Supplementary Material (ESI) for Journal of Materials Chemistry

Electronic Supplementary Information (ESI) for
Manuscript: Org. Biomol. Chem. (Title: Development of a linear type of low molecular weight CXCR4 antagonists based on T140 analogs)

Experimental

Genaral

Melting points are uncorrected. ^{1}H-NMR spectra were recorded using a JEOL EX-270 or a JEOL AL-400 spectrometer at 270 or 400 MHz ^{1}H frequency in $CDCl_3$, respectively. Chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Nominal (LRMS) and exact mass (HRMS) spectra were recorded on a JEOL JMS-01SG-2 or JMS-HX/HX 110A mass spectrometer. Ion-spray (IS)-mass spectrum was obtained with a Sciex API*III*E triple quadrupole mass spectrometer (Toronto, Canada). Optical rotations were measured in $CHCl_3$ or H_2O with a JASCO DIP-360 digital polarimeter (Tokyo, Japan) or a Horiba high-sensitive polarimeter SEPA-200 (Kyoto, Japan). For flash column chromatography, silica gel 60 H (silica gel for thin-layer chromatography, Merck) and Wakogel C-200 (silica gel for column chromatography) were employed. HPLC solvents were H_2O and CH_3CN, both containing 0.1% (v/v) TFA. For analytical HPLC, a Cosmosil 5C18-AR column (4.6 x 250 mm, Nacalai Tesque Inc., Kyoto, Japan) was eluted with a linear gradient of CH_3CN at a flow rate of 1 mL/min on a Waters model 600 (Nihon Millipore, Ltd., Tokyo, Japan). Preparative HPLC was performed on a Waters Delta Prep 4000 equipped with a Cosmosil 5C18-AR column (20 x 250 mm, Nacalai Tesque Inc.) using an isocratic mode of CH_3CN at a flow rate of 15 mL/min.

Synthesis of compound 1 (Scheme S1)

[4-(*N′*-2,4,6-Trimethylphenylsulfonyl-guanidino)-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butyl]-carbamic acid tert-butyl ester 46

To a stirred solution of Boc-Arg(Mts)-OH **44** (1.0 g, 2.2 mmol) in DMF (10 mL) were added HOBt (340 mg, 2.2 mmol), DCC (680 mg, 3.3 mmol) and 1-naphthalen-1(*S*)-yl-ethylamine **45** (350 μL, 2.2 mmol) at room temperature with stirring, and the stirring was continued for 12 h. After filtration, the filtrate was concentrated under reduced pressure. The residue was extracted with EtOAc and the extract was washed successively with aq. 5 % citric acid, brine, aq. 5 % $NaHCO_3$ and

brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with $CHCl_3$–MeOH (9 : 1) gave 1.3 g (2.2 mmol, 100 %) of **46** as a colorless oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.38 (9H, s, *tert*-Bu), 1.40-1.70 (4H, br m, CH_2 x 2), 1.58 (3H, d, *J* = 6.6 Hz, Me), 2.25 (3H, s, Ar-*p*-Me), 2.61 (6H, s, Ar-*o*-Me), 3.02 (2H, br m, 3-CH_2), 4.00-4.20 (2H, br m, CH x 2), 5.56 (1H, br, NH), 5.76 (1H, br, NH), 6.17 (3H, br, guanidino), 6.87 (2H, s, ArH), 7.20-8.00 (7H, s, ArH); *m*/*z* (FAB-LRMS) 610 (MH^+), 554, 510 (base peak), 428, 356, 155, 119; Found (FAB-HRMS): 610.3078. Calcd for $C_{32}H_{44}N_5O_5S$ (MH^+): 610.3063.

{4-(*N′*-2,4,6-Trimethylphenylsulfonyl-guanidino)-1(*S*)-[4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butylcarbamoyl]-butyl}-carbamic acid tert-butyl ester 48

To a mixture of compound **46** (520 mg, 0.850 mmol) and anisole (0.50 mL, 4.6 mmol) at 0 °C were added TFA (10 mL) and 4 M HCl-dioxane (5 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure. To a stirred solution of the residue (HCl salts of **47**) in DMF (5 mL) at 0 °C were added triethylamine (Et_3N) (180 μL, 1.3 mmol), Boc-Arg(Mts)-OH **44** (470 mg, 1.0 mmol), HOBt (140 mg, 0.94 mmol) and DCC (260 mg, 1.3 mmol), and the mixture was allowed to warm to room temperature and stirred at this temperature for 12 h. After filtration, the filtrate was concentrated under reduced pressure. The residue was extracted with EtOAc and the extract was washed successively with aq. 5 % citric acid, brine, aq. 5 % $NaHCO_3$ and brine, and dried over $MgSO_4$. Concentration under reduced pressure followed by chromatography over silica gel with $CHCl_3$–MeOH (9 : 1) gave 440 mg (0.46 mmol, 54 %) of **48** as a colorless oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.40 (9H, s, *tert*-Bu), 1.56 (3H, d, *J* = 6.9 Hz, Me), 1.60-2.00 (8H, br m, CH_2 x 4), 2.25 (6H, s, Ar-*p*-Me x 2), 2.60 (6H, s, Ar-*o*-Me), 2.61 (6H, s, Ar-*o*-Me), 3.06 (4H, br m, 3-CH_2 x 2), 4.00-4.20 (2H, br m, CH x 2), 4.36 (1H, br, CH) 5.65-5.85 (3H, br m, NH), 6.10-6.35 (6H, br, guanidine x 2), 6.87 (4H, s, ArH), 7.20-8.00 (7H, s, ArH); *m*/*z* (FAB-LRMS) 948 (MH^+), 848, 766, 356 (base peak), 294, 155, 119; Found (FAB-HRMS): 948.4462. Calcd for $C_{47}H_{66}N_9O_8S_2$ (MH^+): 948.4476.

2(*S*)-Amino-5-guanidino-pentanoic acid

[4-guanidino-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butyl]-amide 1

To the compound **48** (200 mg, 210 μmol) were added *m*-cresol (0.49 mL, 4.7 mmol), 1, 2-ethanedithiol (200 μL, 2.4 mmol), thioanisole (1.2 mL, 10 mmol), TFA (7.5 mL) and bromotrimethylsilane (1.3 mL, 10 mmol) with stirring at 0 °C, and the stirring was continued at room temperature for 3 h. The solution was concentrated under reduced pressure, followed by the addition of Et_2O to precipitate the product. After washing with Et_2O (20 mL x 3), the crude product was purified by preparative HPLC and lyophilized to give a white powder of compound **1** (99 mg, 120 μmol, 58 %); $[\alpha]^{23.9}_D$ -6.36 (*c* 0.31, aq. 1 M AcOH); *m*/*z* (ISMS) 599.0 ($(MH+TFA)^+$), 485.0 (MH^+). Calcd for $C_{24}H_{37}O_2N_9$ (MH^+): 484.6.

