleum	3405, 3320 (br), 2980 (br), 1750 (br), 1660 (br)	(300 MHz): 0.90 (d, 6H), 1.0–1.9 (2 × m, 4H), 2.59 (dd, 1H, $J_{gem} = 17.6$, $J = 6.0$), 2.85 (dd, 1H, $J_{gem} = 17.6$, $J = 4.0$), 3.38 (m, 1H), 3.50 (s, 2H), 3.80 (m, 2H), 4.45, 4.65, 4.90 (3 × m, 3H), 4.90–5.10 (m, 8H), 6.05, 6.15 (2 × br, 2H), 6.65 (s, 1H), 7.08–7.30 (m, 20H), 7.75, 9.12, 9.30 (3 × m, 3H)	$C_{49}H_{54}N_8O_{11}S$: C, 61.11; H, 5.65; N, 11.64; S, 3.33 (C, 61.18; H, 5.61; N, 11.55; S, 3.40)
13	29.5	84–86, EtOAc–light petroleum	3405, 3300 (br), 2975 (br), 1730 (br), 1660 (br)	(300 MHz): 0.76 (t, 3H), 0.95–1.30, 1.60–1.90 (2 × m, 8H), 2.60 (dd, 1H, $J_{gem} = 17.5$, $J = 7.8$), 2.85 (dd, 1H, $J_{gem} = 17.5$, $J = 4.5$), 3.05 (m, 2H), 3.52 (s, 2H), 3.90 (m, 2H), 4.52, 4.72, 4.98 (3 × m, 3H), 5.0–5.15 (m, 8H), 6.15, 6.48 (2 × br, 2H), 6.63 (s, 1H), 7.13–7.30 (m, 20H), 7.83, 9.25, 9.40 (3 × m, 3H)	$C_{50}H_{56}N_8O_{11}S$: C, 61.46; H, 5.78; N, 11.47; S, 3.28 (C, 61.27; H, 5.88; N, 11.29; S, 3.22)
14	55.95	84–86, EtOAc–light petroleum	3400, 3310 (br), 2970 (br), 1750 (br), 1670–1650 (br)	(300 MHz): 0.78, 0.88 (2 × d, 6H), 1.58–1.78 (m, 5H), 2.70 (dd, 1H, $J_{gem} = 17.7$, $J = 7.8$), 2.89–3.08 (m, 3H), 3.58 (s, 2H), 3.95 (m, 2H), 4.55, 4.80, 5.0 (3 × m, 3H), 5.05–5.25 (m, 8H), 6.15, 6.63 (2 × br, 2H), 6.72 (s, 1H), 7.0 (br, 1H), 7.25–7.38 (m, 20H), 7.70, 8.21, 9.40 (3 × m, 3H)	$C_{50}H_{56}N_8O_{11}S$: C, 61.46; H, 5.78; N, 11.47; S, 3.28 (C, 61.11; H, 6.06; N, 11.14; S, 3.11)

15	42.7	86–88, EtOAc–light petroleum	3400–3300 (br), 2960 (br), 1730 (br), 1690, 1645 (br)	(300 MHz): 0.85 (d, 6H), 1.2, 1.68 (2 × m, 6H), 1.45 (m, 1H), 2.65 (dd, 1H, $J_{gem} = 17.5$, $J = 7.8$), 3.00 (dd, 1H, $J_{gem} = 17.5$, $J = 5.0$), 3.08 (m, 2H), 3.58 (s, 2H), 3.92 (m, 2H), 4.52, 4.72, 4.98 (3 × m, 3H), 5.01–5.20 (m, 8H), 6.13, 6.40 (2 × br, 2H), 6.69 (s, 1H), 7.12–7.32 (m, 20H), 7.75, 9.29, 9.40 (3 × m, 3H)	$C_{51}H_{58}N_8O_{11}S$: C, 61.80; H, 5.89; N, 11.31; S, 3.23 (C, 61.65; H, 5.51; N, 10.93; S, 3.05)
16	47.9	85–87, EtOAc–light petroleum	3420, 3320 (br), 2950 (br), 1740 (br), 1695, 1650 (br)	(300 MHz): 0.85 (d, 6H), 1.36, 1.50, 1.79 (3 × m, 8H), 2.79 (dd, 1H, $J_{gem} = 17.75$, $J = 7.8$), 3.02 (dd, 1H, $J_{gem} = 17.75$, $J = 5.07$), 3.68 (s, 2H), 3.70 (m, 1H), 4.05 (m, 2H), 4.62, 4.92, 5.12 (3 × m, 3H), 5.12–5.30 (m, 8H), 6.15, 6.30 (2 × br, 2H), 6.79 (s, 1H), 7.32–7.50 (m, 20H), 8.05 (br, 1H), 9.39, 9.50 (2 × br, 2H)	$C_{51}H_{58}N_8O_{11}S$: C, 61.80; H, 5.89; N, 11.31; S, 3.23 (C, 61.54; H, 5.58; N, 11.02; S, 3.27)
17	48	89–90, EtOAc–light petroleum	3430, 3335 (br), 2990 (br), 1740 (br), 1695 (br), 1660 (br).	(300 MHz): 0.87 (s, 9H), 1.76–2.08 (2 × m, 4H), 2.78 (dd, 1H, $J_{gem} = 17.2$, $J = 7.8$), 3.03 (m, 3H), 3.66 (s, 2H), 4.02 (m, 2H), 4.63, 4.88, 5.04 (3 × m, 3H), 5.13–5.28 (m, 8H), 6.22, 6.70 (2 × br, 2H), 6.77 (s, 1H), 7.27–7.45 (m, 20H), 7.88 (br, 1H), 9.32, 9.48 (2 × br, 2H)	$C_{51}H_{58}N_8O11_4S$: C, 61.80; H, 5.89; N, 11.31; S, 3.23 (C, 61.72; H, 5.68; N, 11.27; S, 3.09)

(*continued*)

