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Supporting Information

Module Assembly for Protein Surface Recognition: Bivalent Type-I Geranylgeranyltransferase Inhibitors for Simultaneous Targeting of Interior and Exterior Protein Surfaces

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List of Abbreviations:

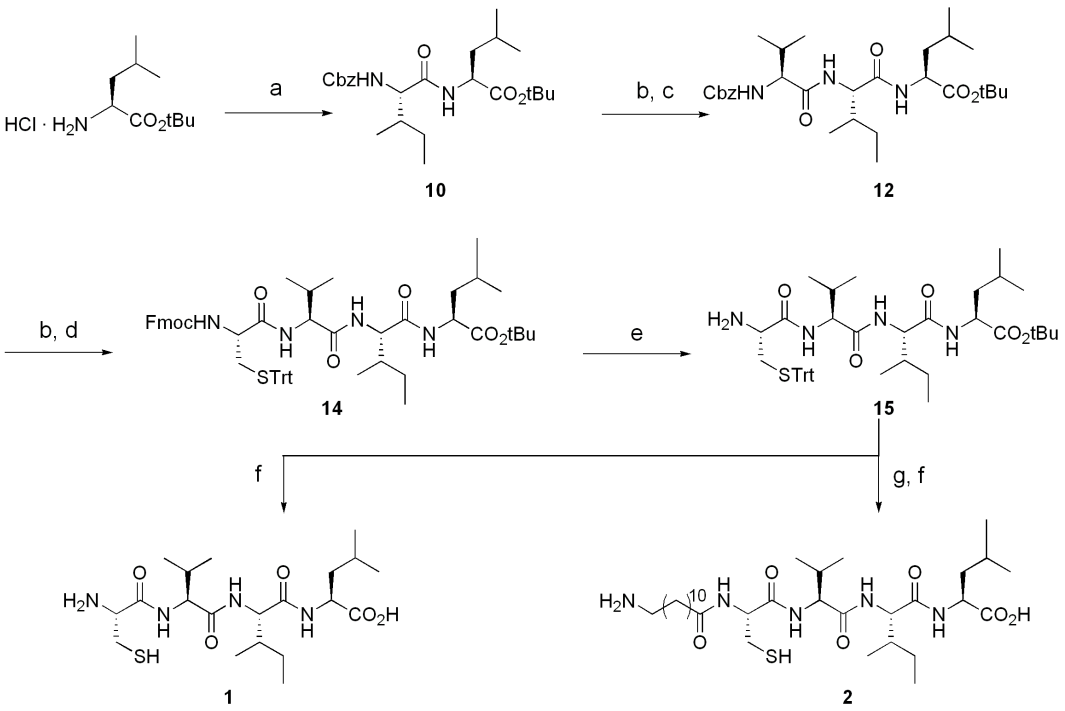
Boc	*tert*-Butoxycarbonyl
Boc_2O	Di-*tert*-butyl dicarbonate
Cbz	Benzyloxycarbonyl
DICI	Diisopropylcarbodiimide
DIEA	Diisopropylethylamine
DMF	Dimethylformamide
DTT	Dithiothreitol
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarboiimide
Fmoc	(9H-Fluoren-9-ylmethoxy)carbonyl
HOBt	1-Hydroxybenzotriazole
PyBop	1-Benzotriazolyloxy-tris-pyrrolidinophosphonium hexafluorophosphate
SPE	Solid phase extraction
SPPS	Solid phase peptide synthesis
TFA	Trifluoroacetic acid
THF	tetrahydrofuran
Trt	Trityl

General Procedures. Reagents and solvents were obtained from commercial sources without further purification unless otherwise noted. Melting points were determined with an Electrothermal capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-LA 400 spectrometer. Chemical shifts were reported in δ (ppm) relative to tetramethylsilane. All coupling constants were described in Hz. Elemental analyses were performed by Perkin Elmer - 2400CHN. Flash column chromatography was performed on silica gel (40-63 μm) under a pressure of about 4 psi. Synthesized final compounds were checked for purity by analytical HPLC, which was performed using a JASCO PU-2086 and a JASCO UV-2075 detector with a GL Science Inertsil 150 mm x 4.6 mm, 5 μm C-18 column, eluted with gradient 10% to 90 % of CH_3CN in 0.1 % TFA in H_2O in 30 min. High-resolution mass spectra (HRMS) and low-resolution mass spectra (LRMS) were taken by Mr. H. Yamada at Material Analysis Center of ISIR using JEOL JMS-700, JEOL JMS-600H and JEOL JMS-T100LC mass spectrometer.

Synthesis of tetrapeptide CVIL and its derivatives (Scheme S1).

***N*-Benzylcarbonyl-L-isoleucyl-L-leucine *t*-Butyl Ester (10)**. To a solution of Cbz-Ile-OH (3.60 g, 13.5 mmol), H-Leu-OtBu·HCl (3.02 g, 13.5 mmol), HOBt·H_2O (3.07 g, 20.0 mmol), Et_3N (2.1 mL) in anhydrous DMF (60 mL) was added EDCI (2.58 g, 13.5 mmol) in CH_2Cl_2 (10 mL) at 0 °C, and the mixture was stirred at room temperature (rt) overnight. After DMF was removed by distillation the residue was dissolved into AcOEt and the organic layer was washed by 10% citric acid, 5% sodium bicarbonate, and brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the product as a cream yellow solid (6.0 g, 100%): Mp 73-77 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.88-0.94 (m, 12H, CH_3 x 4), 1.09-1.19 (m, 1H, CH), 1.45-1.53 (m, 11H, O*t*Bu, CH_2), 1.57-1.65 (m, 2H, CH_2), 1.83-1.89 (m, 1H, CH), 4.03 (dd, *J* = 6.7 and 8.5 Hz, 1H, CH), 4.46-4.52 (m, 1H, *a*-CH), 5.10 (s, 1H, CH_2O), 5.35 (d, *J* = 8.6 Hz, 1H, NH), 6.11 (d, *J* = 8.3 Hz, 1H, NH), and 7.34 (m, 5H, Ph): HR FAB-MS calcd for $C_{24}H_{39}N_2O_5$ $[M+H]^+$ 435.2859, found 435.2857.

Scheme S1



(a) Cbz-Ile-OH, EDCI, HOBt·H_2O, Et_3N, DMF, quant.; (b) H_2 / 10% Pd-C, MeOH, quant.; (c) Cbz-Val-OH EDCI, HOBt·H_2O, DMF, 92%; (d) Fmoc-Cys(Trt)-OH, EDCI, HOBt, DMF, 90%; (e) 10 v/v % DEA, DMF, quant.; (f) 50 v/v% TFA, 5 v/v % TES, CH_2Cl_2, 88% for **1**, 91% for **2**; (g) BocHN-$(CH_2)_{11}$-CO_2H (**17**), PyBop, HOBt·H_2O, DIEA, DMF 97%.

***N*-L-Isoleucyl-L-leucine *t*-Butyl Ester (11).** A solution of **10** (4.02 g, 9.25 mmol) and 10% Pd-C (250 mg) in MeOH (10 mL) was stirred at rt until the starting material disappeared on tlc. Filtration of the catalyst off over celite and evaporation of the solvent afforded the yellow oily product (2.80 g, quant.): ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.88-0.97 (m, 12H, CH_3 x 4), 1.07-1.15 (m, 1H, CH), 1.32-1.68 (m, 13H, O*t*Bu and CH_2 x 2), 1.95-2.01 (m, 1H, CH), 3.29 (d, *J* = 3.8 Hz, 1H, *a*-CH), 4.46-4.52 (m, 1H, *a*-CH) and 7.59 (d, *J* = 8.4 Hz, 1H, NH): HR FAB-MS calcd for $C_{16}H_{33}N_2O_3$ $[M+H]^+$ 301.2491, found 301.2519.

***N*-Benzyloxycarbonyl-L-Valyl-L-isoleucyl-L-leucine *t*-Butyl Ester (12).** This tripeptide was prepared by a method similar to that described for **10**. The crude product was purified by SiO_2 column chromatography (AcOEt: hexane = 1 : 4 to 1:1) to afford the product as a white solid (4.12 g, 92%): Mp 162-163 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.87-0.94 (m, 18H, CH_3 x 4), 1.04-1.15 (m, 1H, CH), 1.43-1.64 (m, 13H, O*t*Bu and CH_2 x2), 1.78-1.84 (m, 1H, CH), 2.02-2.11 (m, 1H, CH), 4.06 (dd, *J* = 7.1 and 8.3 Hz, 1H, *a*-CH), 4.38 (t, *J* = 8.3 Hz, 1H, *a*-CH), 4.49 (m, 1H, *a*-CH), 5.10 (s, 1H, CH_2OCO), 5.75 (d,

J = 8.7 Hz, 1H, NH), 6.66 (m, 2H, NH x 2), and 7.32 (m, 5H, Ph): HR FAB-MS calcd for $C_{29}H_{48}N_3O_6$ $[M+H]^+$ 534.3543, found 534.3561.

***N*-L-Valyl-L-isoleucyl-L-leucyl *t*-Butyl Ester (13).** Deprotection of **12** (3.03 g, 5.68 mmol) was carried out by a method similar to that described for **11** to give the product as a cream yellow solid (2.30 g, quant.): Mp 137-138 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.86-1.00 (m, 18H, CH_3 x 6), 1.12-1.20 (m, 1H, CH), 1.45-1.69 (m, 13H, O*t*Bu CH_2 x 2), 1.88-1.95 (m, 1H, CH), 2.26-2.34 (m, 1H, CH), 3.35 (br, CH) 4.29 (dd, *J* = 7.7 Hz, *a*-CH), 4.45 (m, 1H, *a*-CH), 6.43 (br, 1H, NH), and 7.84 (br, 1H, NH): HR FAB-MS calcd for $C_{21}H_{42}N_3O_4$ $[M+H]^+$ 400.3175, found 400.3174.

***N*-9-Fluorenylmethoxycarbonyl-S(trityl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucyl *t*-Butyl Ester (14).** This tetrapeptide was prepared by the coupling reaction of **13** (2.02 g, 0.5.05 mmol) and Fmoc-Cys(Trt)-OH (3.27 g, 5.59 mmol) by a method similar to that described for **10**. The crude product (4.94 g) was purified SiO_2 column chromatography (AcOEt: hexane = 1:2 to 1:1) to give the product as white solid (90%): Mp 151-154 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.78-0.91 (m, 18H, CH_3 x 6), 1.01-1.10 (m, 1H, CH), 1.44-1.60 (m, 13H, O*t*Bu and CH_2 x 2), 1.79-1.89 (m, 1H, CH), 2.0-2.14 (m, 1H, CH), 2.60-2.70 (m, 2H, CH_2), 3.68 (m, 1H, FmocCH), 4.12-4.39 (m, 5H, a-CH x 3, and FmocCH_2), 4.44-4.49 (m, 1H, *a*-CH), 5.03 (d, *J* = 7.2 Hz, 1H, NH), 6.29-6.50 (m, 3H, NH x 3) and 7.17-7.75 (m, 23H, Aryl H): HR FAB-MS calcd for $C_{58}H_{71}N_4O_7S$ $[M+H]^+$ 967.5043, found 967.5065.

***N*-S(Trityl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-Butyl Ester (15).** A solution of **14** (101 mg, 0.104 mmol) and diethylamine (0.4 mL, 10% v/v) in DMF (4 mL) was stirred at rt for 2h. After removal of DMF by distillation, hexane was added to the residue and the resulting precipitates were collected by centrifugation to afford the product as a white solid (115 mg, 100%): Mp 156-158 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.84-0.92 (m, 18H, CH_3 x 6), 1.04-1.15 (m, 1H, CH), 1.41-1.64 (m, 13H, O*t*Bu and CH_2 x 2), 1.81-1.87 (m, 1H, CH), 2.06-2.15 (m, 1H, CH), 2.59 (m, 2H, CH_2), 3.01 (m, 1H), 4.09 (m, 1H, *a*-CH), 4.16 (m, 2H, *a*-CH), 4.45 (m, 1H, *a*-CH), 6.08 (d, 1H, *J* = 7.8 Hz, NH), 6.36 (d, *J* = 8.6 Hz, 1H, NH) and 7.17-7.45 (m, 15H, Aryl H) and 7.59 (d, *J* = 8.7 Hz, 1H, NH): HR FAB-MS calcd for $C_{43}H_{61}N_4O_5S_1$ $[M+H]^+$ 745.4363, found 745.4366.