Synthesis of compound 2

Boc-Arg(Mts)-Arg(Mts)-NMe(OMe) 51

By use of a procedure identical with that described for the preparation of **47** (HCl salts) from **46**, compound **49** (3.3 g, 6.5 mmol) was converted into HCl salts of **50**. To a stirred solution of the HCl salts of **50** in DMF (10 mL) at 0 °C were added Et_3N (900 μL, 6.5 mmol), Boc-Arg(Mts)-OH **44** (2.7 g, 5.9 mmol), HOBt (0.90 g, 5.9 mmol) and WSCD (1.0 mL, 5.9 mmol), and the mixture was allowed to warm to room temperature and stirred at this temperature for 12 h. After concentration under reduced pressure, the residue was extracted with EtOAc and the extract was washed successively with aq. 5 % citric acid, brine, aq. 5 % $NaHCO_3$ and brine, and dried over $MgSO_4$. The solution was concentrated under reduced pressure, followed by the addition of Et_2O to precipitate the crude product **51**, which was used directly in the following step without further purification.

(4-(*N′*-2,4,6-Trimethylphenylsulfonyl-guanidino)-1(*S*)-{4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-[(1-naphthalen-1(*S*)-yl-ethylamino)-methyl]-butylcarbamoyl}-butyl)-carbamic acid tert-butyl ester 57

To a stirred solution of the protected dipeptide **51** (1.0 g, 1.2 mmol) in CH_2Cl_2 (1.5 mL) was added dropwise a solution of DIBAL-H in toluene (1.0 M, 4.8 mL, 4.8 mmol) at – 78 °C under argon, and the mixture was stirred at – 78 °C for 4 h. Then, a saturated aqueous citric acid was added dropwise with vigorous stirring. The mixture was allowed to warm to 0 °C, and extracted with EtOAc. The extract was washed with brine and

dried over $MgSO_4$. The solution was concentrated under reduced pressure to yield a crude aldehyde **54** (Boc-Arg(Mts)-Arg(Mts)-H), which was used in the following step without further purification. To the stirred solution of **54** in $ClCH_2CH_2Cl$–DMF (1 : 6 (v/v), 5 mL) were added 1-naphthalen-1(*S*)-yl-ethylamine **45** (290 μL, 1.8 mmol) and AcOH (68 μL, 1.2 mmol) at 4 °C, and stirred for 10 min. $NaBH(OAc)_3$ (150 mg, 2.4 mmol) was added to the above mixture at 4 °C and stirred for 8 h with warming to room temperature. The mixture was concentrated under reduced pressure, and the residue was extracted with $CHCl_3$. The extract was washed with aq. 5 % $NaHCO_3$ and brine and dried over $MgSO_4$. Concentration under reduced pressure gave an oily residue, which was purified by chromatography over silica gel with $CHCl_3$–MeOH (19 : 1) to yield 690 mg (0.74 mmol, 62 % yield from **51**) of compound **57** as a yellow oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.37 (9H, s, *tert*-Bu), 1.52 (3H, d, J = 6.3 Hz, Me), 1.60-2.00 (8H, br m, CH_2 x 4), 2.24 (3H, s, Ar-*p*-Me), 2.25 (3H, s, Ar-*p*-Me), 2.60 (6H, s, Ar-*o*-Me), 2.63 (6H, s, Ar-*o*-Me), 3.06-3.20 (6H, br m, CH_2 x 3), 3.90-4.10 (3H, br m, CH x 3), 4.69 (1H, br, NH), 5.80-6.00 (2H, br m, NH), 6.40-6.60 (6H, br, guanidine x 2), 6.85 (2H, s, ArH), 6.87 (2H, s, ArH), 7.20-8.20 (7H, s, ArH); *m*/*z* (FAB-LRMS) 934 (MH^+), 824, 781 (base peak), 172, 155, 119; Found (FAB-HRMS): 934.4715. Calcd for $C_{47}H_{68}N_9O_7S_2$ (MH^+): 934.4683.

2(*S*)-Amino-5-guanidino-pentanoic acid {4-guanidino-1(*S*)-[(1-naphthalen-1(*S*)-yl-ethylamino)-methyl]-butyl}-amide 2

By use of a procedure identical with that described for the preparation of **1** from **48**, compound **57** (64 mg, 69 μmol) was converted into 6.6 mg (7.1 μmol, 10 % yield) of compound **2** as a freeze-dried powder. $[\alpha]^{23.5}_D$ 21.6 (*c* 0.23, aq. 1 M AcOH); *m*/*z* (ISMS) 471.5 (MH^+). Calcd for $C_{37}H_{55}O_6N_5S$ (MH^+): 470.6.

Synthesis of compound 3

(4-(*N'*-2,4,6-Trimethylphenylsulfonyl-guanidino)-1(*S*)-{[4-(*N'*-2,4,6-trimethylphenyl sulfonyl-guanidino)-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butylamino]-meth yl}-butyl)-carbamic acid tert-butyl ester 65

By use of a procedure identical with that described for the preparation of **54** from **51**, compound **49** (380 mg, 0.77 mmol) was converted into a crude aldehyde **62** (Boc-Arg(Mts)-H). By use of a procedure identical with that described for the

preparation of **57** from **54** and **45**, **62** and **47** were converted into 223 mg (0.23 mmol, 31 % yield from **49**) of compound **65** as a yellow oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.39 (9H, s, *tert*-Bu), 1.62 (3H, d, *J* = 6.3 Hz, Me), 1.60-2.00 (8H, br m, CH_2 x 4), 2.60 (3H, s, Ar-*p*-Me), 2.63 (3H, s, Ar-*p*-Me), 2.88 (6H, s, Ar-*o*-Me), 2.96 (6H, s, Ar-*o*-Me), 2.90-3.10 (6H, br m, CH_2 x 3), 3.40-4.00 (3H, br m, CH x 3), 4.50-4.55 (1H, br, NH), 5.12 (1H, br, NH), 5.82 (1H, br, NH), 6.20-6.40 (6H, br, guanidine x 2), 6.85-6.90 (4H, s, ArH), 7.20-8.20 (7H, s, ArH); *m*/*z* (FAB-LRMS) 934 (MH^+), 834, 566, 342, 294 (base peak), 155, 119; Found (FAB-HRMS): 934.4698. Calcd for $C_{47}H_{68}N_9O_7S_2$ (MH^+): 934.4683.