Table 1. Continued

Sub. no.	Yield (%)	Mp (°C)	IR (KBr) (cm^{-1})	^{1}H NMR	Analysis MF; calc. (found) (%)
18	55	88–90, EtOAc–light petroleum	3420 (br), 3320 (br), 2960 (br), 1740 (br), 1700, 1660 (br)	(300 MHz): 0.88–1.20 (m, 10H), 2.10–2.48 (2 × m, 4H), 2.70 (dd, 1H, $J_{gem} = 17.5$, $J = 7.8$), 2.95 (m, 1H), 3.45 (s, 2H), 3.57 (m, 1H), 3.97 (m, 2H), 4.60, 4.77, 5.03 [3 × m, 3H), 5.05–5.23 (m, 8H), 6.17, 6.37 (2 × br, 2H), 6.70 (s, 1H), 7.20–7.38 (m, 20H), 7.80 (br, 1H), 9.25, 9.40 (2 × br, 2H)	$C_{52}H_{58}N_8O_{11}S$: C, 62.26; H, 5.83; N, 11.17; S, 3.19 (C, 62.36; H, 5.87; N, 11.09; S, 3.22)
19	43.56	Glassy solid	3400–3300 (br), 2970 (br), 1740 (br), 1700 (br), 1675 (br)	(300 MHz): 0.75–0.95 (m, 6H), 1.65–1.85 (m, 4H), 2.15 (m, 1H), 2.70 (m, 1H), 3.05 (m, 1H), 3.60 (s, 2H), 4.0 (m, 2H), 4.50, 4.88, 4.96 (3 × m, 4H), 5.02–5.15 (m, 10H), 6.04 (m, 1H), 6.73 (s, 1H), 7.20–7.45 (m, 25H), 9.28, 9.42 (2 × br, 2H)	$C_{58}H_{62}N_8O_5S$: C, 62.69; H, 5.62; N, 10.08; S, 2.89 (C, 62.48; H, 5.39; N, 10.11; S, 2.72)
21	50.7	188–92	3500–2950 (br), 1730 (br), 1665 (br	(D_2O; 300 MHz): 1.15 (m, 6H), 1.69, 2.07 (2 × m, 4H), 2.81 (dd, 1H, $J_{gem} = 17.5$, $J = 7.5$), 2.90 (dd, 1H, $J_{gem} = 17.5$, $J = 4.5$), 3.23 (t, 2H), 3.74 (s, 2H), 3.91 (m, 1H), 4.32, 4.62 (2 × t, 2H, $J = 5.63$, $J = 7.5$), 7.05 (s, 1H)	$C_{18}H_{30}N_8O_5S$: 2HCl, $2H_2O$; C, 36.17; H, 6.41; N, 18.75; Cl, 11.87; S, 5.37 (C, 35.97; H, 6.31; N, 18.59; Cl, 11.86; S, 5.26)
22	70.32	170–75 (d)	3400–3300 (br), 2940 (br), 1720 (br), 1655 (br)	(D_2O; 300 MHz): 0.85 (t, 3H), 1.18, 1.42 (2 × m, 4H), 1.70, 2.06 (2 × m, 4H), 2.88 (m, 2H), 3.0–3.12 (m, 4H), 3.72 (s, 2H), 4.35, 4.68 (2 × m, 2H), 7.08 (s, 1H)	$C_{19}H_{31}N_8O_5S$; 2HCl, $3H_2O$: C, 37.31; H, 6.59; N, 18.32; Cl, 11.60; S, 5.24 (C, 35.44; H, 6.41; N, 18.18; Cl, 11.47; S, 5.32)

23	57	172–74	3450–3300 (br), 2970 (br), 1720 (br), 1680–1660 (br)	(D_2O; 300 MHz): 0.77, 0.80 (2 × d, 6H), 1.58–1.72 (m, 1H), 1.98–2.06 (2 × m, 4H), 2.49–3.25 (3 × m, 6H), 3.70 (s, 2H), 4.29 (t, 1H), 4.51 (m, 1H), 7.03 (s, 1H)	$C_{19}H_{31}N_8O_5S$; HCl, H_2O: C, 42.33; H, 6.50; N, 20.79; Cl, 6.57; S, 5.95 (C, 42.46; H, 6.54; N, 20.54; Cl, 6.42; S, 6.01)
24	77.2	160–63	3400–3200 (br), 2970 (br), 1740 (br), 1670 (br)	(D_2O; 300 MHz): 0.83 (br, 6H), 1.28 (m, 2H), 1.43 (m, 1H), 1.79, 2.10 (2 × m, 4H), 2.78–2.95 (m, 2H), 3.16–3.29 (m, 4H), 3.70 (s, 2H), 4.32, 4.66 (2 × m, 2H), 7.06 (s, 1H)	$C_{20}H_{33}N_8O_5S$; 2HCl: C, 38.95; H, 6.70; N, 18.17; Cl, 11.50; S, 6.20 (C, 38.79; H, 6.35; N, 17.85; Cl, 11.36; S, 5.29)
25	74.1	195–200	3350–3200 (br), 2990 (br), 1745 (br), 1690–1670 (br)	(D_2O; 300 MHz): 0.79 (m, 6H), 1.30, 1.51 (2 × m, 4H), 1.73, 2.06 (2 × m, 4H), 2.77–3.02 (m, 2H), 3.21 (m, 2H), 3.55 (m, 1H), 3.73 (s, 2H), 4.33, 4.68 (2 × m, 2H), 7.09 (s, 1H)	$C_{20}H_{34}N_8O_5S$, 2HCl, H_2O: C, 40.74; H, 6.49; N, 19.09; Cl, 12.03; S, 5.44 (C, 40.87; H, 6.12; N, 18.88; Cl, 12.23; S, 5.41)
26	66	188–191	3500–3300 (br), 2980 (br), 1740 (br), 1685–1660 (br)	(D_2O; 300 MHz): 0.83 (s, 9H), 1.75, 2.05 (2 × m, 4H), 2.80–3.03 (m, 2H), 3.0 (s, 2H), 3.28 (t, 2H), 3.72 (br, 2H), 4.35, 4.79 (2 × m, 2H), 7.08 (s, 1H)	$C_{20}H_{34}N_8O_5S$, 2HCl, $2H_2O$: C, 39.53; H, 6.64; N, 18.44; Cl, 11.67; S, 5.28 (C, 39.57; H, 6.44; N, 18.32; Cl, 11.76; S, 5.36)
27	66.67	195–199	3400–3250 (br), 2910 (br), 1725 (br), 1670–1640 (br)	(D_2O; 300 MHz): 1.0–1.30, 1.50–1.80, 2.01–2.12 (3 × m, 15H), 2.80–2.98 (m, 2H), 3.25 (t, 2H), 3.58 (br, 2H), 4.31, 4.62 (2 × m, 2H), 7.05 (s, 1H).	$C_{21}H_{34}N_8O_5S$, 2HCl, 1.5 H_2O: C, 41.31; H, 6.44; N, 18.35; Cl, 11.61; S, 5.25 (C, 41.22; H, 6.59; N, 18.08; Cl, 11.76; S, 4.98)

(*continued*)