***N*-L-Cysteinyl-L-valyl-L-isoleucyl-L-leucine (1).** To a solution of **14** (28 mg, 0.038 mmol) and triethylsilane (50 µL, 5% v/v) in CH_2Cl_2 (450 µL) was added trifuloroacetic acid (500 µL, 50% v/v) at 0 °C, and the mixture was stirred for 5min at 0 °C and then for another 55 min at 30°C. After evaporating solvents, the residue was washed with Et_2O several times to give the product as a white powder (18 mg, 88%): HPLC purity 85 %: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 0.77-0.87 (m, 18H, CH_3 x 6), 0.99-1.10 (m, 1H, CH), 1.42-1.69 (m, 5H, CH_2 x 2 and CH), 1.88-1.96 (m, 1H, CH), 2.78-2.80 (m, 2H, CH_2), 3.66 (m, 1H, *a*-CH), 4.14-4.26 (m, 3H, *a*-CH), 7.98 (d, *J* = 9.0 Hz, 1H, NH) and 8.15-8.19 (m, 2H, NH):HR FAB-MS calcd for $C_{20}H_{39}N_4O_5S_1$ $[M+H]^+$ 447.2641, found 447.2650.

12-(*N*-*tert*-Butoxycarbonyl)aminododecanoic acid Methyl Ester (16). To a solution of 12-aminododecanoic acid methyl ester hydrochloride (513 mg, 1.912 mmol) and 5N NaOH (10 mL) in 1,4-dioxane / H_2O mixture (20 mL / 10 mL) was added Boc_2O (563 mg, 2.58 mmol) in dioxane at 0 °C, and the mixture was stirred at rt overnight. After concentration, the product was extracted with AcOEt, dried over anhydrous $MgSO_4$, followed by evaporation of solvent to afford the desired product as colorless solid (645 mg, 100%): Mp 76-79 °C: ^{1}H-NMR (400 MHz, CD_3OD) *d* 1.27 (s, 14H, -$(CH_2)_7$), 1.44 (s, 11H, Boc and CH_2), 1.57 (m, 2H, -NHCH_2C$\underline{H}_2$), 2.27 (t, *J* = 7.4 Hz, 2H, C$\underline{H}_2$CO_2CH_3) and 3.00 (t, *J* = 7.0 Hz, 2H, -NHC$\underline{H}_2$).

12-(*N*-*tert*-Butoxycarbonyl)aminododecanoic acid (17). A solution of **16** (335 mg, 1.02 mmol) and 1N LiOH (10 mL) in THF (10 mL) was refluxed for 2 days. After concentration, the product was extracted with $CHCl_3$ (60 mL x 3), 10% citric acid (20 mL), and brine, and the combined organic layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the product as a white solid (43 mg, 100%): Mp 76-79 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.27 (m, 14H,-$(CH_2)_7$), 1.44 (m, 9H, Boc), 1.57 (m, 4H, -NHCH_2C$\underline{H}_2$ and -C$\underline{H}_2$CH_2CO_2CH_3), 2.34 (t, *J* = 7.1 Hz, 2H, C$\underline{H}_2$CO_2CH_3) and 3.04-3.11 (m, 2H, -NHC$\underline{H}_2$).

***N*-{11-(*tert*-butoxycarbonyl)aminoundecanylcarbonyl}-S(trityl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-Butyl Ester (18).** This compound was prepared by EDCI / HOBt coupling reaction of **15** (112 mg, 0.15 mmol) and **17** (52 mg, 0.16 mmol) by a similar procedure to that described for **10**. The crude product (163 mg) was purified by SiO_2 column chromatography ($CHCl_3$: CH_3OH = 50 : 1) to give the product as a white solid (152 mg, 97%): Mp 159-162 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.74-0.91 (m, 18H, CH_3 x 6), 0.99-1.08 (m, 1H, CH), 1.22-1.27 (m, 14H, $(CH_2)_7$), 1.44-1.67 (m, 22H, Boc, O*t*Bu, CH_2 x 2), 1.78-1.85 (m, 1H, CH), 2.01-2.11 (m, 3H, -C$\underline{H}_2$CONH and CH), 2.57 (dd, *J* = 5.7 and 13.2 Hz, 1H, *ß*-CH_{2a}, Cys), 2.68 (dd, *J* = 7.4 and 13.2 Hz, 1H, *ß*-CH_{2b}, Cys), 3.08 (m, 2H, CONHC$\underline{H}_2$), 4.02-4.04 (m, 1H, *a*-CH), 4.18 (dd, *J* = 6.9 and 8.1 Hz 1H, *a*-CH), 4.30 (dd, *J* = 7.8 and 8.7 Hz, 1H, *a*-CH), 4.42-4.51 (m,2H, BocNH and *a*-CH), 6.02, 6.49, 6.71, 6.81 (br, 4H, NH) and 7.19-7.44 (m, 15H, Aryl H): HR FAB-MS calcd for $C_{60}H_{92}N_5O_8S_1$ $[M+H]^+$ 1042.6667, found 1042.6676.

11-Aminoundecanylcarbonyl-S(trityl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine (2). Deprotection of **18** (31 mg, 0.03 mmol) and product isolation were carried out by a similar procedure to that described for **1** to give the product as a white powder (20 mg, 91%): HPLC purity 90 %: ^{1}H-NMR (400 MHz, DMSO-d_6) δ 0.78-0.88 (m, 18H, CH_3 x 6), 1.03-1.10 (m, 1H, CH), 1.23 (br, 14H, $(CH_2)_7$), 1.46-1.52 (m, 9H, -$NH_2CH_2C\underline{H}_2$, -C$\underline{H}_2$CH_2CO_2CH_3, ?-CH_2 Ile and *ß*-CH_2 Leu and *ß*-CH Ile), 1.90-1.98 (m, 1H, CH), 2.08-2.18 (m, 2H, -C$\underline{H}_2$CONH), 2.59-2.77 (m, 4H, *ß*-CH_2, Cys and -CONHC$\underline{H}_2$), 4.15-4.22 (m, 3H, *a*-CH), 4.39-4.45 (m, 1H, *a*-CH), and 7.47-7.86 (m, 4H, NH and NH_3) and 8.04-8.21 (m, 3H, NH): HR FAB-MS calcd for $C_{32}H_{62}N_5O_6S_1$ $[M+H]^+$ 644.4421, found 644.4441.

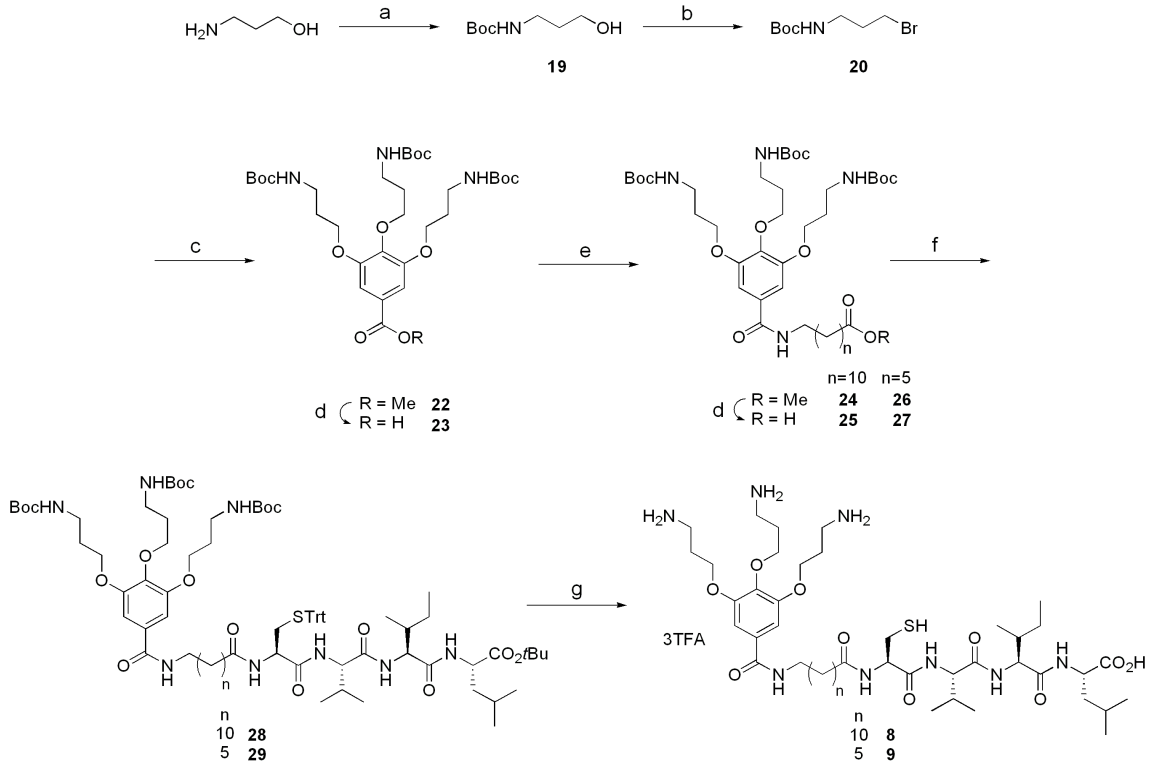
Synthesis of 3,4,5-tris(3-amino-1-propxyl) benzoic acid derivatives and the corresponding bivalent inhibitors (Scheme S2).

3-(*N*-*tert*-Butoxycarbonyl)amino-1-propanol (19). To a solution of 3-amino-1-propanol (5.02 g, 66.9 mmol) and 5N NaOH (15 mL) in dioxane (50 mL) and H_2O (20 mL) mixture was slowly added Boc_2O (16.8 g, 77.0 mmol) in dioxane at 0 °C, and the reaction mixture was stirred at rt overnight. After concentration, the residue was extracted from 10% citric acid with AcOEt, and dried over anhydrous Na_2SO_4. Evaporation of solvents gave the pure product as colorless oil (11.6 g, 99%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H, Boc), 2.05 (tt, *J* = 5.6 and 5.8 Hz, 2H, $C\underline{H}_2CH_2OH$), 3.26 (dd, *J* = 5.8 and 6.2 Hz, 2H, $NHC\underline{H}_2$), 3.44 (t, *J* = 5.6 Hz, CH_2Br), and 4.66 (br, 1H, NH).

3-(*N*-*tert*-butoxycarbonyl)amino-1-bromo-propane (20). To a solution of **19** (1.27 g, 7.21 mmol), CBr_4 (3.60 g, 10.9 mmol) in THF (20 mL) was added Ph_3P (2.94 g, 11.2 mmol) in THF (20 mL) at 0 °C, and the mixture was stirred for 1h at 0°C. Additional CBr_4 (1.20 g, 3.75 mmol) and Ph3P (1.07 g, 4.09 mmol) were added to the solution and the mixture was stirred for 3h at r.t. until the starting material completely disappered on TLC. After evaporating solvents, the residue was purified by SiO_2 column ($CHCl_3$ alone) to give the product as pale yellow oil (1.46 g, 84%): ^{1}H-NMR (400 MHz, $CDCl_3$) *d*1.44 (s, 9H, Boc), 2.05 (quint, *J* = 6.4 Hz, 2H, $C\underline{H}_2CH_2Br$), 3.26 (dt, *J* = 6.3 and 12.8 Hz, 2H, $NHC\underline{H}_2$), 3.44 (t, *J* = 6.5 Hz, $C\underline{H}_2Br$), and 4.64 (br, 1H, NH).

Gallic acid Methyl Ester (21). To a cold MeOH (60 mL) was added thionyl chloride (8.4 mL, 118 mmol) dropwise at 0 °C, and a solution of gallic acid (10.41 g, 61.1 mmol) in MeOH was then added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 10min, at rt for 2h, and refluxed overnight. After quenching reaction with sat. $NaHCO_3$, the mixture was concentrated. Extraction with AcOEt and sat. $NaHCO_3$, and brine, followed by evaporation gave the desired product as cream yellow solid (7.06 g, 62%): Mp 193-195 °C: ^{1}H-NMR (400 MHz, CD_3OD) *d* 3.81 (s, 3H, CO_2CH_3), and 7.03 (s, 2H, Aryl H).

Scheme S2



(a) Boc_2O, 1,4-dioxane, 5N NaOH, H_2O, 99%; (b) CBr_4, PPh_3, CH_2Cl_2, 63%; (c) **21**, K_2CO_3, DMF, 80%; (d) 1M KOH, CH_2Cl_2, CH_3OH, quant. for **23**, 96% for **25**; quant. for **27**; (e) H_2N-$(CH_2)_n$-CO_2CH_3, PyBop, HOBt·H_2O, DIEA, DMF, 79% for **24**; 75% for **26**; (f) H-C(Trt)VIL-O*t*Bu (**15**), PyBop, HOBt·H_2O, DIEA, DMF 83%; (g) 50 v/v% TFA, 5 v/v% TES, CH_2Cl_2, 92%.