2(*S*)-(2(*S*)-Amino-5-guanidino-pentylamino)-5-guanidino-pentanoic acid (1-naphthalen-1(*S*)-yl-ethyl)-amide 3

By use of a procedure identical with that described for the preparation of **1** from **48**, compound **65** (50 mg, 54 μmol) was converted into 9.9 mg (11 μmol, 20 % yield) of compound **3** as a freeze-dried powder. $[\alpha]^{23.9}_D$ 10.1 (*c* 0.40, aq. 1 M AcOH); *m*/*z* (ISMS) 470.0 (MH^+). Calcd for $C_{37}H_{55}O_6N_5S$ (MH^+): 470.6.

Synthesis of compound 4

Boc-Tyr(Cl_2Bn)-Arg(Mts)-Arg(Mts)-NMe(OMe) 52

By use of a procedure identical with that described for the preparation of **48** from **46** and Boc-Arg(Mts)-OH **44**, **51** (1.0 g, 1.20 mmol) and Boc-Tyr(Cl_2Bn)-OH (580 mg, 1.3 mmol) were converted into 1.1 g (0.92 mmol, 77 % yield from **51**) of compound **52** as colorless crystals.

Mp 128-131 °C (from $CHCl_3$-MeOH); found: C, 54.08; H, 6.30; N, 12.05. $C_{53}H_{72}Cl_2N_{10}O_{11}S_2 \cdot H_2O$ requires C, 54.03; H, 6.33; N, 11.89 %); $[\alpha]^{21.9}_D$ – 6.75 (*c* 0.89, $CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.34 (9H, s, *tert*-Bu), 1.40-2.00 (8H, br m, CH_2 x 4), 2.25 (3H, s, Ar-*p*-Me), 2.26 (3H, s, Ar-*p*-Me), 2.64 (6H, s, Ar-*o*-Me), 2.68 (6H, s, Ar-*o*-Me), 2.90-3.30 (6H, br m, CH_2), 3.19 (1H, s, NMe), 3.76 (1H, s, OMe), 3.71 (1H, br m, CH), 4.31 (1H, br m, CH), 4.71 (1H, br m, CH), 5.10-5.25 (1H, br m, NH), 5.20 (2H, br, Cl_2Ph-CH_2), 6.00-6.30 (2H, br, NH x 2), 6.30-6.60 (6H, br, guanidine x 2), 6.86 (2H, s, ArH), 6.89 (2H, s, ArH), 6.90 (2H, d, *J* = 8.1 Hz, ArH), 7.09 (2H, d, *J* = 8.4 Hz, ArH), 7.20-7.40 (3H, m, ArH); *m*/*z* (FAB-LRMS) 1159 (MH^+), 1059 (base peak), 977, 400, 294, 159, 119; Found (FAB-HRMS): 1159.4272. Calcd for $C_{53}H_{73}Cl_2N_{10}O_{11}S_2$

(MH^+): 1159.4278.

[2-[4-(2,6-Dichloro-benzyloxy)-phenyl]-1(*S*)-(4-(*N'*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-{4-(*N'*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-[(1-naphthalen-1(*S*)-yl-ethylamino)-methyl]-butylcarbamoyl}-butylcarbamoyl)-ethyl]-carbamic acid tert-butyl ester 58

By use of a procedure identical with that described for the preparation of **57** from **51**, **52** (1.0 g, 0.86 mmol) was converted into 642 mg (0.51 mmol, 59 % yield from **52**) of compound **58** as colorless crystals.

Mp 149-150 °C (from $CHCl_3$-MeOH); found: C, 55.14; H, 6.31; N, 11.06. $C_{63}H_{80}Cl_2N_{10}O_9S_2 \cdot 5H_2O$ requires C, 56.23; H, 6.75; N, 10.41 %); $[\alpha]^{18.8}_D$ – 10.14 (*c* 0.69, $CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.33 (9H, s, *tert*-Bu), 1.54 (3H, br, Me), 1.60-2.00 (8H, br m, CH_2 x 4), 2.24 (6H, s, Ar-*p*-Me x 2), 2.62 (12H, s, Ar-*o*-Me x 2), 2.80-3.20 (8H, br m, CH_2 x 4), 3.50-4.80 (5H, br m, CH x 4, NH), 5.18 (2H, br, Cl_2Ph-CH_2), 5.10-6.10 (3H, br m, NH x 3), 6.40-6.60 (6H, br, guanidine x 2), 6.86 (4H, s, ArH), 6.89 (2H, d, *J* = 8.6 Hz, ArH), 7.12 (2H, d, *J* = 8.6 Hz, ArH), 7.20-7.60 (10H, m, ArH); *m*/*z* (FAB-LRMS) 1255 (MH^+), 1102, 917 (base peak), 343, 225, 155, 119; Found (FAB-HRMS): 1255.5037. Calcd for $C_{63}H_{81}Cl_2N_{10}O_9S_2$ (MH^+): 1255.5007.

2(*S*)-[2(*S*)-Amino-3-(4-hydroxy-phenyl)-propionylamino]-5-guanidino-pentanoic acid {4-guanidino-1(*S*)-[(1-naphthalen-1(*S*)-yl-ethylamino)-methyl]-butyl}-amide 4

By use of a procedure identical with that described for the preparation of **1** from **48**, compound **58** (110 mg, 90 μmol) was converted into 29 mg (26 μmol, 29 % yield) of compound **4** as a freeze-dried powder. $[\alpha]^{23.5}_D$ 17.0 (*c* 1.35, H_2O); *m*/*z* (ISMS) 633.5 (MH^+). Calcd for $C_{33}H_{49}O_3N_{10}$ (MH^+): 633.8.