Table 1. Continued

Sub. no.	Yield (%)	Mp (°C)	IR (KBr) (cm^{-1})	^{1}H NMR	Analysis MF; calc. (found) (%)
28	63.97	196–202	3400–3300 (br), 2960 (br), 1725 (br), 1670–1640 (br)	(D_2O; 300 MHz): 0.88 (m, 6H), 1.63–1.76 (m, 2H), 2.01–2.19 (m, 3H), 2.83–2.95 (2 × dd, 2H, $J_{gem} = 15.4$, $J = 7.7$, 6.6), 3.23 (t, 2H), 3.72 (br, 2H), 4.22, 4.31, 4.72 (3 × m, 3H), 7.05 (s, 1H)	$C_{20}H_{32}N_8O_7S$, 2HCl, $2H_2O$: C, 37.68; H, 6.01; N, 17.58; Cl, 11.12; S, 5.03 (C, 37.92; H, 6.15; N, 17.35; Cl, 11.07; S, 4.87)
29	94.6	168–69	3300 (br), 3000–2930 (br), 1730, 1705, 1680 (br)	1.21 (t, 3H), 1.46 (s, 9H), 3.63 (s, 2H), 4.08 (s, 2H), 4.09 (quartet, 2H), 6.74 (s, 1H).	$C_{14}H_{21}N_3O_5S$: C, 48.96; H, 6.16; N, 12.24; S, 9.34 (C, 49.13; H, 6.24; N, 12.11; S, 9.57)
30	74.7	148–50	3000–2950 (br), 1745 (br), 1650 (br)	1.31 (t, 3H), 2.63 (br, 4H), 3.74 (s, 2H), 4.09 (quartet, 2H), 6.83 (s, 1H), 7.23–7.54 (m, 15H)	$C_{29}H_{29}N_3O_3S$: C, 69.71; H, 5.85; N, 8.41; S, 6.42 (C, 69.77; H, 5.92; N, 8.24; S, 6.67)
31	89.9	176–78	3365, 1685 (br), 1675	($CDCl_3$ + DMSO-D_6): 1.46 (s, 9H), 3.63 (s, 2H), 4.26 (d, 2H), 6.20 (br, 1H), 6.74 (s, 1H)	$C_{12}H_{17}N_3O_5S$: C, 45.70; H, 5.44; N, 13.33; S, 10.17; (C, 45.76; H, 5.52; N, 13.19; S, 10.40)
32	92.7	205–06	3210 (br), 3075 (br), 1745 (br), 1710, 1565 (br)	($CDCl_3$ + DMSO-D_6): 2.51, 2.57 (2 × t, 4H), 3.53 (s, 2H), 6.73 (s, 1H), 7.11–7.50 (m, 15H)	$C_{27}H_{25}N_3O_3S$: C, 68.77; H, 5.34; N, 8.91; S, 6.80 (C, 68.66; H, 5.29; N, 8.92; S, 6.84)
33	77.24	93–95	3350 (br), 2980 (br), 1730 (br), 1675, 1665, 1655	(300 MHz): 1.45 (s, 9H), 2.69 (dd, 1H), 3.03 (d, 2H), 3.18 (dd, 1H), 3.56 (s, 2H), 3.95 (d, 2H), 4.88, 5.02 (2 × m, 2H), 5.12, 5.23 (2 × s, 4H), 6.61 (s, 1H), 7.03–7.40 (m, 10H)	$C_{39}H_{43}N_2O_7S$: C, 61.81; H, 5.72; N, 9.24; S, 4.23; (C, 61.75; H, 5.61; N, 9.07; S, 4.05)

34	87.2	79–81	3310 (br), 1750 (br), 1700 (br), 1670 (br)	(300 MHz): 2.43 (s, 4H), 2.62 (dd, 1H), 2.94 (d, 2H), 3.08 (dd, 1H), 3.51 (s, 2H), 4.83, 4.96 (2 × m, 2H), 5.0 (br, 4H), 6.60 (s, 1H), 6.93–7.38 (m, 25H)	$C_{59}H_{51}N_5O_7S$: C, 71.03; H, 5.52; N, 7.67; S, 3.51 (C, 70.85; H, 5.66; N, 7.54; S, 3.68)
36	67.88	74–75	3345 (br), 1755, 1745 (br), 1660 (br)	(300 MHz): 2.60 (m, 2H), 2.65 (dd, 1H), 2.94 (d, 2H), 3.13 (dd, 1H), 3.50 (s, 2H), 3.72 (s, 2H), 4.83, 4.94 (2 × m, 2H), 5.04 (s, 6H), 5.12 (s, 2H), 6.63 (s, 1H), 6.93–7.44 (m, 20H)	$C_{52}H_{51}N_7O_{11}S$: C, 63.59; H, 5.24; N, 9.98; S, 3.26; (C, 63.42; H, 5.15; N, 9.94; S, 3.40)
38	65	135–38	3400–3100 (br), 1700–1650 (br)	(D_2O, 300 MHz): 2.63–2.87 (m, 4H), 2.92, 3.08 (m, 2H), 3.45–3.68 (m, 4H), 4.39, 4.58 (2 × m, 2H), 6.90 (s, 1H), 7.06–7.30 (m, 5H)	$C_{22}H_{27}N_7O_7S$, HCl, $2H_2O$: C, 44.78; H, 5.47; N, 16.17; Cl, 6.01; S, 5.43 (C, 44.63; H, 5.49; N, 16.05; Cl, 6.17; S, 5.28)
39	87.5	Oily	(neat): 3400–3000 (br), 3000–2940 (br), 1740, 1700 (br), 1680 (br)	1.27 (t, 3H), 1.47 (s, 9H), 1.58–1.98 (m, 6H), 2.43 (t, 2H), 3.11 (m, 2H), 3.67 (m, 2H), 4.17 (quartet, 2H), 6.77 (s, 1H)	$C_{18}H_{29}N_3O_5S$: C, 54.12; H, 7.32; N, 10.52; S, 8.02; (C, 54.36; H, 7.41; N, 1038; S, 5.16)
40	84.7	122–24	3390, 3370, 2970 (br), 1710–1680 (br)	($CDCl_3$ + DMSO-D_6): 1.46 (s, 9H), 1.49–1.86 (m, 6H), 2.46 (t, 2H), 3.37 (m, 2H), 3.66 (s, 2H), 6.74 (s, 1H)	$C_{16}H_{25}N_3O_5S$: C, 51.74; H, 6.78; N, 11.31; S, 8.63; (C, 51.69; H, 6.95; N, 11.47; S, 8.51)
41	84	112–14	3380–3300, 3000–2950 (br), 1750 (br), 1705, 1690 (br)	(CD_3OD): 1.18–1.65 (2 × m, 6H), 1.31 (s, 9H), 2.33 (t, 2H), 2.79, 2.90 (2 × m, 4H), 3.50 (s, 2H), 4.68 (m, 1H), 4.96 (s, 2H), 6.72 (s, 1H), 7.20, 7.40, 7.60. 7.75 (4 × m, 5H)	$C_{27}H_{36}N_4O_8S$: C, 56.24; H, 6.29; N, 9.72; S, 5.56; (C, 56.15; H, 6.17; N, 9.65; S, 5.41)

(*continued*)