3,4,5-Tris[3-(*N*-*tert*-butoxycarbonyl)amino-1-propanoxy]benzoic acid Methyl Ester (22). A solution of **21** (316 mg, 1.72 mmol), **20** (1.39 g, 5.82 mmol), and K_2CO_3 (1.38 g, 9.98 mmol) in DMF (3 mL) was stirred at 40 °C for 4 h. After concentration, the product was extracted with AcOEt (150 mL), and the organic layer was washed with 10% citric acid, brine, and dried (anhydrous $MgSO_4$). The crude product was purified by SiO_2 column chromatography (AcOEt : hexane = 1 : 2 to AcOEt : hexane = 1 : 1) to afford the product as a colorless oil (1.07 g, 95%): ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.44 (br, 27H, Boc×3), 1.94 (quint, *J* = 6.1 Hz, 2H, 4-$OCH_2C\underline{H}_2$), 2.03 (quint, *J* = 6.1 Hz, 4H, 3 and 5- $OCH_2C\underline{H}_2$), 3.33-3.41 (m, 6H, 3, 4,5-BocNHC$\underline{H}_2$), 3.89 (s, 3H CO_2CH_3) and 4.08-4.12 (m, 6H, 3, 4, 5-OC$\underline{H}_2$), 5.07 (br, 2H, 3 and 5-N$\underline{H}$), 5.34 (br, 1H, 4-N$\underline{H}$) and 7.27 (s, 2H, benzoyl): HR FAB-MS calcd for $C_{32}H_{54}N_3O_{11}$ $[M+H]^+$ 656.3758, found 656.3787.

3,4,5-Tris(3-amino-1-propanoxy)benzoic acid Methyl Ester (4). To a solution of **22** (26 mg, 0.040 mmol) in CH_2Cl_2 (0.5 mL) was added trifluoroacetic acid (500 μL, 50 v/v %), and the mixture was stirred at 0 °C for 1h. After evaporating solvents, the residue was washed with Et_2O several times and

the precipitates were then collected by centrifugation to give the product as colorless amorphous (30 mg, quant): HPLC purity 93%: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 1.87-1.96 (m, 2H, 4-OCH$_2$CH$_2$), 2.01-2.10 (m, 4H, 3 and 5- OCH$_2$CH$_2$), 2.94-3.04 (m, 6H, NCH$_2$×3), 3.84 (s, 3H, OCH$_3$), 4.03 (t, 2H, *J* = 5.6 Hz, 4-OCH$_2$), 4.12 (t, 4H, *J* = 6.3 Hz, 3 and 5-OCH$_2$), 7.27 (s, 2H, benzoyl) and 7.82 (br, 9H, NH$_3$×3): HR FAB-MS calcd for $C_{32}H_{54}N_3O_{11}$ $[M+H]^+$ 356.2185, found 356.2205.

3,4,5-Tris[3-(*N*-*tert*-butoxycarbonyl)amino-1-propanoxy]benzoic acid (23). This compound was prepared by saponification of **22** (203 mg, 0.31 mmol) with 1N KOH (9 mL, 9 mmol) in CH_2Cl_2 (5 mL) by refluxing for 11 h. After concentration, the product was extracted with 10% citric acid and CH_2Cl_2, and dried (anhydrous $MgSO_4$). The desired product was an yellow oil (198 mg, quant.): ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.44 (br, 27H, Boc×3), 1.93-2.05 (m, 6H, 3 and 5- OCH$_2$CH$_2$), 3.34-3.42 (m, 6H, BocNHCH$_3$×3), 4.10-4.13 (m, 6H, 3, 4 and 5-OCH$_2$), 5.15-5.42 (m 3H, BocNH) and 7.30 (s, 2H, benzoyl): HR-FAB MS calcd for $C_{31}H_{52}N_3O_{11}$ $[M+H]^+$ 642.3602, found 642.3596.

12-[3,4,5-Tris{3-(*N*-*tert*-butoxycarbonyl)amino-1-propanoxy}benzoylamino]dodecanoic acid Methyl Ester (24). This compound was prepared by a reaction of **23** (152 mg, 0.24 mmol) and 12-aminododecanoic acid methyl ester (70 mg, 0.26 mmol) using DIEA (diisopropylethylamine, 80 μL, 0.47 mmol), HOBt (73 mg, 0.48 mmol), and PyBop (152 mg, 0.29 mmol). The crude material was purified by SiO_2 column chromatography (hexane alone to AcOEt : hexane = 2 : 1) to afford the desired product as a white solid (16 mg, 79%): Mp 91-92 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.27-1.37 (m, 14H, -(CH$_2$)$_7$CH$_2$CH$_2$CO$_2$CH$_3$), 1.44 (s, 27H, Boc×3), 1.58-1.65 (m, 4H, -CONHCH$_2$CH$_2$ and -CH2CH$_2$CO$_2$CH$_3$), 1.93 (quint *J* = 6.0 Hz, 2H, 4-BocNHCH$_2$CH$_2$,), 2.03 (quint, *J* = 6.1 Hz, 4H, 3,5-BocNHCH$_2$CH$_2$,), 2.30 (t, *J* = 7.5 Hz, 2H, CH$_2$CO$_2$CH$_3$), 3.34-3.45 (m, 8H, BocNHCH$_3$×3 and -CONHCH$_2$), 3.66 (s, 3H, CO$_2$CH$_3$), 4.06-4.11 (m, 6H, 3, 4 and 5-OCH$_2$), 5.05-5.33 (m, 3H, NH), 6.21 (br, 1H, CONH) and 7.00 (s, 2H, benzoyl): HR-FAB MS calcd for $C_{44}H_{77}N_4O_{12}$ $[M+H]^+$ 853.5538, found 853.5524.

12-[3,4,5-Tris{3-(*N*-*tert*-butoxycarbonyl)amino-1-propanoxy}benzoylamino]dodecanoic acid (25). This compound was prepared from **24** (123 mg, 0.14 mmol) with 1N KOH (4.5 mL, 4.5 mmol) by a method similar to that described for **23**. After work up the reaction, evaporation of the solvent gave the product as white amorphous solid (115 mg, 96%). This material was used for the next reaction without further purification.: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.25-1.34 (m, 14H, -(CH$_2$)$_7$CH$_2$CH$_2$CO$_2$CH$_3$), 1.43 (br, 27H, Boc×3), 1.58-1.64 (m, 4H, -CONHCH$_2$CH$_2$ and -CH2CH$_2$CO$_2$CH$_3$), 1.93 (quit *J* = 5.9 Hz, 2H, 4-BocNHCH$_2$CH$_2$,), 2.01 (quit *J* = 6.1 Hz, 4H, 3,5-BocNHCH$_2$CH$_2$,), 2.30 (t, *J* = 7.0 Hz, 2H, CH$_2$CO$_2$CH$_3$), 3.33-3.45 (m, 8H, BocNHCH$_3$×3 and -CONHCH$_2$), 4.05-4.09 (m, 6H, 3, 4 and 5-OCH$_2$), 5.11 (br, 2H, BocNH), 5.32 (br, 1H, NH), 6.34 (br, 1H, CONH) and 7.01 (s, 2H, benzoyl).

6-[3,4,5-Tris{3-(*N*-*tert*-butoxycarbonyl)amino-1-propanoxy}benzoylamino]hexanoic acid Methyl Ester (26). This compound was prepared by a reaction of **23** and 6-aminohexanoic acid methyl ester by a similar method desrribed for **24**, as a white solid (90 mg, 75%): Mp 98-101 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.44 (s, 27H, Boc×3), 1.61-1.72 (m, 6H, -CONHCH$_2$(C$\underline{H}_2$)$_3$), 1.92-2.05 (m, 6H, 4-BocNHCH$_2$C$\underline{H}_2$,), 2.34 (t, *J* = 7.3 Hz, 2H, C$\underline{H}_2$CO$_2$CH$_3$), 3.33-3.47 (m, 8H, BocNHC$\underline{H}_3$×3 and -CONHC$\underline{H}_2$), 3.66 (s, 3H, CO$_2$C$\underline{H}_3$), 4.06-4.12 (m, 6H, 3, 4 and 5-OC$\underline{H}_2$), 5.06 (br, 2H, N$\underline{H}$×2), 5.33 (br, 1H, N$\underline{H}$), 6.34 (br, 1H, CON$\underline{H}$) and 7.02 (s, 2H, benzoyl): HR-FAB MS calcd for $C_{35}H65N_4O_{12}$ $[M+H]^+$ 769.4599 found 769.4593.

6-[3,4,5-Tris{3-(*N*-*tert*-butoxycarbonyl)amino-1-propanoxy}benzoylamino]hexanoic acid (27). This compound was prepared from **26** (80 mg, 0.10 mmol) by a method similar to that described for **25** to the product as white amorphous solid (79 mg, quant.): ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.44-1.66 (m, 33H, Boc×3) -CONHCH$_2$(C$\underline{H}_2$)$_3$), 1.91-2.04 (m, 6H, 4-BocNHCH$_2$C$\underline{H}_2$,), 2.4 (t, *J* = 5.4 Hz, 2H, C$\underline{H}_2$CO$_2$CH$_3$), 3.33-3.40 (m, 6H, BocNHC$\underline{H}_3$×3), 3.52 (m, 2H, -CONHC$\underline{H}_2$), 4.06-4.13 (m, 6H, 3, 4 and 5-OC$\underline{H}_2$), 5.09-5.33 (br×2, 3H, N$\underline{H}$), 6.35 (br, 1H, CON$\underline{H}$) and 6.72-7.19 (m, 2H, benzoyl): HR-FAB MS calcd for $C_{37}H_{63}N_4O_{12}$ $[M+H]^+$ 755.4442, found 755.4446.

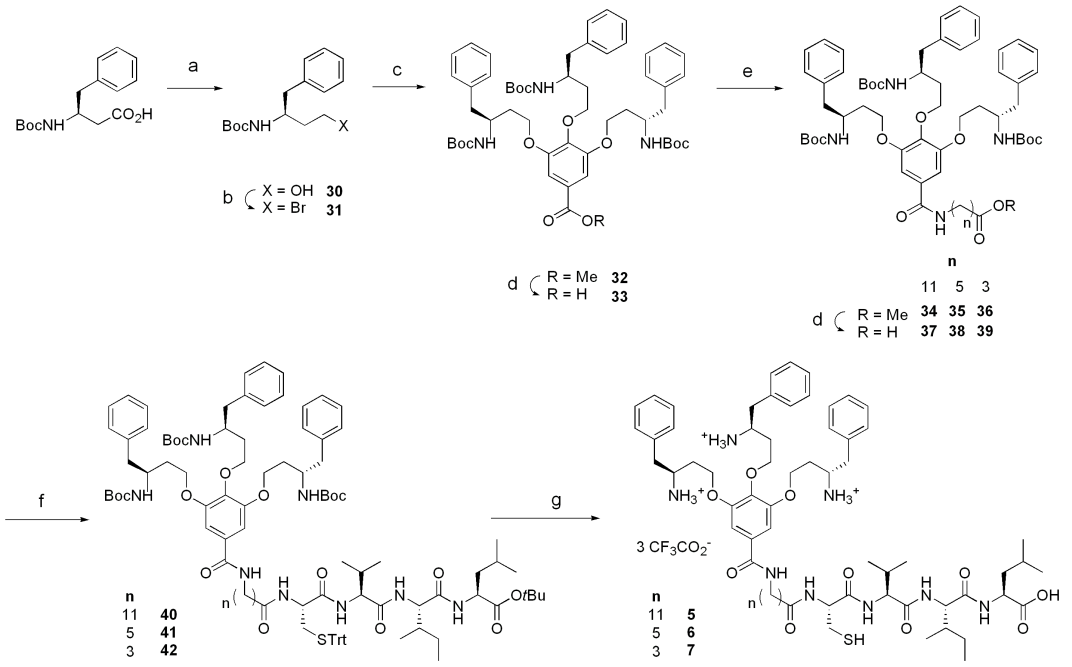
***N*-(11-{3,4,5-Tris[3-*N*-(*tert*-butoxycarbonyl)amino-1-propanoxy]benzoylamino}undecanylcarbonyl)-S(trityl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-Butyl Ester (28).** A solution of **25** (100 mg, 0.12 mmol), H-Cys(Trt)-Val-Ile-LeuOtBu (**15**) (99 mg, 0.13 mmol), HOBt (39 mg, 0.25 mmol), DIEA (42 μL, 0.25 mmol), and PyBop (78 mg, 0.15 mmol) in DMF (10 mL) was stirred at rt for 12h. After removal of DMF by distillation, the residue was dissolved in a large amount of $CHCl_3$ and the organic layer was washed with 10% citric acid, 5% $NaHCO_3$, brine, and dried over anhydrous $MgSO_4$. The crude material was purified by size exclusion column (Sephadex LH-20; $CHCl_3$: CH_3OH = 1 : 1) to give the product as a white solid (154 mg, 83%): Mp 134-138 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.77-0.92 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 1.02-1.10 (m, 1H, ?-CH Leu), 1.24-1.32 (m, 14H, -(C$\underline{H}_2$)$_7$CH$_2$CH$_2$CONH), 1.43-1.45 (m, 36H, 3,4,5-Boc and O*t*Bu), 1.48-1.63 (m, 8H, -CONHCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CH$_2$CONH, ?-CH$_2$ Ile and ß-CH$_2$ Leu), 1.83-2.15 (m, 10H, 3,4,5-BocNHCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CONH and ß-CH Ile and ß-CH Val), 2.59 (dd, 1H, *J* = 5.6 and 13.3 Hz, ß-C$\underline{H}_{2a}$ Cys), 2.70 (dd, 1H, *J* = 7.4 and 13.4 Hz, ß-C$\underline{H}_{2b}$ Cys), 3.32-3.44 (m, 8H, 3,4,5-BocNHC$\underline{H}_2$ and PhCONHC$\underline{H}_2$), 3.97-4.16 (dd, *J* = 6.6 and 12.7 Hz, 2H, *a*-CH Cys), 4.05-4.16 (m, 8H, 3, 4, 5-OC$\underline{H}_2$ and *a*-CH Cys and *a*-CH Ile), 4.28 (dd, *J* = 7.1 and 8.7 Hz, 1H *a*-CH Val), 4.42-4.47 (m, 1H, *a*-CH Leu), 5.07 (br, 2H, BocN$\underline{H}$), 5.33 (br, 1H, BocN$\underline{H}$), 5.84 (br, 1H, CON$\underline{H}$), 6.30-6.36 (m, 2H, CON$\underline{H}$CH$_2$ and CON$\underline{H}$), 6.60 (br, 2H, CON$\underline{H}$), 7.00 (s, 2H, benzoyl) and 7.20-7.44 (m, 15H, Trt): HR-FAB MS calcd for $C_{86}H_{133}N_8O_{16}S_1$ $[M+H]^+$ 1565.956, found 1565.918.