Synthesis of compound 5

Boc-Tyr(Cl_2Bn)-Arg(Mts)-NMe(OMe) 60

By use of a procedure identical with that described for the preparation of **52** from **51**, **49** (1.0 g, 2.0 mmol) was converted into 1.5 g (1.9 mmol, 93 % yield from **49**) of compound **60** as a colorless oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.35 (9H, s, *tert*-Bu), 1.60-2.00 (4H, br m, CH_2 x 2), 2.24 (6H, s, Ar-*p*-Me x 2), 2.66 (12H, s, Ar-*o*-Me x 2), 2.80-3.30 (4H, br m, CH_2 x 2), 3.16

(3H, s, NMe), 3.70 (3H, s, OMe), 4.20-4.50 (2H, m, CH x 2), 4.89 (1H, m, NH), 5.20 (2H, s, Cl_2Ph-CH_2), 5.20-5.30 (1H, m, NH), 6.30-6.50 (3H, br, guanidine), 6.87 (2H, s, ArH), 6.89 (2H, d, *J* = 8.6 Hz, ArH), 7.09 (2H, d, *J* = 8.6 Hz, ArH), 7.15-7.40 (3H, m, ArH); *m*/*z* (FAB-LRMS) 821 (MH^+), 721 (base peak), 643, 294, 225, 159, 119; Found (FAB-HRMS): 821.2846. Calcd for $C_{38}H_{51}Cl_2N_6O_8S$ (MH^+): 821.2866.

[2-[4-(2,6-Dichloro-benzyloxy)-phenyl]-1(*S*)-(4-(*N'*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-{[4-(*N'*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butylamino]-methyl}-butylcarbamoyl)-ethyl]-carbamic acid tert-butyl ester 66

By use of a procedure identical with that described for the preparation of **57** from **51**, **60** (410 g, 0.50 mmol) was converted into 400 mg (0.32 mmol, 63 % yield from **60**) of compound **66** as a colorless oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.27-1.31 (9H, m, *tert*-Bu), 1.57 (3H, br, Me), 1.40-1.80 (8H, br m, CH_2 x 4), 2.22 (6H, s, Ar-*p*-Me x 2), 2.61 (12H, s, Ar-*o*-Me x 2), 2.80-3.20 (8H, br m, CH_2 x 4), 3.40-4.80 (5H, br m, CH x 4, NH), 5.14 (2H, br, Cl_2Ph-CH_2), 5.10-5.90 (3H, br m, NH x 3), 6.30-6.50 (6H, br, guanidine x 2), 6.84 (4H, s, ArH), 7.05 (2H, d, *J* = 8.9 Hz, ArH), 7.22 (2H, d, *J* = 8.9 Hz, ArH), 7.20-8.10 (10H, m, ArH); *m*/*z* (FAB-LRMS) 1255 (MH^+), 764 (base peak), 664, 294, 155, 119; Found (FAB-HRMS): 1255.4978. Calcd for $C_{63}H_{81}Cl_2N_{10}O_9S_2$ (MH^+): 1255.5007.

2(*S*)-{2(*S*)-[2(*S*)-Amino-3-(4-hydroxy-phenyl)-propionylamino]-5-guanidino-pentylamino}-5-guanidino-pentanoic acid (1-naphthalen-1(*S*)-yl-ethyl)-amide 5

By use of a procedure identical with that described for the preparation of **1** from **48**, compound **66** (67 mg, 53 μmol) was converted into 29 mg (26 μmol, 50 % yield) of compound **5** as a freeze-dried powder. $[\alpha]^{23.2}_D$ 7.45 (*c* 0.939, H_2O); *m*/*z* (ISMS) 633.5 (MH^+). Calcd for $C_{33}H_{49}O_3N_{10}$ (MH^+): 633.8.

Synthesis of compound 6

4-Fluoro-*N*-{4-(*N'*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-[1(*S*)-(methoxy-methyl-carbamoyl)-4-(*N'*-2,4,6-trimethylphenylsulfonyl-guanidino)-butylcarbamoyl]-butyl}-benzamide, 4-Fluorobenzoyl-Arg(Mts)-Arg(Mts)-NMe(OMe) 53

By use of a procedure identical with that described for the preparation of **52** from **51**, **51**

(740 mg, 0.85 mmol) was converted into 1.5 g (0.85 mmol, 100 % yield from **51**) of a crude compound **53** as a colorless oil, using WSDI instead of DCC. The crude **53** was used in the following step without further purification.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.40-2.00 (8H, br m, CH_2 x 4), 2.25 (6H, s, Ar-*p*-Me x 2), 2.61 (6H, s, Ar-*o*-Me), 2.63 (6H, s, Ar-*o*-Me), 2.90-3.30 (2H, br m, CH_2), 3.15 (1H, s, NMe), 3.74 (1H, s, OMe), 4.00-4.20 (2H, br m, CH), 4.70-4.90 (2H, br m, NH), 6.30-6.60 (6H, br, guanidine x 2), 6.86 (2H, s, ArH), 6.87 (2H, s, ArH), 6.90-7.00 (2H, m, ArH), 7.70-7.90 (2H, m, ArH).

4-Fluoro-*N*-(4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-{4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-[(1-naphthalen-1(*S*)-yl-ethylamino)-methyl]-butylcarbamoyl}-butyl)-benzamide 59

By use of a procedure identical with that described for the preparation of **57** from **51**, **53** (0.70 g, 0.81 mmol) was converted into 330 mg (0.35 mmol, 43 % yield from **53**) of compound **59** as colorless crystals.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.55 (3H, br, Me), 1.58-1.80 (8H, br m, CH_2 x 4), 2.24 (3H, s, Ar-*p*-Me), 2.25 (3H, s, Ar-*p*-Me), 2.54 (6H, s, Ar-*o*-Me), 2.57 (6H, s, Ar-*o*-Me), 2.60-3.30 (6H, br m, CH_2 x 3), 3.80-4.90 (6H, br m, CH x 3, NH x 3), 6.40-6.60 (6H, br, guanidine x 2), 6.82 (2H, s, ArH), 6.84 (2H, s, ArH), 6.80-6.90 (2H, m, ArH), 7.40-8.10 (9H, m, ArH); *m*/*z* (FAB-LRMS) 956 (MH^+), 802, 307 (base peak), 155, 119. Calcd for $C_{49}H_{63}FN_9O_6S_2$ (MH^+): 956.4.

4-Fluoro-*N*-(4-guanidino-1(*S*)-{4-guanidino-1(*S*)-[(1-naphthalen-1(*S*)-yl-ethylamino)-methyl]-butylcarbamoyl}-butyl)-benzamide 6

By use of a procedure identical with that described for the preparation of **1** from **48**, compound **59** (160 mg, 160 μmol) was converted into 74 mg (79 μmol, 48 % yield) of compound **6** as a freeze-dried powder. $[\alpha]^{27.2}_D$ 8.99 (*c* 2.4, H_2O); *m*/*z* (ISMS) 592.5 (MH^+); *m*/*z* (FAB-LRMS) 592 (MH^+), 155, 123 (base peak); Found (FAB-HRMS): 592.3539. Calcd for $C_{31}H_{43}FN_9O_2$ (MH^+): 592.3524.