Table 1. Continued

Sub. no.	Yield (%)	Mp (°C)	IR (KBr) (cm^{-1})	^{1}H NMR	Analysis MF; calc. (found) (%)
42	88	Oily	3370–3300, 3000–2950 (br), 1755 (br), 1710, 1695 (br)	(CD_3OD): 0.78 (t, 3H), 1.19, 1.32, 1.43, 1.69 (4 × m, 10H), 1.37 (s, 9H), 2.39 (t, 2H), 2.65, 2.95 (2 × dd, 2H), 3.02–3.13 (m, 4H), 3.54 (s, 2H), 4.55 (br, 1H), 4.77 (m, 1H), 5.06 (s, 2H), 6.61 (s, 1H), 7.26 (m, 5H), 8.24 (br, 1H)	$C_{31}H_{45}N_5O_7S$: C, 58.93; H, 7.18; N, 11.09; S, 5.07(C, 58.77; H, 7.02; N, 11.17; S, 5.25)
43	60	Oily	3500–3300, 1730 (br), 1660 (br), 1650–1630 (br)	(300 MHz): 0.79 [t, 3H, $J = 7.8$, $N(CH_2)_3CH_3$], 1.15–1.78 [4 × m, 10H, $NHCH_2(CH_2)_2CH_3$, -$NHCH_2(CH_2)_3CH_2CO$-], 2.37 (t, 2H, $J = 7.8$, $(CH_2)_4CH_2CO$], 2.63, 2.95 [2 × dd, 2H, $J_{gem} = 17.5$, $J = 7.5$, 5.5, $CH(CH_2COO)$ of Asp], 3.12–3.33 (2 × m, 4H, 2 × NCH_2), 3.52 (s, 2H, CH_2CO of Tha), 4.73 (m, 1H, -$C^{\alpha}H$ of Asp), 5.05 (m, 6H, 3 × OCH_2Ph), 6.62 (s, 1H, *H*-5 of Tha), 7.18–7.37 (m, 5H, Ph*H*), 8.14, 8.26 (br, 1H, N*H*)	$C_{45}H_{51}N_7O_9S$: C, 61.34; H, 6.11; N, 11.65; S, 3.81; (C, 61.07; H, 6.16; N, 11.44; S, 4.03)
44	60	172–75	3310, 2980, 1690–1670 (br), 1655	(300 MHz, D_2O): 0.79 [t, 3H, $J = 7.63$, $N(CH_2)_3CH_3$], 1.15, 1.48, 1.62, 1.73 [4 × m, 10H, $NHCH_2(CH_2)_2CH_3$, -$NHCH_2(CH_2)_3CH_2$ CO-], 2.55 [t, 2H, $J = 7.62$, $(CH_2)_4CH_2CO$], 2.82, 2.88 [2 × dd, 2H, $J_{gem} = 17.5$, $J = 7.6$, 5.02, -$CH(CH_2COOH)$- of Asp], 3.12–3.19 (m, 4H, 2 × NCH_2), 3.66 (s, 2H, CH_2CO of Tha), 4.65 (m, 1H, -$C^{\alpha}H$ of Asp), 6.96 (s, 1H, *H*-5 of Tha)	$C_{21}H_{34}N_7O_8S2F_3$: C, 40.12; H, 5.41; N, 15.47; F, 8.99; S, 10.12 (C, 39.96; H, 5.33; N, 15.22; F, 8.82; S, 9.99)

The same procedure was utilized to prepare amides of the general structure Boc-Asp (OBzl)-NHR, starting from Boc-Asp (OBzl)-OH and the required amine. The final product was characterized by ^{1}H NMR (data not included).

4.3 General Method for Deblocking Boc-Asp (OBzl)-NHR

A clear solution of Boc-Asp (OBzl)–NHR (2.5 mmol) in formic acid (15 ml) and anisole (0.5 ml) was kept at room temperature. TLC monitored the progress of the reaction. When the reaction was over (~4 h), slight excess of HCl in dry ether was added and stirred for 5 min to convert the formate salt into hydrochloride salt. Solvent was removed under reduced pressure. The residue was triturated with dry ether to obtain a white solid of hydrochloride salt. It was filtered and washed with dry ether. The solid was dried in vacuum desiccators over KOH pellets overnight. The solid material was sufficiently pure to carry out the coupling reaction.

4.4 Benzyl 3-S-Propylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thiazol-4-yl) Pentanoate (2)

A solution of Trit-Tha-OH (1.2 g; 3 mmol) and HOBt (0.41 g; 3.5 mmol) in CH_2Cl_2 (5 ml) and DMF (15 ml) was chilled to 0°C. To this solution, DCC (0.72 g; 3.5 mmol) was added under vigorous stirring. After 5 min, Asp-NHPr (*n*) was obtained by neutralizing AspNHPr (*n*). HCl (0.77 g; 2.55 mmol) by adding Et_3N (0.36 ml; 2.58 mmol) in DMF (2 ml) at 0°C. The reaction mixture was stirred at 0°C for 3 h. It was kept in the freezer overnight. DCU was filtered off. The filtrate was successively with cold 1N HCl, water, 1N $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. Solvent was removed, and the residue was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% $CH_3CN + 0.2\%$ MeOH in $CHCl_3$. Yield 1.01 g (61.74%), semisolid; IR (KBr): 3300 (br), 3050 (br), 2960, 1745 (br), 1735, 1670 (br) cm^{-1}; ^{1}H NMR: 0.83 (t, 3H, $J = 7.1$, $NHCH_2CH_2CH_3$), 1.23–1.60 (m, 2H, $NHCH_2CH_2CH_3$), 2.66 [dd, 1H, $J_{gem} = 17.2$, $J = 6.1$, -CH ($CH_\alpha H_\beta COO$-) of Asp], 2.95–3.26 [m, 3H, -CH ($CH_\alpha H_\beta COO$-) of Asp & $NHCH_2CH_2CH_3$], 3.46 (s, 2H, CH_2CO of Tha), 4.79 (m, 1H, -$C_\alpha H$ of Asp), 5.0 (s, 2H, $PhCH_2O$), 6.09 (s, 1H, *H*-5 of Tha), 7.26–7.39 (m, 20H, Ph*H*); anal. calc. for $C_{38}H_{38}O_4N_4S$: C, 70.56; H, 5.92; N, 8.66; S, 4.96; found: C, 70.51; H, 5.90; N, 8.58; S, 4.84%.

4.5 Benzyl 3-S-Isopropylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thiazol-4-yl) Pentanoate (3)

This compound was prepared by coupling **1** with Asp (OBzl)-NHPr (i) following the method described for the synthesis of **2**. The crude product

was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% CH_3CN + 0.2% MeOH in $CHCl_3$.

4.6 Benzyl 3-S-Butylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thiazol-4-yl) Pentanoate (4)

This was obtained by coupling **1** with Asp (OBzl)-NHBu (i) following the method described for the synthesis of **2**. The crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% CH_3CN + 0.2% MeOH in $CHCl_3$.

4.7 Benzyl 3-S-Isobutylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thiazol-4-yl) Pentanoate (5)

This was obtained by coupling **1** with Asp (OBzl)-NHBu (i) following the method described for the synthesis of **2**. The crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% CH_3CN + 0.2% MeOH in $CHCl_3$.

4.8 Benzyl 3-S-Isopentylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thiazol-4-yl) Pentanoate (6)

A solution of Trit-Tha-OH (2.2 g; 5.5 mmol) in DMF (20 ml) was chilled to -30°C and 1-methyl morpholine (0.61 ml; 5.5 mmol) and isobutyl chloroformate (0.72 ml; 5.5 mmol) were added under vigorous stirring. A solution of HCl-Asp (OBzl) $NHCH_2CH_2CHMe_2$ (1.64 g; 5 mmol) in DMF (10 ml) was neutralized with Et_3N (0.7 ml; 5 mmol) at -30°C and then added to the mixed anhydride generated previously. The reaction mixture was stirred at -20°C to -30°C for 1 h and then kept in the freeze at -15°C overnight. The solvent was removed, and the residue was partioned between EtOAc (25 ml) and aqueous 1 N $NaHCO_3$ (10 ml) and stirred for 5 min. The organic layer was separated. It was washed successively with water, 1N citric acid, and brine. The organic layer was dried over anhydrous Na_2SO_4. Solvent was removed and the crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% CH_3CN + 0.2% MeOH in $CHCl_3$. Yield 74.4%; semisolid; IR (KBr): 3300 (br), 2970 (br), 1755 (br), 1745 (br) cm^{-1}; ^{1}H NMR: 0.86, [d, 6H, $J = 6.1$, -CH (CH_3)$_2$], 1.19–1.60 (m, 3H, $NCH_2CH_2CHMe_2$), 2.62 [dd, 1H, $J_{gem} = 17.2$, $J = 6.08$, -CH ($CH_\alpha H_\beta COO$-) of Asp], 3.04 [dd, 1H, $J_{gem} = 17.2$, $J = 4.05$, -CH ($CH_\alpha H_\beta COO$-) of Asp], 3.09–3.30 (m, 2H, $NHCH_2CH_2$-), 3.49 (s, 2H, CH_2CO of Tha), 4.77 (m, 1H, -$C_\alpha H$ of Asp), 4.99 (s, 2H, $PhCH_2O$), 6.09 (s, 1H, *H*-5 of Tha), 6.53 (br, 1H, N*H*), 6.86 (br, 1H, N*H*), 7.23–7.39