***N*-(5-{3,4,5-Tris[3-*N*-(*tert*-butoxycarbonyl)amino-1-propanoxy]benzoylamino}hexanylcarbonyl)-S(trityl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-Butyl Ester (29).** This compound was prepared by reaction of **27** (58 mg, 0.077 mmol), H-Cys(Trt)-Val-Ile-LeuOtBu (**15**) (66 mg, 0.089 mmol), HOBt (27 mg, 0.18 mmol), DIEA (28 µL, 0.16 mmol), and PyBop (61 mg, 0.11 mmol) in DMF (1 mL) to give the product as a white solid (95 mg, 84%): Mp 106 -111 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.75-0.91 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 0.99-1.07 (m, 1H, ?-CH Leu), 1.39-1.47 (m, m, 36H, 3,4,5-Boc and O*t*Bu), 1.54-1.65 (m, 10H, -CONHCH$_2$(C$\underline{H}_2$)$_3$, ?-CH_2 Ile and ß-CH_2 Leu), 1.76-1.82 (m, 1H, ß-CH Ile) 1.91-2.15 (m, 9H, 3,4,5-BocNHCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CONH and and ß-CH Val), 2.54-2.72 (m, 2H, ß-C$\underline{H}_2$ Cys), 3.32-3.40 (m, 8H, 3,4,5-BocNHC$\underline{H}_2$ and PhCONHC$\underline{H}_2$), 4.05-4.09 (m, 7H, 3, 4, 5-OC$\underline{H}_2$ and *a*-CH Cys), 4.15-4.19 (m, 1H, *a*-CH Ile), 4.24-4.28 (m, 1H *a*-CH Val), 4.41-4.47 (m, 1H, *a*-CH Leu), 5.12 (br, 2H, BocN$\underline{H}$), 5.35 (br, 1H, BocN$\underline{H}$), 5.97 (br, 1H, CON$\underline{H}$), 6.53-6.82 (m, 4H, CON$\underline{H}$CH$_2$ and CON$\underline{H}$), 7.04 (s, 2H, benzoyl) and 7.19-7.43 (m, 15H, Trt): HR-FAB MS calcd for $C_{75}H_{113}N_8O_{14}S_1$ $[M\text{-Boc} +H]^+$ 1381.8097, found 1381.8099.

***N*-(11-{3,4,5-Tris[3-amino-1-propanoxy]benzoylamino}undecanylcarbonyl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine Trifluoroacetic acetate (8).** Deprotection of **28** (31 mg, 0.02 mmol) was carried out by a method similar to that described for **1**. The crude material was washed with Et_2O several times, and the resulting precipitates were collected by centrifugation to afford the product as a white powder (23 mg, 92%): HPLC purity 91%: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 0.77-0.87 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 1.01-1.10 (m, 1H, ?-CH Leu), 1.23-1.27 (m, 14H, -(C$\underline{H}_2$)$_7$CH$_2$CH$_2$CONH), 1.47-1.70 (m, 9H, -CONHCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CH$_2$CONH, ß-CH and ?-CH_2 Ile and ß-CH_2 Leu), 1.87-2.14 (m, 9H, 3,4,5-NHCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CONH and ß-CH Val), 2.59 (dd, 1H, *J* = 8.1 and 13.3 Hz, ß-C$\underline{H}_{2a}$ Cys), 2.72 (dd, 1H, *J* = 5.4 and 13.0 Hz, ß-C$\underline{H}_{2b}$ Cys), 2.96-3.04 (m, 6H, 3,4,5-NHC$\underline{H}_2$), 3.97-4.12 (m, 9H, 3, 4, 5-OC$\underline{H}_2$ and *a*-CHx3), 4.39 (dd, *J* = 7.1 and 13.6 Hz, 1H, *a*-CH), 7.21 (s, 2H, benzoyl), 7.80-7.85 (m, 2H CON$\underline{H}$) 8.05-8.09 (m, 2H CON$\underline{H}$) and 8.41 (t, *J* = 5.3 Hz, 1H, PhCON$\underline{H}$): HR-FAB MS calcd for $C_{48}H_{87}N_8O_{10}S_1$ $[M+H]^+$ 967.6266, found 967.6244.

***N*-(5-{3,4,5-Tris[3-amino-1-propanoxy]benzoylamino}hexanylcarbonyl)-L-cysteinyl-L-valyl-L-isoleucyl-L-leucine Trifluoroacetic acetate (9).** Deprotection of **29** (19 mg, 0.0128 mmol) was carried out by a method similar to that described for **1**. The crude material was washed with Et_2O several times, and the resulting precipitates were collected by centrifugation to afford the product as a white powder (16 mg, 87%): HPLC purity 97%: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 0.76-0.86 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 1.02-1.10 (m, 1H, ?-CH Leu), 1.23-1.29 (m, 2H, -C$\underline{H}_2$(CH$_2$)$_2$CONH), 1.44-1.68 (m, 9H, -CONHCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CH$_2$CONH, ß-CH and ?-CH_2 Ile and ß-CH_2 Leu), 1.86-2.06 (m, 7H, 3,4,5-NHCH$_2$C$\underline{H}_2$ and ß-CH Val), 2.13 (dd, 2H *J* = 6.9 and 11.1 Hz, -C$\underline{H}_2$CONH), 2.58 (dd, 1H, *J* = 7.8 and 13.4 Hz, ß-C$\underline{H}_{2a}$ Cys), 2.72 (dd, 1H, *J* = 5.4 and 13.4 Hz, ß-C$\underline{H}_{2b}$ Cys), 2.95-3.03 (m, 6H, 3,4,5-

Scheme S3



(a) EtOCOCl, $NaBH_4$, 80%; (b) CBr_3, PPh_3, 77%; (c) **21**, K_2CO_3, DMF, 91%; (d) 1M KOH, CH_2Cl_2, CH_3OH; (e) H_2N-$(CH_2)_n$-CO_2CH_3 (n=11, 5, 3), PyBop, HOBt·H_2O, DIEA, DMF, 85-98%; (f) **15**, PyBop, HOBt·H_2O, DIEA, DMF, 70-90%; (g) 50 v/v% TFA, 5 v/v% TES, CH_2Cl_2, quant.

NHC<u>H</u>$_2$), 3.97 (t, *J* = 5.7 Hz, 2H, 4-OCH_2) 4.10 (t, *J* = 6.0 Hz, 4H, 3, 5-OC<u>H</u>$_2$), 4.15-4.21 (m, 3H a-CH), 4.39 (dd, *J* = 7.8 and 13.4 Hz, 1H, *a*-CH), 7.21 (s, 2H, benzoyl), 7.80-7.84 (m, 2H CONH) 8.04 (d, *J* = 8.1 Hz, 1H, CONH), 8.10 (d, *J* = 7.5 Hz, 1H CONH) and 8.41 (t, *J* = 5.3 Hz, 1H, PhCONH): HR-FAB MS calcd for $C_{42}H_{75}N_8O_{10}S_1$ $[M+H]^+$ 883.5327, found 883.5334.

Synthesis of 3,4,5-tris(3-amino-3-(*S*)-benzyl-1-propoxyl)benzoic acid derivatives and the corresponding bivalent inhibitors (Scheme S3).

3-(*N*-*tert*-butoxy)amino-1-bromo-4-phenyl-butanol (30). To a solution of (L)-homophenylalanine (2.01 g, 7.18 mmol) and Et_3N (1.2 mL, 8.61 mmol) in THF (40 mL) was added ethyl chloroformate (0.82 mL, 8.61 mmol) dropwise at -10 °C, and the mixture was stirred at -10 °C for 2.5 h. The resulting Et_3N·HCl salt was removed by filtration, and the solid was washed with THF (10 mL). The organic layer was combined, and reacted with $NaBH_4$ (1.38 g, 36.34 mmol) at rt. After stirring for 3h at rt, THF was removed by evaporation, and the residue was dissolved in AcOEt. The organic layer was washed with 10% citric acid, and brine, and dried (Na_2SO_4). The crude material was purified by SiO_2 column

chromatography (AcOEt : hexane = 1:2) to give the desired product as a white solid (1.53 g, 80%): Mp 55-57 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.40 (s, 9H, Boc), 1.81-1.89 (m, 1H, -C$\underline{H}_{2a}$CH$_2$OH), 2.80 (d, *J* = 6.7 Hz, 2H, -C$\underline{H}_2$Ph), 3.19 (m, 1H, -C$\underline{H}_{2b}$CH$_2$OH), 3.63 (m, 2H, -C$\underline{H}_2$OH), 4.09-4.14 (m, 1H -C$\underline{H}$), 4.45 (d, *J* = 8.0 Hz, 1H, N$\underline{H}$) and 7.17-7.32 (m, 5H, Ph): LR ESI-MS calcd for $C_{15}H_{23}NO_3Na$ $[M+Na]^+$ 288, found 288.

3-(*N*-*tert*-butoxy)amino-1-bromo-4-phenyl-butane (31). To a solution of **30** (198 mg, 0.75 mmol), CBr_4 (353 mg, 1.06 mmol) in CH_2Cl_2 (2 mL) was added dropwise PPh_3 (303 mg, 1.15 mmol) in CH_2Cl_2 solution at 0 °C, and the mixture was stirred at for 1h at 0 °C. After evaporation, the crude product was purified by SiO_2 chromatography to give a white solid (172 mg, 77%). This compound was immediately used for the next reaction: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 1.41 (s, 9H, Boc), 1.95-2.05 (m, 2H, -C$\underline{H}_2$CH$_2$Br), 2.75-2.88 (m, 2H, -CH$_2$Ph), 3.36-3.47 (m, 2H, -CH$_2$Br), 3.90-3.99 (m, 1H C$\underline{H}$), 4.34-4.36 (d, *J* = 7.0 Hz, 1H, NH) and 7.16-7.32 (m, 5H, Ph): LR ESI-MS calcd for $C_{15}H_{22}BrNO_2Na$ $[M+Na]^+$ 350, found 350.

3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzoic acid Methyl Ester (32). See the article, experimental section.

3,4,5-Tris{3-amino-4-phenyl-1-butoxy}benzoic acid Methyl Ester (3). See the article, experimental section.

3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzoic acid (33). See the article, experimental section.

12-[3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzolyamino]dodecanoic acid Methyl Ester (34). This compound was prepared by a coupling reaction of **33** (95 mg, 0.10 mmol) and 12-aminododecanoic acid methyl ester (34 mg, 0.13 mmol) by a method similar to that described for **24**. The crude product was purified by SiO_2 chromatography ($CHCl_3$: AcOEt : MeOH = 20: 3: 1) to afford the product as white solid (100 mg, 85%): Mp 187-190 °C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 1.13-1.29 (m, 41H, 3,4,5,-Boc and -(C$\underline{H}_2$)$_7$CH$_2$CH$_2$CO$_2$CH$_3$), 1.44-1.50 (m, 4H, -C$\underline{H}_2$(CH$_2$)$_7$CH$_2$C$\underline{H}_2$CO$_2$CH$_3$) 1.76-1.86 (m, 6H, 3,4,5-OCH$_2$C$\underline{H}_2$ and -C$\underline{H}_2$CH$_2$CO$_2$CH$_3$), 2.26 (t, *J* = 7.2 Hz, 2H, -C$\underline{H}_2$CO$_2$CH$_3$), 2.67-2.79 (m, 6H, -C$\underline{H}_2$Ph), 3.18-3.23 (m, 2H, -CONHC$\underline{H}_2$), 3.56 (3H, s, - CO$_2$C$\underline{H}_3$), 3.84 – 3.98 (9H, m, 3, 4 ,5 - OC$\underline{H}_2$CH$_2$C$\underline{H}$), 6.55 (d, *J* = 8.3 Hz, 1H, 4-N$\underline{H}$Boc), 6.79 (d, *J* = 8.6 Hz, 2H, 3,5-N$\underline{H}$Boc), 7.08 (s, 2H, 2,6-H of benzoyl), 7.16 – 7.24 (15H, m, Aryl H), and 8.32 (m, 1H, CONH): LR ESI-MS calcd for $C_{65}H_{94}N_4O_{12}Na$ $[M+Na]^+$ 1145, found 1145: Anal. calcd for $C_{57}H_{78}N_4O_{12}$: C, 69.49; H, 8.43; N, 4.99. found: C, 69.40; H, 8.35; N, 4.88.

