SUPPORTING INFORMATION

Small molecule inhibitors of the neuropilin-1 VEGF-A interaction

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 Statistical analysis
Data were analysed using Prism (version 4.0) statistical packages. Comparisons of two sets of variables were performed using the Student's $t$ test or $t$ test with Welch's correction where appropriate. Differences among three or four concentrations of compounds were evaluated using the one-way analysis of variance (ANOVA) with Bonferroni’s multiple comparison tests. Differences between two treatment groups at various concentrations were analysed using the two-way ANOVA with Bonferroni’s post-tests. Values represent means ± SEM determined from the results of three independent experiments each performed in duplicates or triplicates unless where stated. A value of $p < 0.05$ was taken as statistically significant.

 Purification and characterization of linear peptides
Peptides were purified by reverse-phase HPLC (Gilson) using a preparative C-18 column. The relevant fractions were collected, evaporated, lyophilized (-54 °C, 0.08 mbar) and stored
at 4 °C. Confirmation of the structure was performed by reverse-phase LC-MS (Gilson/Waters ZQ) using an analytical C-18 column (Phenomenex Columbus, 250 x 4 mm, 5µm or Luna, 50 x 2 mm, 5µm or Thermobetabasic, 100 x 21.2 mm, 5 µm) and an AB gradient of 5 –95% for B, over 25 - 35 minutes, at a flow rate of 1 mL/minute, where eluent A was 0.1% trifluoroacetic/water and eluent B was 0.1% trifluoroacetic acetonitrile. Peptides 16, 23 and 24 were synthesised by Pepceuticals Ltd, Nottingham, UK.

Ac-RXDKPAR-OH (X = 2-aminobutyric acid) 16
White solid, 20 mg,  HPLC Rₜ 4.56 min.; purity > 95%; MS m/z – 868.9 [M]+.

H-kPAR-OH, 18
White solid, 29 mg,  HPLC Rₜ 4.81 min.; purity > 95%; MS m/z – 471 [M + 1]+.

H-KPaR-OH, 19
White solid, 30 mg,  HPLC Rₜ 5.47 min.; purity > 95%; MS m/z – 471 [M + 1]+.

H-KPPR-OH, 20
White solid, 20 mg,  HPLC Rₜ 8.26 min.; purity > 95%; MS m/z – 497.7 [M + 1]+.

H-KPFR-OH, 21
White solid, 30 mg,  HPLC Rₜ 11.43 min.; purity > 95%; MS m/z – 547.6 [M + 1]+.

H-KPAr-OH, 22
White solid, 37 mg,  HPLC Rₜ 4.21 min.; purity > 95%; MS m/z – 471 [M + 1]+.

**Small Molecule Experimental Section**

All commercially available solvents and reagents were used without further treatment as received unless otherwise noted. NMR spectra were measured with a Bruker DRX 400 or 600 MHz spectrometer; chemical shifts are expressed in ppm relative to TMS as an internal standard and coupling constants (J) in Hz. Mass spectra were obtained using a Waters ZQ2000 single quadrupole mass spectrometer with electrospray ionisation (ESI). High
resolution mass spectra were acquired on a Waters LCT time of flight mass spectrometer with electrospray ionisation (ESI). All novel final compounds were analysed by reverse-phase LCMS using an analytical C18 column (Thermo Betabasic, 100 x 4.6 mm, 5 μm) and an AB gradient of 5–95 % for B at a flow rate of 1 mL/minute, where eluent A was 0.1 % formic acid/water and eluent B was 0.1 % formic acid/acetonitrile. Analysis of intermediates by reverse-phase LCMS was carried out on an analytical C18 column (Phenomenex Gemini, 50 x 3.0 mm, 5 μm) and an AB gradient of 5–95 % for B at a flow rate of 1 mL/minute, where eluent A was 0.1 % formic acid/water and eluent B was 0.1 % formic acid/acetonitrile. Mass-directed preparative LCMS was carried out using a C18 column (Phenomenex Gemini, 50 x 21.2 mm, 5 μm) and a linear AB gradient of 5–95% for B at a flow rate of 20 mL/minute, where eluent A was 0.1% formic acid/water and eluent B was 0.1% formic acid/acetonitrile unless otherwise stated. Silica gel (60 Å, 40-63 μm, Fisher) was used for flash column chromatography. Routine analytical thin layer chromatography was performed on pre-coated plates (60F254, Machery-Nagel).

**Abbreviations**

br = broad; δ = chemical shift; d = doublet; DCM = dichloromethane; DIC = N, N'-diisopropylcarbodiimide; DIPEA = diisopropylethylamine; DMF = N,N-dimethylformamide; DMSO = dimethyl sulfoxide; ESI = electrospray ionisation; HATU = O-(7-Azabenzotriazol-1-yl)-N,N',N,N'-tetramethyluronium hexafluorophosphate; HCl = hydrochloric acid; HOBt = 1-hydroxybenzotriazole; Hz = Hertz; HRMS = high resolution mass spectrometry; IR= infrared; J = coupling constant; LCMS = liquid chromatography-mass spectrometry; M = molarity; m = multiplet; MS = mass spectrometry; m/z = mass to charge ratio; M⁺ = molecular ion; MHz = megahertz; NMP = N-Methyl-2-pyrrolidone; NMR = nuclear magnetic resonance; Pd₂(dba)₃ = Tris[dibenzyldeneacetone]dipalladium(0); Pd(Ph₃)₄ = Tetrakis(triphenylphosphine)palladium(0); PyBrop = bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; q = quartet; quant. = quantitative; Rf = retention factor; RT = room temperature; Rt = retention time; s = singlet; t = triplet; tert = tertiary; TFA = trifluoroacetic acid; THF = tetrahydrofuran; TLC = thin layer chromatography; TMS = tetramethylsilane.