(m, 20H, Ph*H*) 8.49 (d, 1H, N*H*, exchangeable); anal. calc. for $C_{40}H_{42}O_4N_4S$: C, 71.19; H, 6.27; N, 8.30; S, 4.75; found: C, 70.76; H, 6.21; N, 8.19; S, 4.63%.

4.9 Benzyl 3-S- (1-Ethylpropylcarbamoyl-4-oxo-5-(2-tritylamino-1,3-thiazol-4-yl) Pentahyphennoate (7)

This compound was prepared by coupling **1** with Asp (OBzl)-$NHCHEt_2$ following the method described for the synthesis of **6**. The crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% $CH_3CN + 0.2\%$ MeOH in $CHCl_3$.

4.10 Benzyl 3-S-Neopentylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thiazol-4-yl) Pentanoate (8)

This compound was prepared by coupling **1** with Asp (OBzl)-$NHCH_2CMe_3$ following the method described for the synthesis of **6**. The crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% $CH_3CN + 0.2\%$ MeOH in $CHCl_3$

4.11 Benzyl 3-S-Cyclohexylcarbamoyl-4-oxo-5-(2-tritylamino-1,-3-thazol-4-yl) Pentanoate (9)

Compound **10** was prepared by coupling **1** with Asp (OBzl)-NHC_6H_{11} following the method described for the synthesis of **2**. The crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% $CH_3CN + 0.2\%$ MeOH in $CHCl_3$.

4.12 Benzyl 3-S-(1-S-Benzyloxycarbonyl-2-methylpropylcarbamoyl-4-oxo-5-(2-tritylami-no1,3-thazol-4-yl) Pentanoate (10)

This compound was prepared by coupling **1** with Asp (OBzl)-Val-OBzl following the method described for the synthesis of **2**. The crude product was purified by flash chromatography with 10% CH_3CN in $CHCl_3$ followed by 10% $CH_3CN + 0.2\%$ MeOH in $CHCl_3$.

4.13 Benzyl 5-{2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-propylcar-bamoylpentanoate (11)

Compound **2** (3.23 g; 5 mmol) was dissolved in 85% AcOH (40 ml) and heated on a steam bath for 10 min. It was brought back to room

temperature, and 1 N HCl (5 ml) was added. It was stirred for 2 min. Solvent was removed, and the residue was triturated with dry ether to obtain a white solid. The solid was filtered and washed with dry ether. The resulting hydrochloride salt was crystallized from dry MeOH–ether. Before the next step, it was dried in vacuum desiccators over NaOH pellets to give a yield of 2.0 g (90.5%).

Tri-Z-Arg (1.9 g; 2.3 mmol) was dissolved in $CHCl_3$ (20 ml) and chilled to $-20^{\circ}C$. To this solution was added 1-methyl morpholine (0.36 ml; 2.3 mmol) and isobutyl chloroformate (0.42 ml; 2.3 mmol) under vigorous stirring conditions. Deprotected **2** (1.32 g; 3 mmol) was dissolved in $CHCl_3$ (10 ml) and chilled to $-20^{\circ}C$. Et_3N (0.42 ml; 3 mmol) was added under stirring. After 1 min, the free amine was added to the reaction mixture containing the mixed anhydride. The reaction mixture was stirred for 1 h at $-20^{\circ}C$ and then kept in the freezer overnight. The reaction mixture was then stirred with 1N $NaHCO_3$ for 5 min. The organic layer was separated and successively washed with water, 1N HCl, and brine. After drying over anhydrous Na_2SO_4, the solvent was removed. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

Compounds **12**–**19** were prepared from **3**–**10** after deblocking the trityl group followed by coupling with tri-Z-Arg using a mixed anhydride method. The experimental details for both the steps were same as described for the preparation of **11**.

4.14 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1, 3-thazol-4-yl)-4-oxo-3-S-isopropyl-carbamoylpentanoate (12)

This compound was prepared from **3** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. Two different solvents were tried in this case. The residue was purified by flash chromatography with 5% MeOH in $CHCl_3$. Yield was 21.8% when DMF was used as solvent for mixed anhydride reaction and 52.07% when THF–CH_2Cl_2 2:1 was as solvent for coupling reaction.

4.15 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-butylcarbamoylpentanoate (13)

This compound was prepared from **4** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. The reaction was

carried out in CH_2Cl_2. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.16 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1, 3-thazol-4-yl)-4-oxo-3-S-isobutylcarbamoylpentanoate (14)

This compound was prepared from **5** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. The reaction was carried out in $CHCl_3$. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.17 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxyca rbonylimino)-methylaminobutylcarboxamido]-1, 3-thazol-4-yl)-4-oxo-3-S-isopentyl-carbamoylpentanoate (15)

This compound was prepared from **6** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. The reaction was carried out in $CHCl_3$. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.18 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-(1-ethylproplcarbamoyl) Pentanoate (16)

This was prepared from **7** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. The reaction was carried out in $CHCl_3$. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$

4.19 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-neopentyl-carbamoylpentanoate (17)

This was prepared from **8** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. The reaction was carried out in $CHCl_3$. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.20 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-cyclohexyl-carbamoylpentanoate (18)

This compound was prepared from **9** and Tri-Z-Arg using the mixed anhydride procedure as described for the synthesis of **11**. The reaction was carried out in $CHCl_3$. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.21 Benzyl 5-(2-[1-S-Benzyloxycarbonylamino-4-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-3-S-(1-S-benzyloxycarbonyl-2-methylpropylcarbamoyl)-4-oxo-pentanoate (19)

The reaction was carried out in $CHCl_3$. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.22 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-propylcarbamoylpentanoic Acid (20)