6-[3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzolyamino]hexanoic acid Methyl Ester (35). See the article, experimental section.

4-[3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzolyamino]butanoic acid Methyl Ester (36). This compound was prepared by a reaction of **33** (96 mg, 0.11 mmol) and 4-aminobutanoic acid methyl ester hydrochloride (21 mg, 0.14 mmol) in a similar manner described for **24**. The crude product was purified by SiO_2 chromatography ($CHCl_3$: AcOEt : MeOH = 10: 3: 1) to afford the product as a white solid (95 mg, 90%): Mp 189-195 °C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 1.14-1.29 (m, 27H, 3, 4, 5, - Boc), 1.72-1.91 (m, 8H, 3,4,5-$OCH_2C\underline{H}_2$ and -$C\underline{H}_2CH_2CO_2CH_3$), 2.35 (t, *J* = 7.4 Hz, 2H, -$C\underline{H}_2CO_2CH_3$), 2.64-2.79 (m, 6H, -CH_2Ph), 3.45-3.53 (m, 2H, -CONHC$\underline{H}_2$), 3.57 (s, 3H,-CO_2CH_3), 3.84-4.00 (m, 9H, 3,4,5-$OC\underline{H}_2CH_2C\underline{H}$), 6.55 (d, *J* = 8.8 Hz, 1H, 4-N$\underline{H}$Boc), 6.79 (d, *J* = 8.6 Hz, 2H, 3, 5-N$\underline{H}$Boc), 7.09 (s, 2H, 2,6-H of benzoyl), 7.14-7.26 (m, 15H, 3, 4, 5 -Ph), and 8.38 (t, *J* = 5.5 Hz, 1H, CON$\underline{H}$): LR ESI-MS calcd for $C_{57}H_{78}N_4O_{12}Na$ $[M+Na]^+$ 1034, found 1034: Anal. calcd for $C_{57}H_{78}N_4O_{12}$: C, 67.70; H, 7.77; N, 5.54. found: C, 67.77; H, 7.84; N, 5.39.

12-[3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzolyamino]dodecanoic acid (37). This compound was prepared by hydrolysis of **34** (84 mg, 0.075 mmol) by a method similar to that described for **25** to give a yellow amorphous solid (93 mg, quant.). This compound was used for the next reaction without further purification: Mp 174-176 °C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 1.14-1.30 (m, 41H, 3,4,5-Boc and -$(C\underline{H}_2)_7CH_2CH_2CO_2CH_3$), 1.45-1.48 (m, 4H, -$C\underline{H}_2CH_2CO_2CH_3$ and -$CONHCH_2C\underline{H}_2$), 1.77-1.89 (m, 6H, 3,4,5-$OCH_2C\underline{H}_2$), 2.17 (t, 2H, *J* = 7.3 Hz, -$C\underline{H}_2CO_2H$), 2.67-2.73 (m, 6H, 3,4,5-Ph$C\underline{H}_2$), 3.18-3.24 (m, 2H, - CONHC$\underline{H}_2$), 3.85-4.00 (m, 9H, 3,4,5 -$OC\underline{H}_2CH_2C\underline{H}$), 6.56 (d, *J* = 8.6 Hz, 1H, 4-N$\underline{H}$Boc), 6.77 (d, *J* = 8.9 Hz, 2H, 3 and 5 -N$\underline{H}$Boc), 7.09-7.26 (m, 17H, benzoyl and 3,4,5-Ph), 8.32 (m, 1H, benzoyl-CON$\underline{H}$) and 11.9 (br, 1H, $CO_2\underline{H}$): HR FAB-MS calcd for $C_{64}H_{93}N_4O_{12}$ $[M+H]^+$ 1109.679, found 1109.6803.

6-[3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzolyamino]hexanoic acid (38). See the article, experimental section.

4-[3,4,5-Tris{3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy}benzolyamino]butanoic acid (39). This compound was prepared by hydrolysis of **32** (30 mg, 0.029 mmol) by a method similar to that described for **25**, pale yellow amorphous solid (31 mg, quant.). This compound was used for the next reaction without further purification: Mp 163-168 °C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d*1.14-1.29 (m, 27H, 3,4,5-Boc), 1.71-1.86 (m, 8H, 3,4,5-$OCH_2C\underline{H}_2$, -$CONHCH_2C\underline{H}_2$), 2.25 (t, *J* = 7.2 Hz, 2H, -$C\underline{H}_2CO_2H$), 2.68-2.74 (m, 6H, 3,4,5- $C\underline{H}_2$Ph), 3.23 (m, 2H, -CONHC$\underline{H}_2$), 3.84-3.99 (m, 9H, 3,4,5,-$OC\underline{H}_2CH_2C\underline{H}$), 6.56 (d, *J* = 8.8 Hz, 1H, 4-BocN$\underline{H}$), 6.81 (d, *J* = 8.5 Hz, 2H, 3,4,-BocN$\underline{H}$), 7.10-7.26 (m, 17H, 3,4,5-Ph and benzoyl), 8.41 (m, 1H, benzoyl-CON$\underline{H}$) and 12.0 (br, 1H, $CO_2\underline{H}$): HR FAB-MS calcd for $C_{56}H_{77}N_4O_{12}$ $[M+H]^+$ 997.5538, found 997.5557.

***N*-[12-{3,4,5-Tris(3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy)benzolyamino}dodecanylcarbonyl]-S(trityl)-L-cisteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-butyl ester (40).** This compound was

prepared by a coupling reaction of **37** (83 mg, 0.075 mmol) and **15** (68 mg, 0.091 mmol) in a similar manner to that described for **28**, white solid (110 mg, 80%): Mp 183-185 °C: ^{1}H-NMR (400 MHz, $CDCl_3$) *d* 0.69-0.87 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 0.97-1.06 (m, 1H, ?-CH Leu), 1.12-1.86 (m, 66H, ?-CH_2 Ile and ß-CH_2 Leu, ß-CH Ile, ß-CH Val, 3,4,5-$OCH_2C\underline{H}_2$, -$CONHCH_2(C\underline{H}_2)_9$, O*t*Bu and 3,4,5-Boc), 1.98-2.12 (m, 2H, ß-CH_2, Cys), 2.27-2.38 (m, 2H, -$C\underline{H}_2CONH$), 2.66-2.78 (m, 6H, 3,4,5-$C\underline{H}_2Ph$), 3.17 (m, 2H, -$CONHC\underline{H}_2$), 3.82-3.97 (m, 9H, 3,4,5-$OC\underline{H}_2CH_2C\underline{H}$), 4.11-4.26 (m, 4H, *a*-CH Cys, Val, Ieu and Leu), 6.55 (d, *J* = 8.2 Hz, 4-N<u>H</u>Boc), 6.78 (d, *J* = 8.7 Hz, 2H, 3 and 5-N<u>H</u>Boc), 7.08-7.32 (m, 32H, benzoyl, 3,4,5-Ph and Trt), 7.49 (d, *J* = 8.7 Hz, 1H, N<u>H</u>), 7.87 (d, *J* = 8.7 Hz, 1H, N<u>H</u>), 8.08 (d, *J* = 8.3 Hz, 1H, N<u>H</u>) 8.15 (d, *J* = 7.7 Hz, 1H, N<u>H</u>) and 8.31 (m, 1H, CONH): HR FAB-MS calcd for $C_{107}H_{151}N_8O_{16}S_1$ $[M+H]^+$ 1836.0969, found 1836.0977: Anal. calcd for $C_{107}H_{150}N_8O_{16}S\cdot1.5$ H_2O: C, 68.97; H, 8.28; N, 6.01. found: C, 68.96; H, 8.08; N, 5.87.

***N*-[6-{3,4,5-Tris(3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy)benzolyamino}hexylcarbonyl]-S(trityl)-L-cisteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-butyl ester (41).** See the article, experimental section.

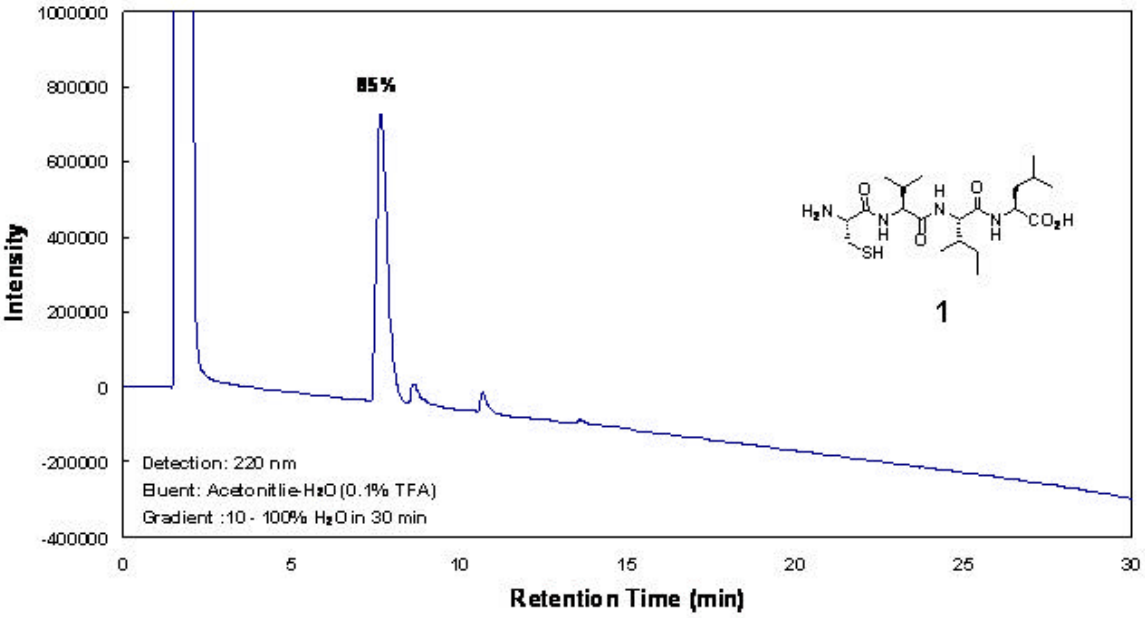
***N*-[4-{3,4,5-Tris(3-*N*-(*tert*-butoxy)amino-4-phenyl-1-butoxy)benzolyamino}butylcarbonyl]-S(trityl)-L-cisteinyl-L-valyl-L-isoleucyl-L-leucine *tert*-butyl ester (42).** This compound was prepared by a coupling reaction of **39** (25 mg, 0.025 mmol) and **15** (28 mg, 0.039 mmol) in a similar manner to that described for **28**. The crude material was purified by size exclusion chromatography (Sephadex LH-20, $CHCl_3$: MeOH = 1:1) to give the product as a white solid (39 mg, 90%): Mp 186-188 °C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 0.68-0.86 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 0.96-1.03 (m, 1H, ?-CH Leu), 1.13-1.86 (m, 50H, ?-CH_2 Ile and ß-CH_2 Leu, ß-CH Ile, ß-CH Val, 3,4,5-$OCH_2C\underline{H}_2$, -$CONHCH_2C\underline{H}_2$, O*t*Bu and 3,4,5-Boc), 2,12-2.17 (m, 2H, ß-$C\underline{H}_2$, Cys), 2.32-2.34 (m, 2H, -$C\underline{H}_2CONH$), 2.66-2.72 (m, 6H, 3,4,5-$C\underline{H}_2Ph$), 3.21-3.26 (m, 2H, -$CONHC\underline{H}_2$), 3.83-3.98 (m, 9H, 3,4,5-$OC\underline{H}_2CH_2C\underline{H}$), 4.11-4.27 (m, 4H, *a*-CH Cys, Val, Ieu and Leu), 6.55 (d, *J* = 8.2 Hz, 4-N<u>H</u>Boc), 6.78 (d, *J* = 8.5 Hz, 2H, 3 and 5-N<u>H</u>Boc), 7.10-7.31 (m, 32H, benzoyl, 3,4,5-Ph and Trt), 7.55 (d, *J* = 8.8 Hz, 1H, N<u>H</u>), 7.87 (d, *J* = 9.1 Hz, 1H, N<u>H</u>), 8.15 (m, 2H, N<u>H</u>) and 8.38 (m, 1H, benzoyl-CON<u>H</u>): Anal. calcd for $C_{99}H_{134}N_8O_{16}S\cdot2H_2O$: C, 67.55; H, 7.90; N, 6.37. found: C, 67.81 ; H, 7.78 ; N, 6.20: HR FAB-MS calcd for $C_{99}H_{135}N_8O_{16}S_1$ $[M+H]^+$ 1723.9717 found 1723.9657.