Synthesis of compound 7

4-Fluoro-*N*-[1(*S*)-(methoxy-methyl-carbamoyl)-4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-butyl]-benzamide, 4-Fluorobenzoyl-Arg(Mts)-NMe(OMe) 61

By use of a procedure identical with that described for the preparation of **53** from **51**, **49** (470 mg, 0.95 mmol) was converted into 450 mg (0.85 mmol, 90 % yield from **49**) of a crude compound **61** as a colorless oil. The crude **61** was used in the following step without further purification.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.40-2.00 (4H, br m, CH_2 x 2), 2.26 (3H, s, Ar-*p*-Me), 2.62 (6H, s, Ar-*o*-Me), 3.15 (1H, s, NMe), 3.20-3.30 (1H, m, CH_2), 3.78 (1H, s, OMe), 4.00-4.20 (1H, m, CH), 5.09 (1H, m, NH), 6.20-6.60 (3H, br, guanidine), 6.86 (2H, s, ArH), 7.02 (2H, t, *J* = 8.6 Hz, ArH), 7.78 (2H, dd, *J* = 8.9, 5.4 Hz, ArH).

4-Fluoro-*N*-(4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-{[4-(*N′*-2,4,6-trimethylphenylsulfonyl-guanidino)-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butylamino]-methyl}-butyl)-benzamide 67

By use of a procedure identical with that described for the preparation of **57** from **51**, **61** (410 mg, 0.79 mmol) was converted into 410 mg (0.43 mmol, 54 % yield from **61**) of compound **67** as a colorless oil.

^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.52 (3H, d, *J* = 6.2 Hz, Me), 1.58-1.80 (8H, br m, CH_2 x 4), 2.24 (3H, s, Ar-*p*-Me), 2.25 (3H, s, Ar-*p*-Me), 2.57 (6H, s, Ar-*o*-Me), 2.59 (6H, s, Ar-*o*-Me), 2.60-3.30 (6H, br m, CH_2 x 3), 3.80-4.70 (4H, br m, CH x 3, NH), 5.70-5.90 (2H, m, NH x 2), 6.20-6.60 (6H, br, guanidine x 2), 6.85 (4H, s, ArH), 6.80-6.90 (2H, m, ArH), 7.40-8.10 (9H, m, ArH); *m*/*z* (FAB-LRMS) 956 (MH^+), 802, 464 (base peak), 155, 119. Calcd for $C_{49}H_{63}FN_9O_6S_2$ (MH^+): 956.4.

4-Fluoro-*N*-(4-guanidino-1(*S*)-{[4-guanidino-1(*S*)-(1-naphthalen-1(*S*)-yl-ethylcarbamoyl)-butylamino]-methyl}-butyl)-benzamide 7

By use of a procedure identical with that described for the preparation of **1** from **48**, compound **67** (110 mg, 120 μmol) was converted into 45 mg (48 μmol, 40 % yield) of compound **7** as a freeze-dried powder. $[\alpha]^{27.4}_D$ 2.80 (*c* 2.2, H_2O); *m*/*z* (ISMS) 593.0 (MH^+); *m*/*z* (FAB-LRMS) 592 (MH^+), 154, 136, 123 (base peak); Found (FAB-HRMS): 592.3528. Calcd for $C_{31}H_{43}FN_9O_2$ (MH^+): 592.3524.

Synthesis of compounds 8-27, witch were contained in library 1 (Figure S1)

Representative compound 8 (4-fluorobenzoyl-Arg-Arg-Nal-NH_2)

The protected resin was manually constructed using Fmoc-based solid-phase synthesis on a 4-(2',4'-dimethoxyphenylaminomethyl)-phenoxy resin (0.34 meq/g, 0.05 mmol scale). Fmoc-Nal-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH and 4-fluorobenzoic acid (2.5 equiv.) were successively condensed using 1,3-diisopropylcarbodiimide (DIPCDI) (2.5 equiv.) in the presence of *N*-hydroxybenzotriazole (HOBt) (2.5 equiv.) in DMF. The Fmoc-group was deprotected by treatment of the resin with 20% (v/v) piperidine/DMF for 1 and 15 min. The resulting protected resin (50 μmol) was treated with aqueous 95% TFA (10 mL) at room temperature for 2 h. After removal of the resin by filtration, the filtrate was concentrated *in vacuo*. Ice-cold dry diethyl ether (30 mL) was added to the residue. The resulting powder was collected by centrifugation and then washed three times with ice-cold dry diethyl ether (20 mL x 3). The crude product was dissolved in H_2O (5 mL), and purified by preparative HPLC to afford a fluffy white powder of the desired compound **8**. Characterized data of all the synthetic compounds are listed in ESI, Table S1.

Synthesis of compounds 28-39 (Scheme 1), witch were contained in library 2 (Figure S2)

Representative compound 39 (4-(4-fluorobenzoylamino-methyl)-*N*-[4-guanidino-1(*S*)-(1-naphthalen-1(*S*)-yl-ethyl carbamoyl)-butyl]-benzamide)

On a 4-sulfamylbutyryl AM resin **40** (1.12 mmol/g, 3.56 g, 3.98 mmol), Fmoc-Arg(Pbf)-OH (4.0 equiv.) was loaded using DIPEA (6.0 equiv.) and PyBOP (3.0 equiv.) in $CHCl_3$ at –20 □ for 8h. After this condensation reaction was repeated twice, the resin **41** (5.59 g, 1.96 mmol) was obtained. Resin **41** was successively condensed with Fmoc-(4-aminomethyl)benzoic acid (3.0 equiv.) and 4-trifluoromethylbenzoic acid (3.0 equiv.) using standerd Fmoc-based solid phase synthesis. After the resin **42** was swollen with THF, 2 M $TMSCHN_2$/hexane (40.0 equiv.) was added, and the mixture was stirred at room temperature for 4h. After washing the resin with THF, DMF and $CHCl_3$

(40 mL × 5 each), (*R*)-(+)-1-(1-naphthyl)ethylamine (100 equiv.) and DMF (2 mL) were added to the resin (225 mg, 0.084 mmol), and the mixture was stirred at 70□ for 24h. After removal of the resin by filtration, the filtrate was concentrated under reduced pressure to give the residue, which was extracted with AcOEt and washed with saturated aq. citric acid and saturated aq. NaCl and then dried over $MgSO_4$. Concentration under reduced pressure gave the protected compound. After removal of a protecting group by treatment with thioanisole/TFA (5 : 95 (v/v), 40 mL) at room temperature for 2h, the reaction solution was concentrated under reduced pressure. Ice-cold dry diehtyl ether (30 mL) was added to the residue. The resulting powder was collected by centrifugation and then washed three times with ice-cold dry diethyl ether (30 mL x 3). The crude product was extracted with water and purified by preparative HPLC to afford a white powder of the desired compound **39** (6.82 mg, 9.13 μmol, 12.8% from **41**). Characterized data of all the synthetic compounds are listed in ESI, Table S1.