Compound **11** (0.6 g; 0.62 mmol) was dissolved in TFA (15 ml) and anisole (0.3 ml). To this solution, trifluorosulfonic acid (0.8 ml) was added under stirring at room temperature. After stirring at room temperature for 3 h, excess dry ether was added. The precipitated solid (highly hygroscopic) was filtered and washed with dry ether. It was dried under vacuum overnight. The crude material was dissolved in ice-cold water (2 ml) and made alkaline to pH$\sim$10 by adding cold dilute NaOH. The mixture was immediately loaded on an RP-18 MPLC column. The column was first eluted with water followed by water–*n*BuOH–AcOH 90:2.5:2.5 (v/v). The fractions containing the required compound were pulled together and evaporated to dryness. The pure compound was dissolved in dry MeOH (2 ml), and HCl in ether was added. The white solid was filtered and crystallized from dry MeOH–dry ether. Yield 0.14 g (41.7%); mp 185–90°C (d); IR (KBr): 3500–3300 (br), 2950 (br), 1745 (br), 1680–1660 (br) cm^{-1}; ^1H NMR (D_2O; 300 MHz): 0.78 (t, 3H, $J = 7.3$, -$NCH_2CH_2CH_3$), 1.43 (m, 2H, $NCH_2CH_2CH_3$), 1.68, 2.01 (2 x m, 4H, β-CH_2 and γ-CH_2 of Arg), 2.58 [dd, 1H, $J_{gem} = 14.5$, $J = 7.3$, -$CH(CH_\alpha H_\beta COO$-) of Asp], 2.66 [dd, 1H, $J_{gem} = 14.5$, $J = 4.8$, -$CH(CH_\alpha H_\beta COO$-) of Asp], 3.10, 3.20 (2 $\times$ m, 4H, δ-CH_2 of Arg + NCH_2CH_2-CH_3), 3.59 (s, 2H, CH_2CO of Tha), 4.28, 4.49 [2 $\times$ t, 2H, $J = 7.0$, 2 $\times$ -$C_\alpha H$- of Arg, Asp), 7.05 (s, 1H, *H*-5 of Tha); anal. calc. for $C_{18}H_{30}O_5N_8S$,

2HCl, $3H_2O$: C, 36.17; H, 6.41; N, 18.75; Cl, 11.87; S, 5.37; found: C, 36.03; H, 6.33; N, 18.58; Cl, 12.07; S, 5.15%.

The final compounds **21**–**28** were obtained from **12**–**19** by following the same procedure including purification, formation of hydrochloride salt, and final crystallization as described for the synthesis of compound **20**.

4.23 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-isopropylcarbamoylpentanoic Acid (21)

This compound was prepared from **12** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.24 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-butylcarbamoylpentanoic Acid (22)

This compound was prepared from **13** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.25 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-isobutylcarbamoylpentanoic Acid (23)

This compound was prepared from **14** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.26 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-isopentylcarbamoylpentanoic Acid (24)

This compound was prepared from **15** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.27 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-(1-ethylpropylcarbamoyl)pentanoic Acid (25)

This compound was prepared from **16** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.28 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-neopentylcarbamoylpentanoicAcid (26)

This compound was prepared from **17** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.29 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-4-oxo-3-S-cyclohexylcarbamoylpentanoic Acid (27)

This compound was prepared from **18** as described for the synthesis of **20**. The crude product was purified in a similar manner.

4.30 5-(2-[1-S-Amino-4-amino(imino)-methylaminobutylcarboxamido]-1,3-thazol-4-yl)-3-S-(1-S-carboxy-2-methylcarbamoyl)-4-oxo-pentanoic Acid (28)

This compound was prepared from **19** as described for the synthesis of **20**. The crude product was purified in a similar manner. Yield 63.97%; mp 196–202°C (d); IR (KBr): 3400–3300 (br), 2960 (br), 1725 (br), 1670–1640 (br) cm^{-1}; ^1H NMR (D_2O; 300 MHz): 0.88 (m, 6H, 2 × CH_3), 1.63–1.76 (2H), 2.01–2.19 (3H) [2 × m, 5H, -CHMe$_2$, and β-CH_2 and γ-CH_2 of Arg), 2.83–2.95 [2 × dd, 2H, $J_{gem} = 15.4$, $J = 7.7$, 6.6, CH(CH_2COOH)], 3.23 (t, 2H, $J = 6.7$, δ-CH_2 of Arg], 3.72 (br, 2H, CH_2CO of Tha), 4.22, 4.31, 4.72 (3 × m, 3H, 3 × -C$_\alpha H$- of Val, Arg and Asp), 7.05 (s, 1H, H-5 of Tha); anal. calc. for $C_{20}H_{32}O_7N_8S$, 2HCl, $2H_2O$: C, 37.68; H, 6.01; N, 17.58; Cl, 11.12; S, 5.03; found: C, 37.92; H, 6.15; N, 17.35; Cl, 11.07; S, 4.87%.

4.31 Ethyl 2-(2-Tertiarybutyloxycarbonylaminomethylcarboxamido-1,-3-thiazol-4-yl)acetate (29)

A solution of Boc-Gly (7.0 g; 40 mmol) and HOBt (6.8 g; 50 mmol) in CH_2Cl_2 (50 ml) was chilled at 0°C. To this solution, DCC (10.3 g; 50 mmol) in CH_2Cl_2 (10 ml) was added under vigorous stirring. After 5 min, ethyl-2-amino-thiazol-4-yl-acetate (9.3 g; 50 mmol) was added, and stirring continued for 2 h at 0°C. The reaction mixture was kept in the freezer overnight. DCU was filtered off. The filtrate was washed successively with 1N citric acid, water, 1 N $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. Solvent was removed, and the residue was crystallized from CH_2Cl_2–light petroleum.

4.32 Ethyl 2-[2-(2-Tritylaminoethylcarboxamido)-1,3-thiazol-4-yl)acetate (30)

This compound was prepared from Trit-β-Ala and Tha-OEt using the same procedure as described previously. The reaction was carried out in EtOAc–DMF instead of CH_2Cl_2. The crude product was purified by flash chromatography with 2% MeOH in $CHCl_3$. It was crystallized from EtOAc–light petroleum.

4.33 2-(2-Tertiarybutyloxycarbonylaminomethylcarboxamido)-1,-3-thiazol-4-yl)acetic Acid (31)

Compound **29** (10.29 g; 30 mmol) was suspended in MeOH (132 ml), and 1N NaOH (33 ml; 33 mmol) was added at room temperature under vigorous stirring conditions. Within 2 min, the entire solid was dissolved. The stirring continued for 1 h. The solvent was removed. The solid was filtered, and the filtrate was extracted with EtOAc (2×10 ml) and discarded. The aqueous layer was acidified and extracted with EtOAc (3×30 ml). The EtOAc layer was washed with brine and dried over anhydrous Na_2SO_4. Solvent was removed. The residue was crystallized from hot EtOAc–light petroleum.

4.34 2-[2-(2-Tritylaminoethylcarboxamido)-1,3-thiazol-4-yl]-acetic Acid (32)

Compound **30** was hydrolyzed according to the method described for the preparation of **31**. The residue was crystallized from hot EtOAc.