***N*-[12-{3,4,5-Tris(3-amino-4-phenyl-1-butoxy)benzolyamino}dodecanylcarbonyl]-L-cisteinyl-L-valyl-L-isoleucyl-L-leucine (5).** Deprotection of **40** (25 mg, 0.013 mmol) was carried out by a similar method described for **8** to give the desired product as a white powder (22 mg, quant.): Mp 149-154 °C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 0.78-0.87 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 1.01-1.10 (m, 1H, ?-CH Leu), 1.23-1.96 (m, 30H, 3,4,5-$OCH_2C\underline{H}_2$, -$CONHCH_2(C\underline{H}_2)_9$, ß-CH Val, ß-CH and ?-$CH_2$ Ile and ß-CH_2 Leu), 2.11-2.15 (m, 2H, -$C\underline{H}_2CONH$), 2.62-2.78 (m, 2H, ß-$C\underline{H}_2$ Cys), 2.83-2.97

(m, 6H, 3,4,5-PhC$\underline{H}_2$), 3.90-3.93 (m, 2H, *a*-CHx2), 4.09-4.21 (m, 7H, 3,4,5-OC$\underline{H}_2$ and *a*-CH), 4.39-4.45 (m, 1H, *a*-CH), 7.16-7.35 (m, 17H, 3,4,5-Ph and benzoyl), 7.79-7.85 (m, 2H, -N$\underline{H}$CH), 8.04-8.15 (m, 2H -N$\underline{H}$C$\underline{H}$) and 8.36-8.38 (m, 1H, PhCON$\underline{H}$): HPLC purity 87%: HR FAB-MS calcd for $C_{69}H_{104}N_8O_{10}S_1$ $[M]^+$ 1236.7596 found 1236.7600.

***N*-[6-{3,4,5-Tris(3-amino-4-phenyl-1-butoxy)benzolyamino}hexylcarbonyl]-L-cisteinyl-L-valyl-L-isoleucyl-L-leucine (6).** See the article, experimental section.

***N*-[6-{3,4,5-Tris(3-amino-4-phenyl-1-butoxy)benzolyamino}butylcarbonyl]-L-cisteinyl-L-valyl-L-isoleucyl-L-leucine (7).** Deprotection of **42** (25 mg, 0.015 mmol) was carried out by a similar method described for **8** to give the desired product as a white powder (21 mg, quant.): HPLC purity 88%: Mp 151-157°C: ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 0.77-0.87 (m, 18H, 2?-CH_3 Val, ?-, *d*-CH_3 Ile and 2d-CH_3 Leu), 1.02-1.11 (m, 1H, ?-CH Leu), 1.34-1.98 (m, 14H, 3,4,5-OCH$_2$C$\underline{H}_2$, -C$\underline{H}_2$CH$_2$CONH, ß-CH Val, ß-CH and ?-CH_2 Ile and ß-CH_2 Leu), 2.19-2.23 (m, 2H, -C$\underline{H}_2$CONH), 2.63-2.79 (m, 2H, ß-C$\underline{H}_2$ Cys), 2.83-2.96 (m, 6H, 3,4,5-PhC$\underline{H}_2$), 3.90-3.93 (m, 2H, *a*-CHx2), 4.10-4.19 (m, 7H, 3,4,5-OC$\underline{H}_2$ and *a*-CH), 4.42-4.47 (m, 1H, *a*-CH), 7.18-7.35 (m, 17H, 3,4,5-Ph and benzoyl), 7.82-7.89 (m, 2H, -N$\underline{H}$CH), 8.10-8.12 (m, 2H -N$\underline{H}$C$\underline{H}$) and 8.42-8.44 (m, 1H, PhCON$\underline{H}$): HR FAB-MS calcd for $C_{61}H_{89}N_8O_{10}S_1$ $[M+H]^+$ 1125.6422 found 1125.6399.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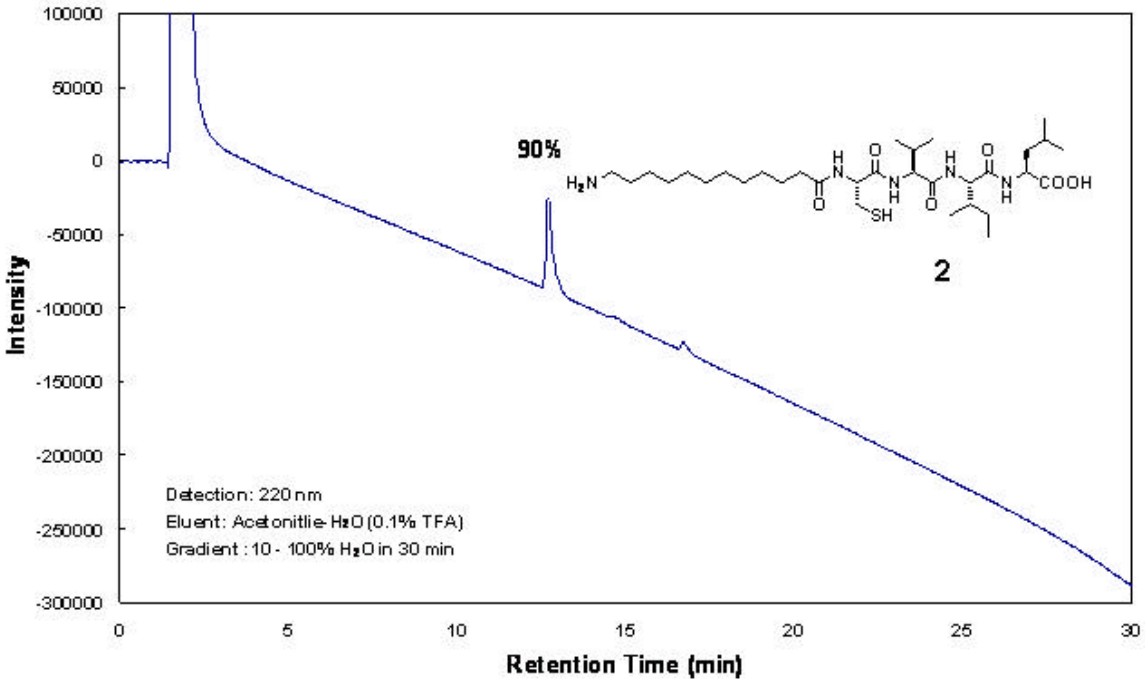
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Intensity
Retention Time (min)
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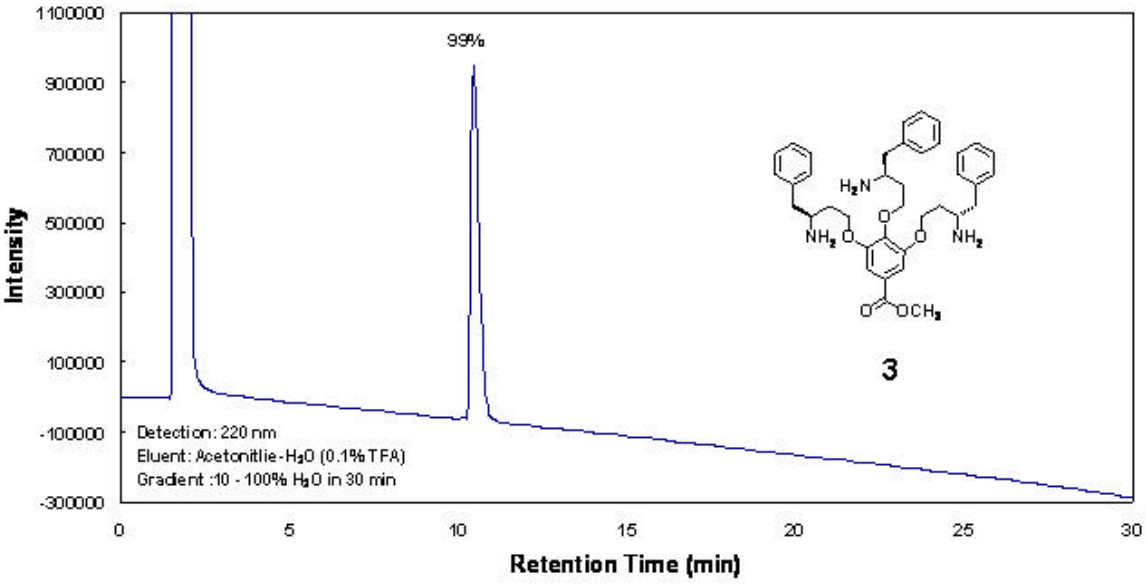
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99%
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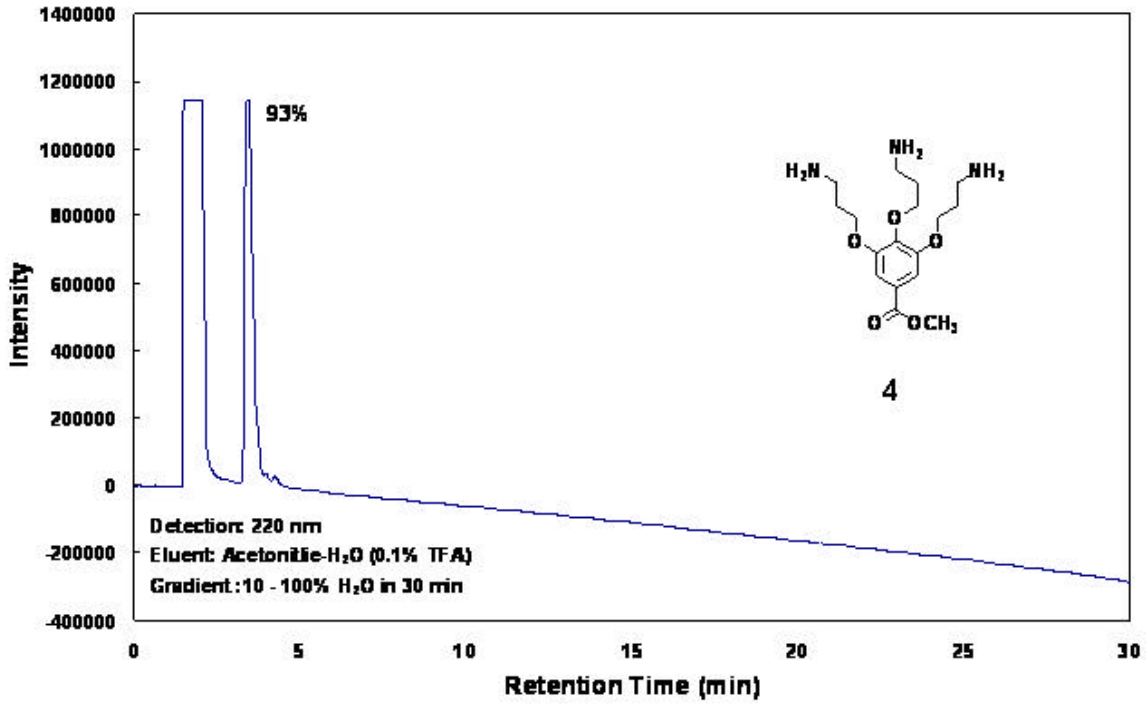
5
10
15
20
25
30
Retention Time (min)
3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200000
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-400000
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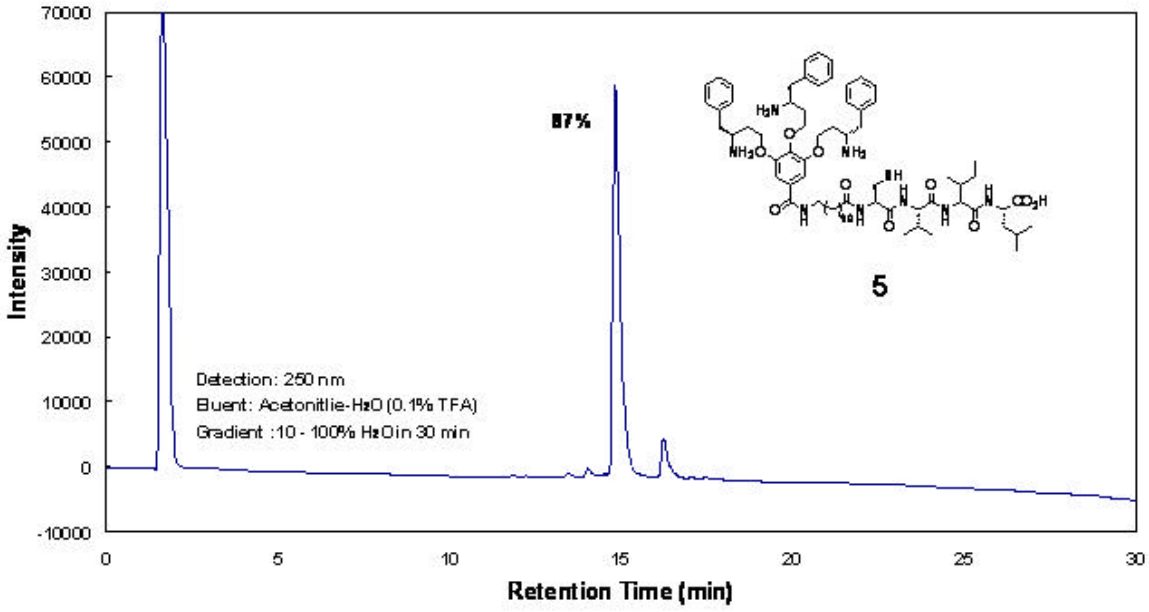
5
10
15
20
25
30
Retention Time (min)
4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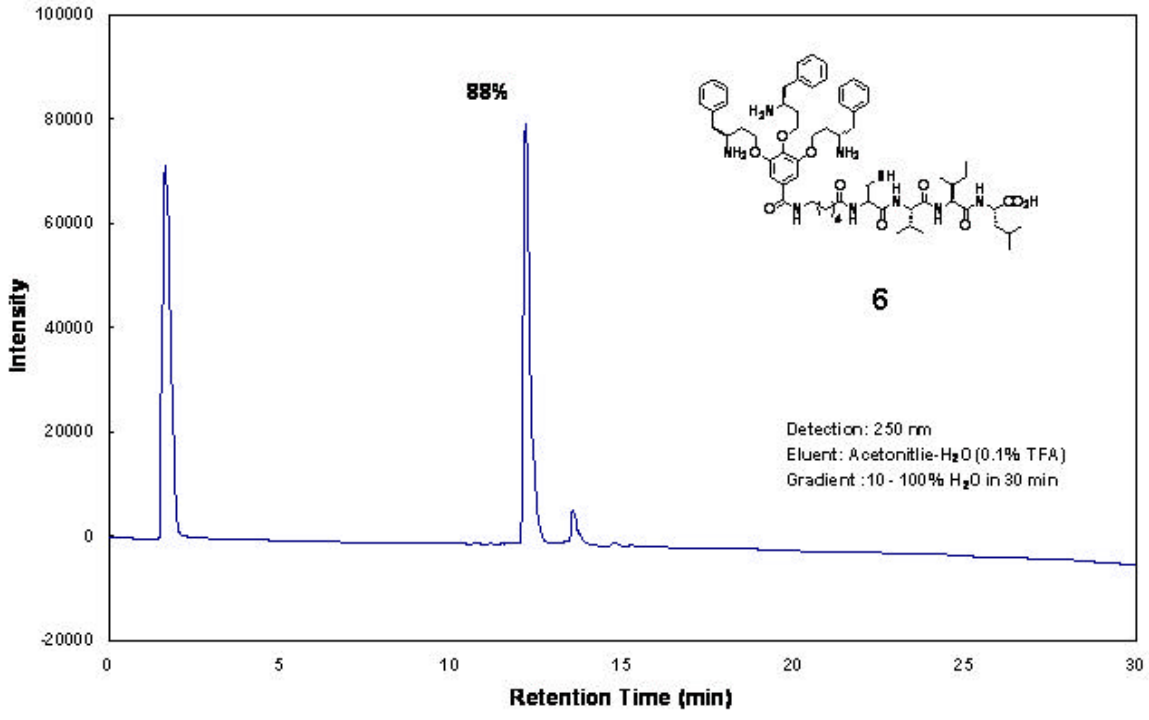
Intensity
Retention Time (min)