Compound 39

$[\alpha]^{27.2}_{D}$ + 146 (*c* 0.04, H_2O); ^{1}H NMR (400 MHz, CD_3OD) δ 1.55 (3H, d, *J* = 6.8 Hz, Me), 1.67-1.82 (4H, m, CH_2 x 2), 3.22 (2H, pentaplet, *J* = 1.6 Hz, CH_2), 4.56 (2H, s, Ar-CH_2), 4.72 (1H, br, CH), 5.79 (1H, br, CH), 7.32-7.55 (6H, m, ArH), 7.68-7.84 (5H, m, ArH), 7.95 (2H, d, *J* = 8.0 Hz, ArH), 8.02 (2H, d, *J* = 8.0 Hz, ArH); *m/z* (ISMS) 634.0 (MH^+); *m/z* Found (FAB-HRMS): 633.2814. Calcd for $C_{34}H_{36}F_3N_6O_3$ (MH^+): 633.2801.

Cell culture

Human T-cell lines, MT-4 and MOLT-4 cells were grown in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 100 IU/mL penicillin and 100 μg/mL streptomycin.

Virus

A strain of X4-HIV-1, HIV-1_{IIIB}, was used for the anti-HIV assay. This virus was obtained from the culture supernatant of HIV-1 persistently infected MOLT-4/HIV-1_{IIIB}

cells, and stored at –80 °C until used.

Anti-HIV-1 assay

Anti-HIV-1 activity was determined based on the protection against HIV-1-induced cytopathogenicity in MT-4 cells. Various concentrations of test compounds were added to HIV-1-infected MT-4 cells at a multiplicity of infection (MOI) of 0.01, and placed in wells of a flat-bottomed microtiter tray (1.5 x 10^4 cells/well). After 5 days' incubation at 37 °C in a CO_2 incubator, the number of viable cells was determined using the MTT method (EC_{50}). Cytotoxicity of compounds was determined based on the viability of mock-infected cells using the MTT method (CC_{50}).

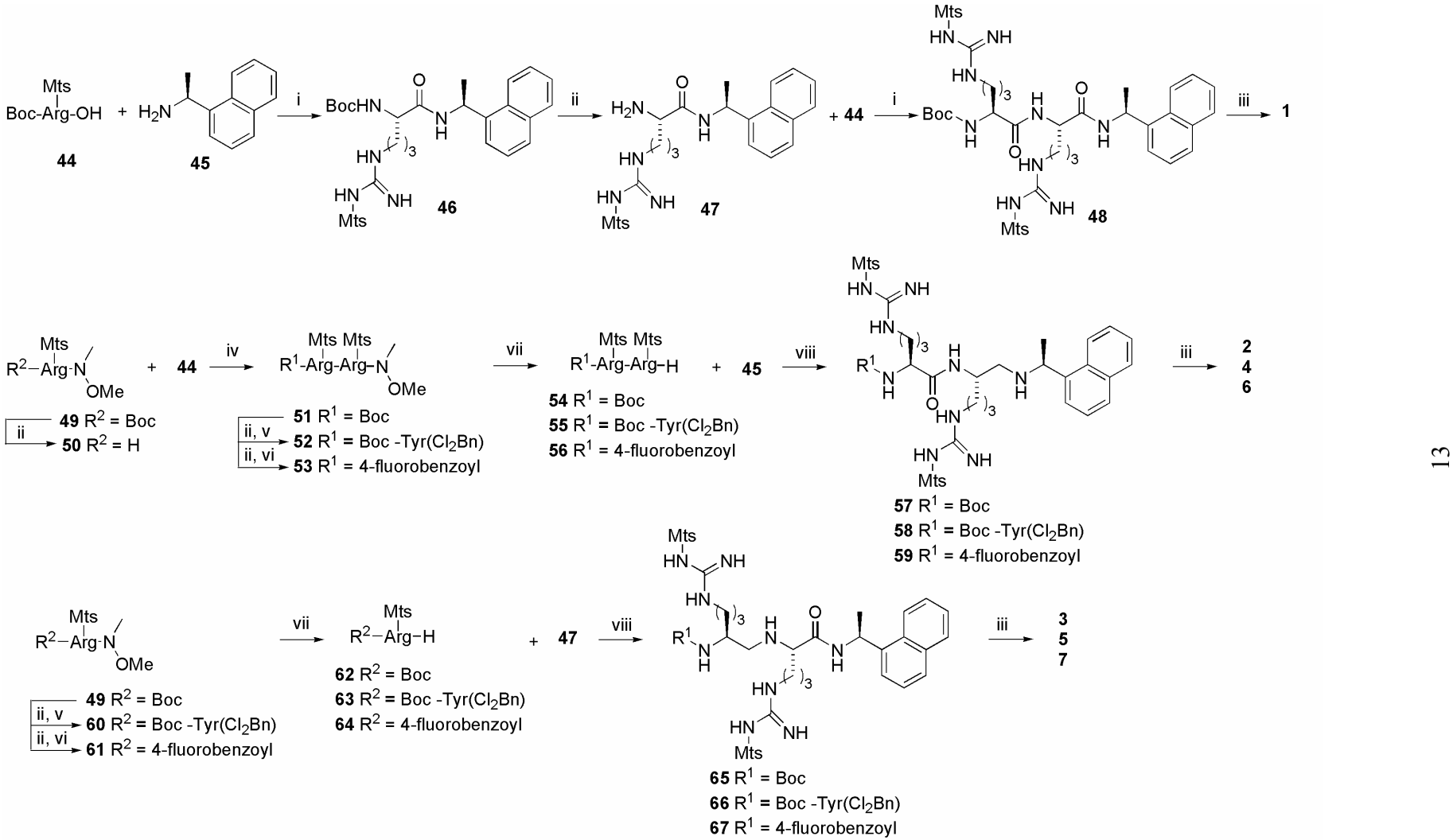
[^{125}I]-CXCL12 binding and displacement