4.35 Boc-Asp(OBzl)-Phe-OBzl

To a solution of Boc-Asp (OBzl)-OH (3.23 g; 10 mmol) and HOBt (1.62 g; 12 mmol) in CH_2Cl_2 (60 ml) chilled at 0°C, DCC (2.47 g; 12 mmol) was added under vigorous stirring conditions. In a separate round-bottomed flask HCl-PheOBzl[24] (3.5 g; 12 mmol) was dissolved in CH_2Cl_2 (20 ml) and DMF (5 ml) and neutralized with Et_3N (1.7 ml; 12 mmol) at 0°C. After 5 min, the amine component was added to the reaction mixture and stirring continued for 1 h at 0°C and stored in the freezer overnight. DCU was filtered off. The filtrate was successively washed with 1N $NaHCO_3$, water, 1N citric acid, and brine. It was dried over anhydrous Na_2SO_4. Solvent was removed, and the residue was purified by flash chromatography using 5% CH_3CN in $CHCl_3$. The pure compound was crystallized from EtOAc–light petroleum. Yield 4.0 (71.4%), mp 102–4°C; IR (KBr): 3380, 3220, 1770, 1755, 1705, 1695 cm^{-1}; ^1H NMR: 1.43 [s, 9H, C (CH_3)$_3$], 2.64, 3.14 ($2 \times$ dd, 2H, $J_{gem} = 17.5$, $J = 6.08$, $J_s = 5.06$, CH (CH_2COO-)], 3.06 (d, 2H, $J = 6.08$, CH (CH_2Ph)], 4.51, 4.80 ($2 \times$ m, 2H, $2 \times C_\alpha H$), 5.09 (s, 4H, $2 \times OCH_2$Ph),

6.83–7.34 (m, 15H, Ph*H*); anal. calc. for $C_{32}H_{36}N_2O_7$: C, 68.55; H, 6.47; N, 4.99; found: C, 68.34; H, 6.41; N, 4.69%.

4.36 Benzyl 3-(2-Tertiarybutyloxycarbonylaminomethylcarboxamido-1,3-thiazol-4-yl-methylcarboxamido)-4-(1-benzyloxycarbonyl-2-phenylethylamino)-4-pentanoate (33)

This was prepared by treating **31** with Asp (OBzl)-Phe (OBzl)-HCl by DCC–HOBt as described for the preparation of **2**. The crude product was purified by flash chromatography with 2.5% MeOH in $CHCl_3$. It was crystallized from EtOAc–light petroleum

4.37 Benzyl 4-(1-benzyloxycarbonyl-2-phenylethylamino)-3-[2-(2-tritylaminoethylcarbohyphen;xamido)-1,3-thiazol-4-yl-methylcarboxamido)-4-pentanoate (34)

This was prepared by coupling **32** with Asp (OBzl)-Phe (OBzl)-HCl by DCC–HOBt as described for the preparation of **2**. The crude product was purified by flash chromatography with 2.5% MeOH in $CHCl_3$. It was crystallized from EtOAc–light petroleum.

4.38 Benzyl 4-(1-Benzyloxycarbonyl-2-phenylethylamino)-3-[2-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminomethylcarboxamido-1,3-thiazol-4-yl-methyl-carboxamido]-4-pentanoate (35)

A solution of compound **33** (1.52 g; 2 mmol) in formic acid (23 ml) and anisole (0.75 ml) was kept at room temperature for 3 h. Dry HCl in ether (1 ml) was added, and stirring continued for 5 min. The solvent was removed under reduced pressure. The oily residue was triturated with dry ether, and the solid obtained was filtered. It was dried in vacuum desiccators over KOH pellets for 12 h. Yield 1.28 g (92.2%). The amine hydrochloride (1.0 g; 1.44 mmol) was dissolved in dichloromethane (15 ml) and acetonitrile (5 ml). After neutralizing with Et_3N (0.21 ml; 1.5 mmol), dicarbobenzoxy-S-methylisothiourea (0.53 g; 1.5 mmol) was added, and the mixture was refluxed for 12 h. The solvent was distilled off. The residue was taken up in EtOAc and washed with water. The organic layer was dried over anhydrous Na_2SO_4. Solvent was removed. The residue was purified by flash chromatography with 2% MeOH in $CHCl_3$. The crude product was crystallized from MeOH. Yield 0.86 g, (63.9%); mp 75–78°C; IR (KBr): 3320 (br), 1735 (br), 1640 (br) cm^{-1}; ^1H NMR (300 MHz): 2.65 [dd, 1H, $J_{gem} = 17.5$, $J_{trans} = 6.5$, -CH ($CH_\alpha H_\beta COO$) of Asp], 2.95 [d, 2H, $J = 6.7$, -CH (CH_2Ph)- of Phe], 3.02 [dd, 1H,

$J_{gem} = 17.5$, $J_{cis} = 5.3$, -CH ($CH_{\alpha}H_{\beta}$COO-) of Asp], 3.48 (s, 2H, CH_2CO of Tha), 4.21 (s, 2H, NHCH_2CO), 4.80, 4.93 (2 × m, 2H, 2 × $C_{\alpha}H$ of Asp and Phe), 4.96, 5.0, 5.12 (m), 5.18 (m, 8H, 4 × PhCH_2O-), 6.62 (s, 1H, *H*-5 of Tha), 6.98–7.43 (m, 20H, Ph*H*); anal. calc. for $C_{51}H_{49}N_7O_{11}S$: C, 63.27; H, 5.10; N, 10.13; S, 3.31; found: C, 62.97; H, 4.96; N, 9.94; S, 3.63%.

4.39 Benzyl 4-(1-Benzyloxycarbonyl-2-phenylethylamino)-3-{2-[2-benzyloxycarbonylamino(benzyloxycarbonylimino)-methylaminoethylcarboxamido]-1,3-thiazol-4-ylmethyl-carboxamido}-4-pentanoate (36)

Compound **34** (2 g; 2.19 mmol) was dissolved in 85% aqueous AcOH and heated on a steam bath for 10 min. Acetic acid was removed, and the residue was triturated with ether. The solid obtained was filtered and washed with dry ether. The dried material was crystallized from MeOH–dry ether to yield 1.47 g (91.87%). The amine acetate obtained previously was reacted with dicarbobenzoxy-S-methylisothiourea as described for the synthesis of compound **35**. The crude product was purified by flash chromatography with 2% MeOH in $CHCl_3$ and was crystallized from MeOH.

4.40 3-[2-Amino(imino)methylaminomethylcarboxamido]-1,-3-thiazol-4-yl-methylcarboxamido]-4-(1-carboxy-2-phenylethylamino)-4-pentanoic Acid (37)

Compound **35** (0.74 g; 0.76 mmol) was dissolved in TFA (20 ml). Anisole (0.3 ml) and trifluoromethansulfonic acid (1 ml) were added at room temperature under vigorous stirring. After 3 h excess dry ether was added. The solid separated was filtered and was washed with dry ether. The hygroscopic solid was dissolved in water (2 ml), had its pH adjusted to ~10, was filtered through celite, and was loaded on a RP-18 MPLC column. The column was first eluted with water (to remove trifluoromethansulfonate salt) followed by *n*BuOH: AcOH–water: 5:5:90. The fractions containing the required compound were pulled together and evaporated to dryness. The residue was dissolved in dry MeOH, and dry HCl in ether was added followed by excess ether. The white solid was filtered, washed with ether, and finally crystallized from dry MeOH–dry ether. Yield 0.236 g (55.53 %); mp 186–89°C; IR (KBr): 3400–3300 (br), 1750–1700, 1685–1660 (br) cm^{-1}; ^{1}H NMR (D_2O, 300 MHz): 2.72, 2.83 [2 × dd, 2H, $J_{gem} = 17.5$, $J = 6.4$, 5.2, -CH (CH_2Ph)-of Phe], 2.8, 3.10 [2 × dd, 2H, $J_{gem} = 16.5$, $J = 7.7$, 5.2, -CH (CH_2COOH)-of Asp], 3.59 (br, 2H, CH_2CO of Tha), 4.18 (br, 2H, NHCH_2CO), 4.50, 4.62 (2 × m, 2H, 2 × $C^{\alpha}H$ of Asp and Phe), 6.93 (s, 1H, *H*-5 of Tha), 7.09–7.28 (m, 5H, Ph*H*); anal. calc. for $C_{21}H_{25}N_7O_7S.HCl$, $2H_2O$: C, 42.60; H, 5.10; N, 16.56; Cl, 5.99; S, 5.42; found: C, 42.69; H, 4.96; N, 16.48; Cl, 6.17; S, 5.64%.