88%
6
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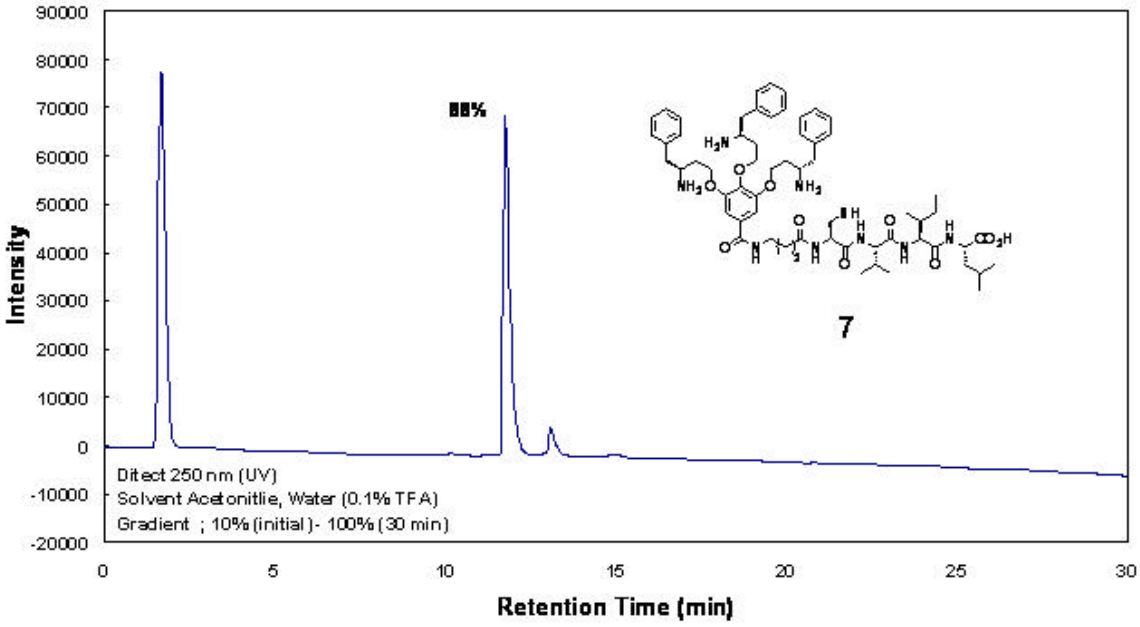
Intensity
Retention Time (min)


88%
7
Ditect 250 nm (UV)
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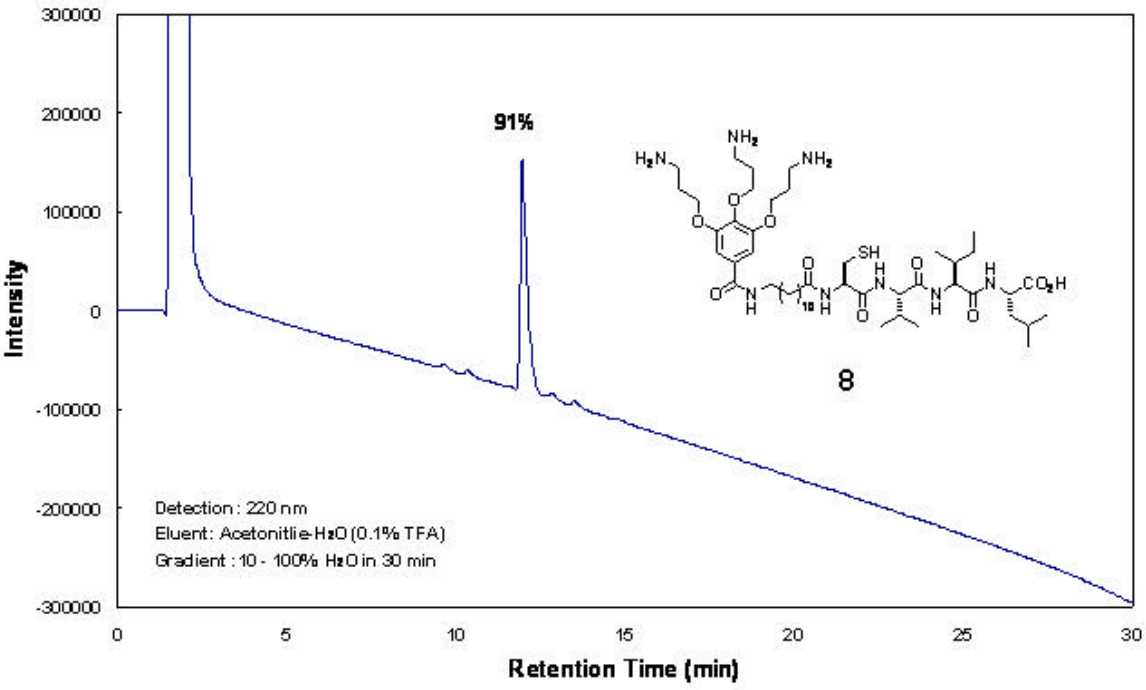
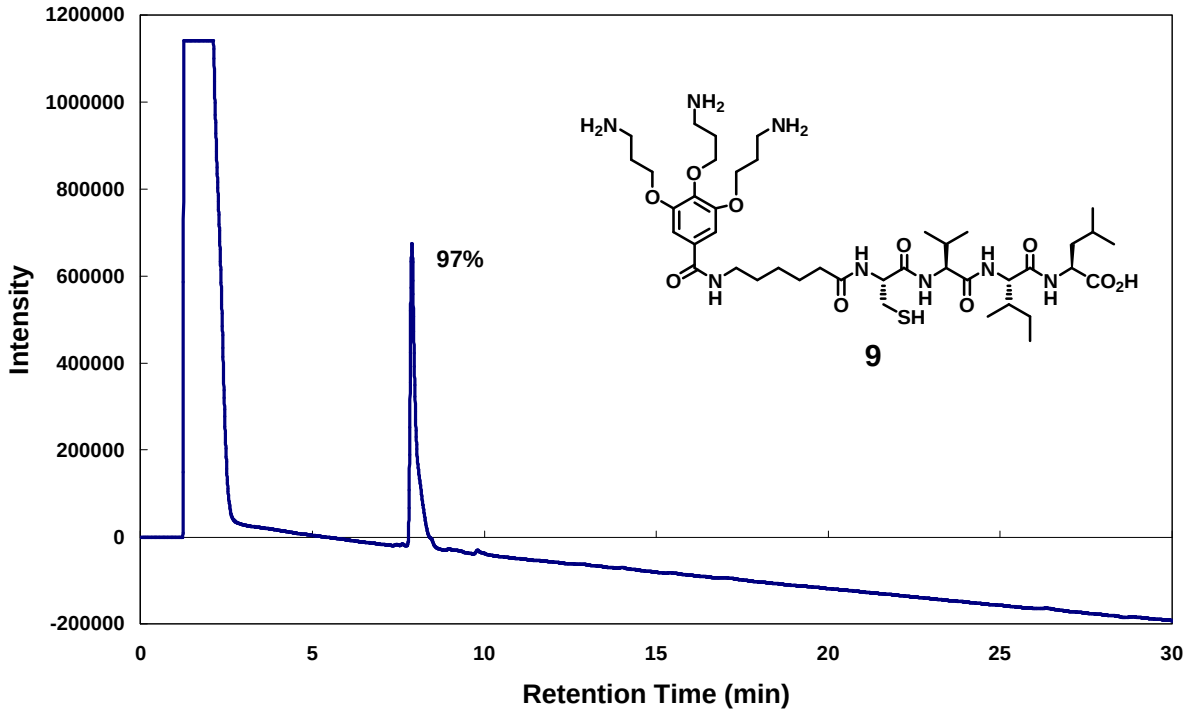
Gradient ; 10% (initial) - 100% (30 min)
Intensity
Retention Time (min)


91%
8
Detection : 220 nm
Eluent : Acetonitlie-H2O (0.1% TFA)
Gradient : 10 - 100% H2O in 30 min
Intensity
Retention Time (min)



Preparation of *N*-dansyl-glycine. To a solution of glycine (184 mg, 2.45 mmol) and 1N NaOH (3 mL) in H2O was added dansyl chloride (600 mg, 2.22 mmol) in THF (20 mL) at rt, and the mixture was stirred for 1h. The pH was adjusted to >9 adding 1N NaOH during the reaction. After concentration and acidification with 5N HCl, the resulting yellow solid was colletcted by filtration. Recrystallizaion from aqueous EtOH gave the pale yellow solid (600 mg, 88%): ^{1}H-NMR (400 MHz, DMSO-d_6) *d* 2.84 (s, 6H, CH_3 x 2), 3.59 (s, 2H, CH_2), 7.23 (d, *J* = 7.6 Hz, 1H, Aryl H), 7.55 (d, *J* = 6.4 Hz, 1H, Aryl H), 7.59 (d, *J* = 7.2 Hz, 1H, Aryl H), 8.09 (d, *J* = 7.2 Hz, 1H, Aryl H), 8.27 (d, *J* = 7.6 Hz, 1H, Aryl H), 8.36 (br, 1H, NH), 8.43 (d, J = 7.6 Hz, 1H, Aryl H).