Stable CHO cell transfectants expressing CXCR4 were prepared as described previously. CHO transfectants were harvested by treatment with trypsin-EDTA, allowed to recover in complete growth medium (MEM-α, 100 μg/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B, 10 % (v/v)) for four to five hours and then washed in cold binding buffer (PBS containing 2 mg/mL BSA). For ligand binding, the cells were resuspended in binding buffer at 1×10^7 cells/mL, and 100 μL aliquots were incubated with 0.1 nM of [^{125}I]-CXCL12 (PerkinElmer Life Sciences) for two hours on ice under constant agitation. Free and bound radioactivities were separated by centrifugation of the cells through an oil cushion and bound radioactivity was measured with a gamma-counter (Cobra, Packard, Downers Grove, IL). Inhibitory activity of the test compounds was determined based on the inhibition of [^{125}I]-CXCL12-binding to CXCR4 transfectants (IC_{50}).

Inhibition (%) = (Et – Ea) / (Et – Ec) x 100

Et: the quantity of radioactivity in the absence of a test compound

Ec: the quantity of radioactivity in the presence of cold SDF-1α as a test compound
Ea: the quantity of radioactivity in the presence of a test compound



Scheme S1 Mts = 2,4,6-trimethylphenylsulfonyl, Cl_2Bn = 2,6-dichlorobenzyl; *Reagents*. i, DCC, HOBt, DMF; TFA, anisole, 4M HCl-Dioxane, then Et_3N, DMF; iii, *m*-cresol, 1,2-ethanedithiol, thioanisole, TFA, TMSBr; iv, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD), HOBt, Et_3N, DMF; v, Boc-Tyr(Cl_2Bn)-OH, DCC, HOBt, DMF; vi, 4-fluorobenzoic acid, WSCD, HOBt, Et_3N, DMF; vii, 1.01M DIBAL-toluene, CH_2Cl_2; viii, $NaB(CN)H_3$ or $NaBH(OAc)_3$, AcOH, $ClCH_2CH_2Cl$, DMF

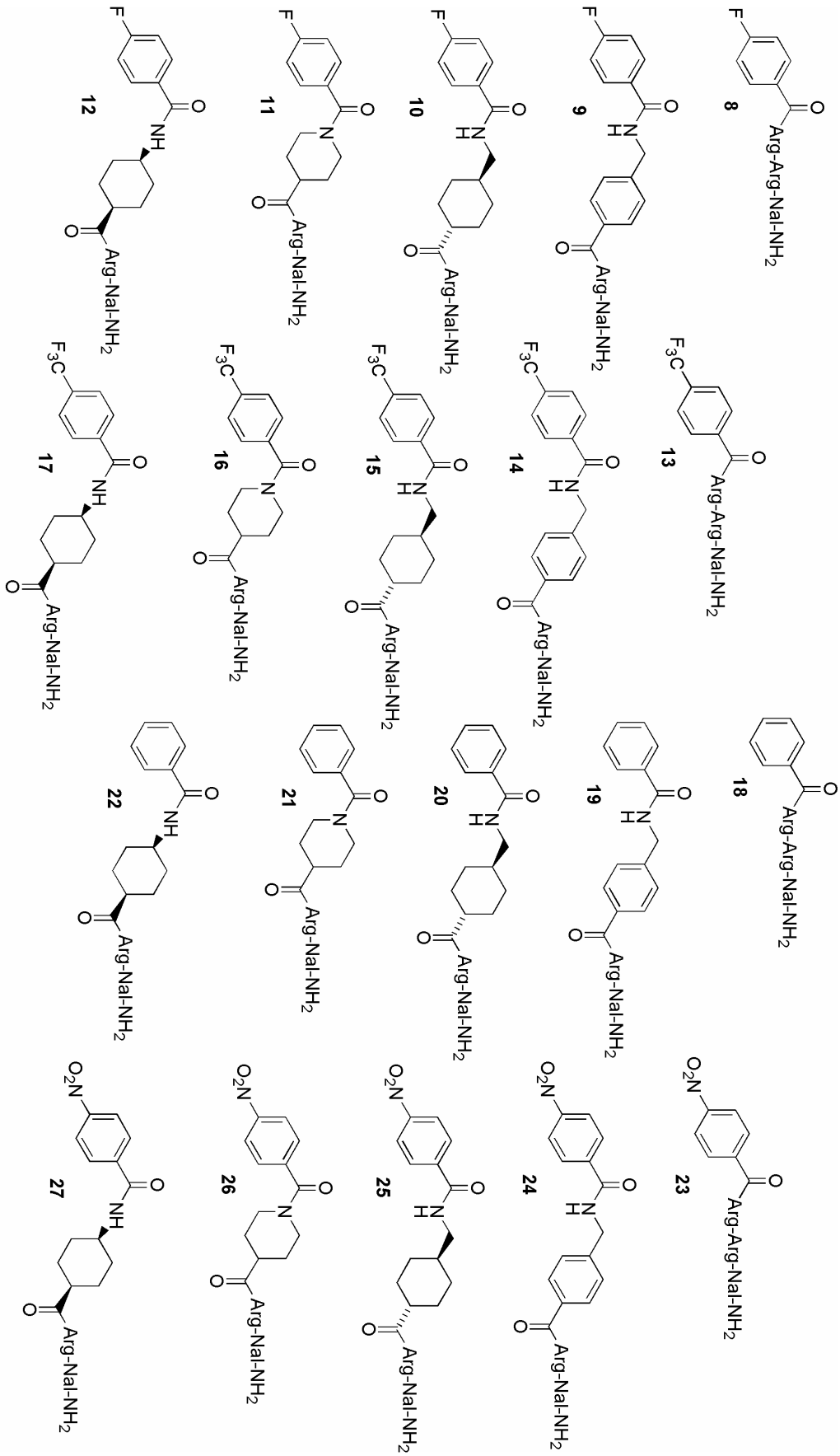


Figure S1 Structures of compounds contained in library 1 (**8-27**).