4.41 3-{2-[2-Amino(imino)methylaminoethyl-1-carboxamido]-1,-3-thiazol-4-yl-methylcarboxamido}-4-(1-carboxy-2-phenylethylamino)-4-pentanoic Acid (38)

This compound was prepared from **36** by following the method described for compound **37**. The purification procedure was also same with only exception being the use of solvent system *n*BuOH: AcOH:–water 2.5: 2.5: 95 v/v.

4.42 Ethyl 2-[2-(5-Tertiarybutyloxycarbonylaminopentylcarboxamido)-1,3-thiazol-4-yl]acetate (39)

This compound was prepared from Boc-protected 6-amino hexanoic acid and Tha-OEt in a similar fashion as described for the synthesis of compound **2**. The crude product was purified by flash chromatography with 2% MeOH in $CHCl_3$.

4.43 2-[2-(5-Tertiarybutyloxycarbonylaminopentylcarboxamido)-1,-3-thiazol-4-yl]acetic Acid (40)

Compound **39** was hydrolyzed according to the method described for the synthesis of compound **31**. The crude product was crystallized from EtOAc.

4.44 Benzyl 3-Carboxy-3-[2-(5-tertiarybutyloxycarbonylaminopentylcarboxamido-1,3-thiazol-4-ylmethylcarboxamido)propanoate (41)

To a suspension of Asp (OBzl)-OH (6.2 g; 28 mmol) in CH_2Cl_2 (50 ml), trimethylsilyl chloride (3.6 ml; 28 mmol) was added under vigorous stirring (5 min). After 2 h, the reaction mixture was chilled to 0°C, and Et_3N (4.2 ml; 30 mmol) was added to generate free amines. This was added after 10 min to a well-stirred solution of **40** (5.19 g; 14 mmol), DCC (3.3 g; 16 mmol), and HOBt (2.2 g; 14 mmol) in CH_2Cl_2 (50 ml) at 0°C. The resulting reaction mixture was stirred at 0°C for 1 h and then stored in the freezer overnight. Excess methanol (~10 ml) was added to the reaction mixture under stirring at room temperature (5 min). Solvent was removed under reduced pressure, and the residue was partitioned between EtOAc and aqueous 1N $NaHCO_3$. The aqueous layer was seperated and acidified with dil. $NaHSO_4$ solution. The separated oily compound was extracted with EtOAc (3 × 15 ml). The combined organic layer was washed with brine and dried over anhydrous Na_2SO_4, and the solvent was evaporated. The crude product was purified by flash chromatography with 10% MeOH + 0.1% AcOH in $CHCl_3$. It was finally crystallized from EtOAc–light petroleum.

4.45 Benzyl 3-Butylcarbamoyl-3-[2-(5-tertiarybutyloxycarbonylaminopentylcarboxamido-1,3-thiazol-4-yl-methylcarboxamido)propionate (42)

A solution of compound **41** (1.26 g; 2.18 mmol) in DMF (10 ml) was chilled to -30°C, and 1-methyl morpholine (0.24 ml; 2.18 mmol) was added, followed by isobutylchloroformate (0.28 ml; 2.18 mmol), under vigorous stirring. After stirring for 5 min, $nBuNH_2$. (0.4 ml; 4.96 mmol) was added, and the bath temperature was raised to -20°C. The mixture was kept in the freezer overnight after stirring at -20°C for 1 h. It was treated with 1N $NaHCO_3$ solution to decompose unreacted mixed anhydride. DMF was removed, and the residue was extracted with EtOAc. The EtOAc layer was washed successively with water, dilute 1N citric acid, and brine. It was dried over anhydrous Na_2SO_4. Solvent was removed, and the residue was purified by flash chromatography with 2% MeOH in $CHCl_3$. Yield 1.15 g (oily). IR (KBr): 3370–3300, 3000–2950 (br), 1755 (br), 1710, 1695 (br) cm^{-1}; ^{1}H NMR (CD_3OD): 0.78 [t, 3H, $J = 8.0$, N $(CH_2)_{3CH3}$], 1.19, 1.32, 1.43, 1.69 [4 × m, 10H, $NHCH_2$ $(CH_2)_{2CH3}$, -$NHCH_2$ $(CH_2)_{3CH2CO}$-], 1.37 [s, 9H, -C $(CH_3)_3$], 2.39 (t, 2H, $J = 8.0$, $(CH_2)_{4CH2CO}$], 2.65, 2.95 [2 × dd, 2H, $J_{gem} = 17.5$, $J = 7.5$, 5.5, -CH (CH_2COO)- of Asp], 3.02–3.13 (m, 4H, 2 × NCH_2), 3.54 (s, 2H, CH_2CO of Tha), 4.55 (br, 1H, N*H*), 4.77 (m, 1H, -$C_\alpha H$ of Asp), 5.06 (s, 2H, OCH_2Ph), 6.61 (s, 1H, *H*-5 of Tha), 7.26 (m, 5H, Ph*H*), 8.24 (br, 1H, N*H*); anal. calc. for $C_{31}H_{45}N_5O_7S$: C, 58.93; H, 7.18; N, 11.09; S, 5.07; found: C, 58.77; H, 7.02; N, 11.17; S, 5.25%.

4.46 Benzyl 3-{2-[5-Benzyloxycarbonylamino-(benzyloxycarbonylimino)methylaminopentylcarboxamido]-1,3-thiazol-4-yl-methylcarboxamido}-3-butylcarbamoylpropanoate (43)

This was prepared from **42** after deblocking the Boc group and subsequent refluxing with dicarbobenzoxy-S-methylisothiourea in CH_2Cl_2–CH_3CN 2:1, as described for the synthesis of **35**. The crude product was purified by flash chromatography with 2.5% MeOH in $CHCl_3$.

4.47 3-{2-[5-Amino(imino)methylaminopentylcarboxamido]-1,-3-thiazol-4-yl-methylcarbohyphen;xamido}-3-butylcarbamoylpropanoic Acid (44)

This was obtained by deprotection of carbobenzoxy and benzyl groups from **43** as described for the synthesis of **37**. The crude product was crystallized from dry MeOH–ether. This compound was characterized as trifluoromethanesulfonic acid salt.

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