SPPS for DansylGCVIL. N-Dansyl-Gly-Cys-Val-Ile-Leu-OH (DansylGCVIL) used in this study was prepared by solid phase synthetic method. The first amino acid residue, Fmoc-Leu-OH, was loaded on the resin surface as follows. To a 20 mL tube with a frit (Bond Elut Resrvoir, Varian, or SPE tubes, Spelco) 1 g of Sasrin Resin (Bachem, 200-400 mesh, 1.02 mmol / g) was placed and swollen in CH_2Cl_2 for at least 30 min. The resin was washed with CH_2Cl_2 several times. To the tube was added pyridine (5 mL) and CH_2Cl_2 (2 mL), and the mixture of Fmoc-Leu-OH (706 mg, 2.0 mmol), HOBt (298 mg, 2.2 mmol) and DICI (306 μl, 2.0 mmol) in CH_2Cl_2 (8 mL) and DMF (2 mL) that was stand at 4 °C for 15 min for pre-activation. The tube was shielded carefully with a plastic top and a rubber cap with Para film, and shaken at rt overnight. After the coupling reaction, the solution was drained off, and the resin was then washed with DMF (~10 mL x 3), CH_2Cl_2 (~10 mL x 3), and MeOH (~10 mL x 3). The resin

was dried under reduced pressure at rt, and the loading percentage was calculated by measuring the weight. The resin was swollen in CH_2Cl_2 again, and acetic anhydride (1.9 mL) and pyridine (1.7 mL) in CH_2Cl_2 (10 mL) was added. The mixture was stand at rt for 30 min, and the solution was drained off. The resin was washed with CH_2Cl_2 (10 mL x 3), MeOH (10 mL x 3), ^{i}PrOH (10 mL x 3), and dried under reduced pressure at rt, and the resulting Fmoc-Leu-Sasrin resin was stored in -20 °C until use.

In a 20 mL tube with a frit Fmoc-Leu-Sasrin resin (200 mg, 0.15 mmol) was placed and swollen in CH_2Cl_2 (10 mL) at least for 30 min. The solvent was drained off, and 20 % piperidine in DMF (2 mL) was added into the tube. The mixture was gently stirred for 15 min at rt. The solution was drained off, and the resin was washed with DMF and CH_2Cl_2. Residual DMF shouldn't be remained before the Kaiser test. Several beads were taken into a small test tube, and checked by Kaiser test. Three solutions were prepared for the test. Solution I contains 500 mg of ninhydrin in 10 mL of EtOH, Solution II contains 40 g of phenol in 10 mL of EtOH, and Solution III was prepared with 0.5 mL of 1 mM aq. KCN in 25 mL of pyridine. To the resin was added DMF (1 mL) and DIEA (105 μl, 0.6 mmol), and mixture of Fmoc-Ile-OH (106 mg, 0.3 mmol), HOBt (41 mg, 0.3 mmol), and PyBop (156 mg, 0.3 mmol) in DMF (1 mL). The mixture was gently stirred for 1h at rt After the coupling reaction, the mixture was drained off, and the resin was washed with DMF and CH_2Cl_2, and checked by Kaiser test. If the coupling reaction was incomplete, the procedure was repeated until the Kaiser test turned negative. Once the coupling reaction was complete, the removal of Fmoc group of Fmoc-Ile-Leu-Sasrin, followed by the coupling reaction was carried out with Fmoc-Cys(Trt)-OH (176 mg, 0.30 mmol), and Dansyl-Gly-OH (93 mg, 0.30 mmol) by the similar procedure that described above except that the reaction time for the coupling with Dansyl-Gly-OH for overnight instead of 1 h.

After all the reaction was complete, the product was cleaved from the resin. First, the resin was washed well with DMF, CH_2Cl_2, MeOH, ^{i}PrOH, and Dichloromethane again. To the resin was added 10 mL of mixture of 1 % TFA and 1% triethylsilane (TES) in CH_2Cl_2, and the mixture was stand at rt for 15 min, and the solution was collected. This procedure was repeated five times until complete cleavage was confirmed by tlc. The combined solution was concentrated to approximately 1 mL, and dry Et_2O was added into the residue to precipitate the product. The precipitates were collected by filtration with a tube with frit, and dissolved in mixture of 1 mL of CH_2Cl_2, 1 mL of TFA, and two drops of TES. The solution was stand at rt for 30 min, and concentrated. The residue was treated with dry Et_2O (20-30 mL), and the resulting precipitates were collected by centrifugation to afford the desired product as a yellow powder, 28 mg (the purity based on HPLC ~100%): LR FAB-MS calcd for $C_{34}H_{52}N_6O_8S_2Na$ $[M+Na]^+$ 759, found 759.

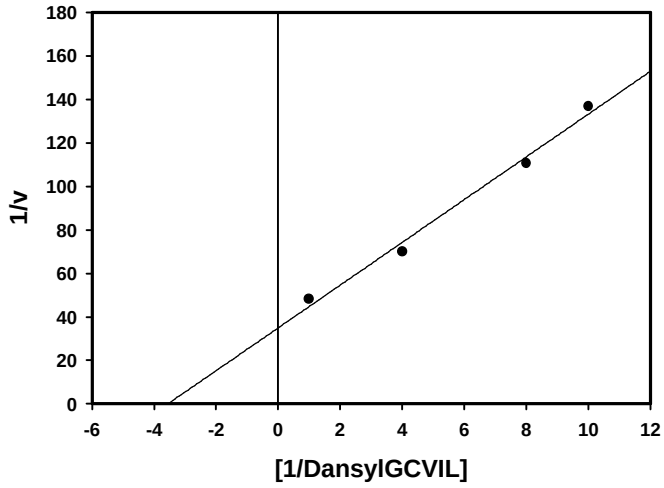


Figure S1. Plot of 1/[DansGCVIL] (mM) versus 1/v (dFL / sec)$^{-1}$. 50 mM Tris·HCl, 5.0 mM DTT, 1.0 mM $MgCl_2$, 10 µM $ZnCl_2$, 0.020% *n*-Dodecyl-ß-D-maltoside: [DansyGCVIL] = 0.1, 0.125, 0.25, 1 µM, [GGPP] = 5 µM [GGTase I] = 31 nM. Ex = 340 nm, Em = 520 nm, T = 293 K (30ºC).

Determination of K_m Value for DansGCVIL. The Km value (0.28 ± 0.04 µM) of DansGCVIL for GGTase I was determined by the fluorescence enzyme assay, changing the concentration of DansGCVIL from 0.1 to 1.0 mM. The kinetic experiment was run in the same condition as described in the experimental in the article, except that the reaction was monitored for 45 sec. This experiment was repeated for three times to obtain the mean value. The data was analysed by SigmaPlot 10.

Lineweaver-Burk Analysis for 8.

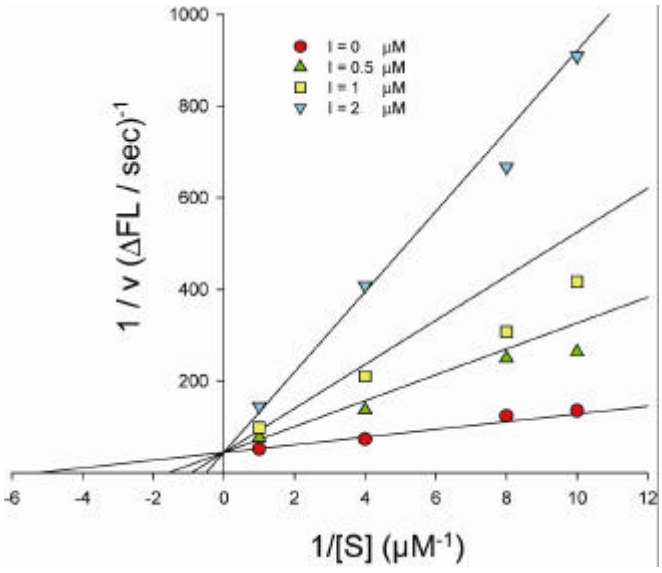


Figure S2. Kinetic analysis of the inhibition of GGTase I by CVIL tetrapeptide **1** and bivalent inhibitor **8**. GGTase I was treated with varying concentrations of the bivalent compound **8** (0.5, 1, and 2 µM) with the substrate concentration increasing from 0.1 to 1 µM. [GGPP] = 5 µM, T = 293 K. The data sets were fit to a competitive inhibition model.

HPLC Analysis of the enzyme reaction.

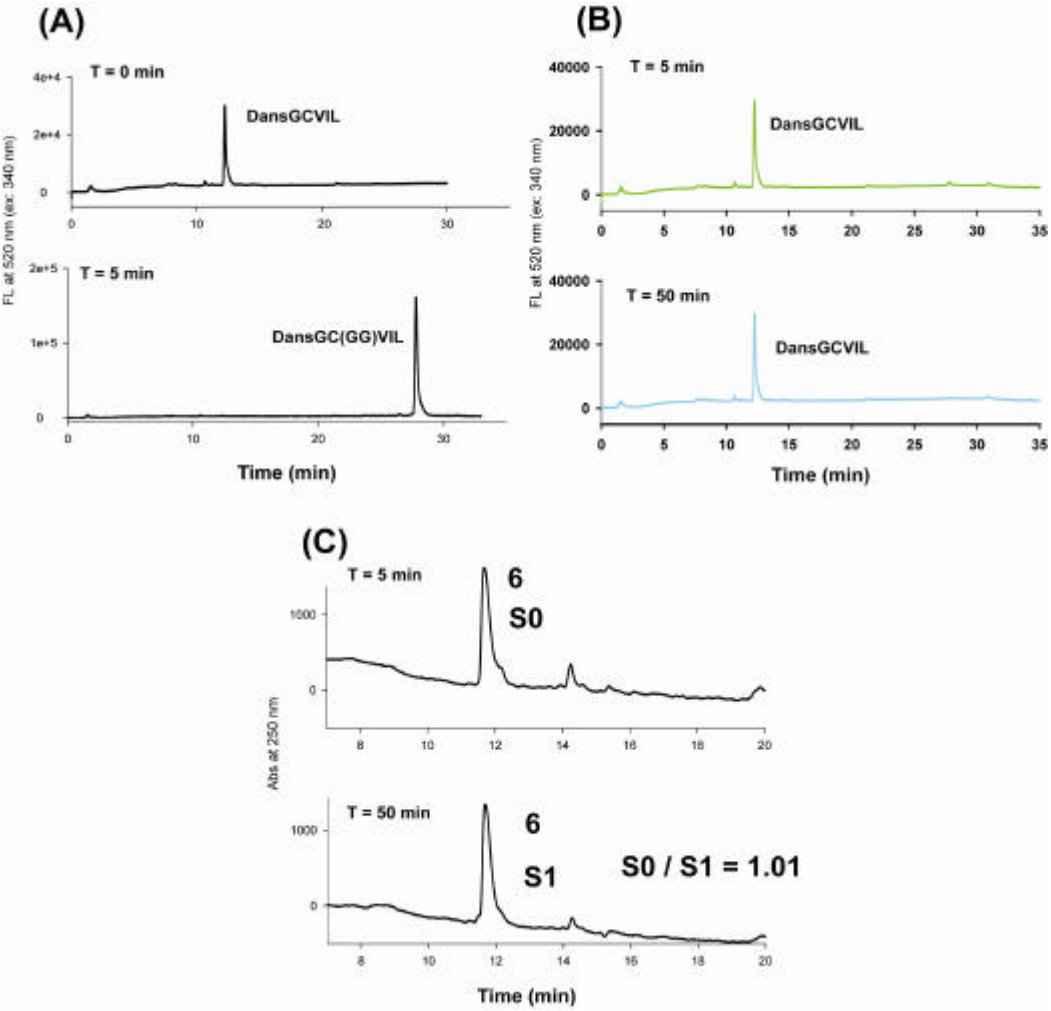


Figure S3. HPLC analysis of the reaction mixture of (A) DansylGCVIL (1 μM), GGPP (5 μM), GGTase I (31 nM) at 0, and 5 minutes, respectively after adding the enzyme; monitored by fluorescence emission at 520 nm (ex: 340 nm): (B) DansylGCVIL (1 μM), GGPP (5 μM), **6** (20 μM), GGTase I (31 nM) at 5, and 50 minutes, respectively after adding the enzyme; monitored by fluorescence emission at 520 nm (ex: 340 nm): (C) **6** (20 μM), GGPP (5 μM), GGTase I (31 nM) at 5 and 50 minutes, respectively, after adding the enzyme; monitored by absorbance at 250 mm.