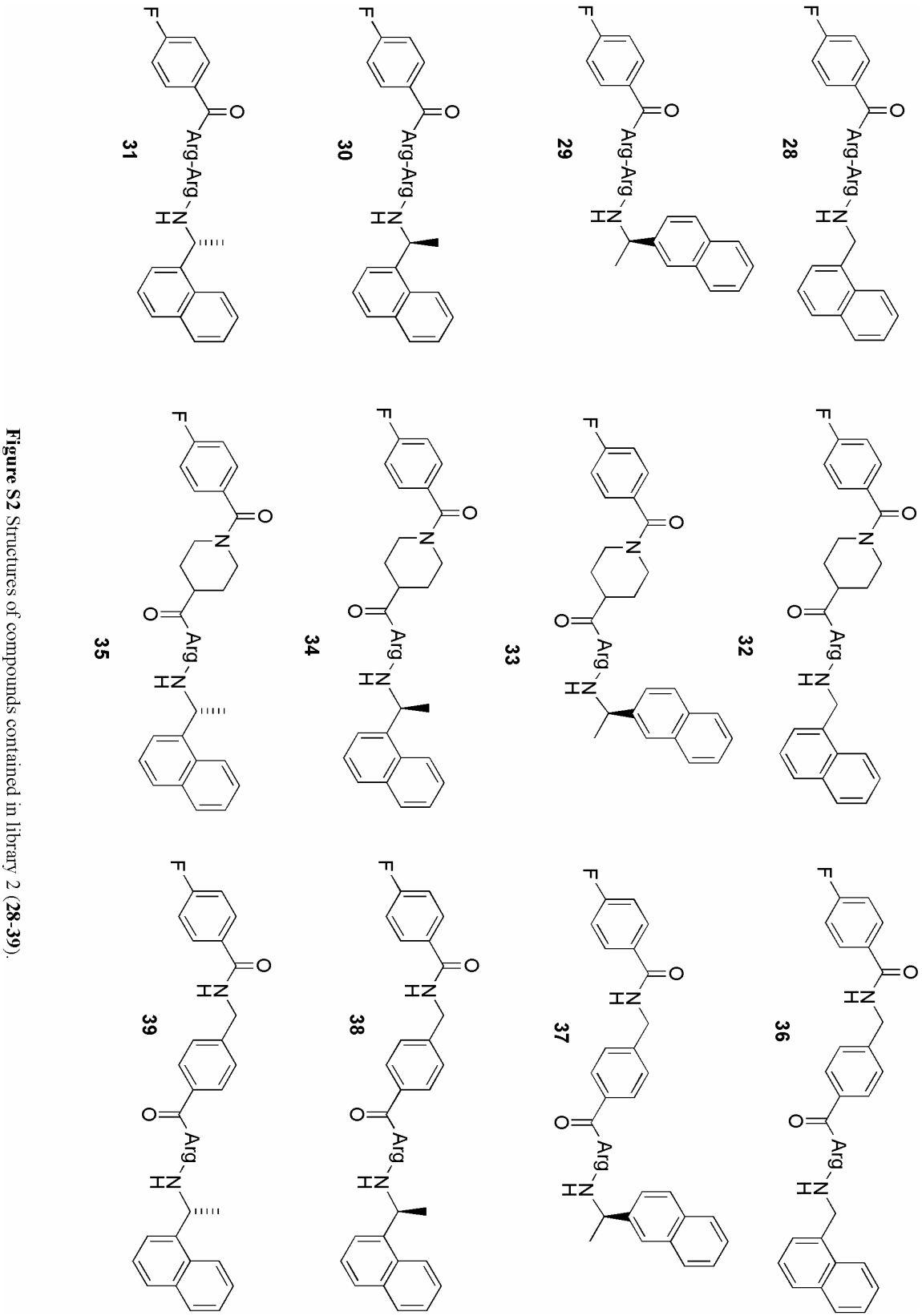


Figure S2 Structures of compounds contained in library 2 (**28-39**).

Table S1 Characterization data (MS) of novel synthetic compounds

Compd.	Formula	ISMS $(MH)^{+}$ [a] Calcd.	Found
8	$C_{32}H_{41}FN_{10}O_4$	649.3	650.0
9	$C_{34}H_{36}FN_7O_4$	626.2	627.5
10	$C_{34}H_{42}FN_7O_4$	632.3	633.0
11	$C_{32}H_{38}FN_7O_4$	604.3	606.0
12	$C_{33}H_{40}FN_7O_4$	618.3	618.5
13	$C_{33}H_{41}F_3N_{10}O_4$	699.3	700.0
14	$C_{35}H_{36}F_3N_7O_4$	676.2	677.0
15	$C_{35}H_{42}F_3N_7O_4$	682.3	683.0
16	$C_{33}H_{38}F_3N_7O_4$	654.3	654.5
17	$C_{34}H_{40}F_3N_7O_4$	668.3	669.0
18	$C_{32}H_{42}N_{10}O_4$	631.3	632.0
19	$C_{34}H_{37}N_7O_4$	608.2	608.5
20	$C_{34}H_{43}N_7O_4$	614.3	614.5
21	$C_{32}H_{39}N_7O_4$	586.3	587.5
22	$C_{33}H_{41}N_7O_4$	600.3	600.5
23	$C_{32}H_{41}N_{11}O_6$	676.3	677.0
24	$C_{34}H_{36}N_8O_6$	653.2	653.5
25	$C_{34}H_{42}N_8O_6$	659.3	660.5
26	$C_{32}H_{38}N_8O_6$	631.3	632.5
27	$C_{33}H_{40}N_8O_6$	645.3	646.0
28	$C_{30}H_{39}FN_9O_3$	592.3	593.0
29	$C_{31}H_{41}FN_9O_3$	606.3	607.0
30	$C_{31}H_{41}FN_9O_3$	606.3	606.5
31	$C_{31}H_{41}FN_9O_3$	606.3	607.0
32	$C_{30}H_{36}FN_6O_3$	547.2	547.5
33	$C_{31}H_{38}FN_6O_3$	561.3	562.0
34	$C_{31}H_{38}FN_6O_3$	561.3	562.0
35	$C_{31}H_{38}FN_6O_3$	561.3	561.5
36	$C_{33}H_{34}F_3N_6O_3$	619.2	619.5
37	$C_{34}H_{36}F_3N_6O_3$	633.2	634.0
38	$C_{34}H_{36}F_3N_6O_3$	633.2	634.0
39	$C_{34}H_{36}F_3N_6O_3$	633.2	634.0

[a] Ion-spray (IS)-mass spectra were obtained with a Sciex API*III*E triple quadrupole mass spectrometer (Toronto, Canada).