**General syntheses of analogues**
1-[4-(4-tert-Butoxycarbonylamino-butylamino)-6-chloro-[1,3,5]triazin-2-yl]-piperidine-4-carboxylic acid ethyl ester

Amine (1.1 eq), ethyl 1-(4,6-dichloro-1,3,5-triazin-2-yl)piperidine-4-carboxylate (1 eq) and sodium carbonate were suspended in acetone and water (1:1; 10 mL) and stirred at 20 °C for 20 h. The solvents were removed in vacuo and the resulting slurry diluted with dichloromethane/water (1:1; 30 mL). The layers were shaken, separated and the aqueous layer further extracted with dichloromethane (2 x 15 mL). The organic layers were combined, washed with brine (15 mL) and the solvent removed in vacuo. The resulting residue was purified using flash column chromatography (eluent: ethyl acetate:iso-hexane; 40:60) to give the title compound as a white solid (360 mg, 96%).

$1^\text{H} \text{NMR (400 MHz; CDCl}_3\text{)}$: δ 6.53 (1H, t, $J = 5.9$ Hz), 4.77-4.43 (3H, m), 4.13 (2H, q, $J = 7.2$ Hz), 3.39 (2H, q, $J = 6.5$ Hz), 3.17-2.95 (4H, m), 2.61-2.48 (1H, m), 1.98-1.90 (2H, m), 1.72-1.47 (6H, m), 1.41 (9H, s), 1.24 (3H, t, $J = 7.2$ Hz).

**General Suzuki coupling procedure**

The nitro-halide (1 eq), boronic acid (1 eq), Pd$_2$(dba)$_3$ (1.5 mol%), Pd(PPh)$_3$$_4$ (3 mol%) and potassium fluoride (3 eq) were heated at reflux, in 1,4-dioxane, for 24 hours, under a nitrogen atmosphere. The resultant brown solution was filtered through Celite and the solvent removed in vacuo. Dichloromethane and water were added to the residue, the phases separated and the aqueous phase extracted with dichloromethane (3 x 15 mL). The combined organic extracts were washed with water (10 mL), brine (10 mL), dried (MgSO$_4$) and the solvent removed in vacuo. The crude product was purified by flash column chromatography using ethyl acetate:iso-hexane (10:90), affording the desired products.

4’-Methyl-3-nitro-biphenyl
The general method described above was used to give the title compound as a yellow oil (411 mg, 88%).

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 8.45 (1H, t, $J = 2.0$ Hz), 8.18 (1H, ddd, $J = 1.0$, 2.0 and 8.1 Hz), 7.92 (1H, m), 7.52 (1H, t, $J = 8.1$ Hz), 7.46 (2H, app d, $J = 8.1$ Hz), 7.31 (2H, app d, $J = 8.1$ Hz), 2.43 (3H, s).

3'-Methyl-3-nitro-biphenyl (5)

The general method described above was used to give the title compound as a yellow oil (370 mg, 79%).

$^1$H NMR (400 MHz; CDCl$_3$) $\delta$ 8.45 (1H, t, $J = 2.0$ Hz); 8.19 (1H, ddd, $J = 1$, 2 and 8 Hz); 7.91 (1H, ddd, $J = 1$, 2 and 8 Hz), 7.60 (1H, t, $J = 8$ Hz), 7.44 – 7.37 (3H, m), 7.24 (1H, m), 2.31 (3H, s). Reference. Bumagin, N. A.et al.; Bull. Russ. Acad. Sci. Div. Chem. Sci. (Engl. Transl.); English; 41; 11.2; 1992; 2128 - 2129

**General tolyl oxidation procedure**

The tolyl-nitrobenzene (1 eq) was suspended in pyridine (3 ml) and water (3 ml) and the heterogeneous mixture heated to reflux. Potassium permanganate (5 eq) was added in three portions and the resulting purple solution heated at reflux for 3 hours and then stirred at room temperature for 1 hour. The reaction mixture was filtered while still hot and the resultant brown (manganese) precipitate washed with hot water (20 ml). The yellow filtrate was acidified with concentrated hydrochloric acid, providing a precipitate which was collected by filtration, washed with hot water and dried in vacuo to afford the desired compound.

3'-Nitro-biphenyl-4-carboxylic acid

The general method described above was used to give the title compound as a pale yellow solid (230 mg, quant.).
1H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 8.51 (1H, m), 8.27 (1H, ddd, $J = 1.0, 2.5$ and 8.1 Hz), 8.22 (1H, m), 8.07 (2H, app d, $J = 8.6$ Hz), 7.93 (2H, app d, $J = 8.6$ Hz), 7.81 (1H, t, $J = 8.1$ Hz).

Reference. Hey; W.; *Journal of the Chemical Society*; 1948, 2213, 2216.
Berliner; E. *Journal of the American Chemical Society*; 75; 1953; 2417,2420

3’-Nitro-biphenyl-3-carboxylic acid (6)

The general method described above was used to give the title compound as a pale yellow solid (171 mg, 75 %); 2213,2216

$^1$H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 8.47 (1H, m), 8.27 (2H, m), 8.21 (1H, m), 8.04 (2H, m), 7.80 (1H, t, $J = 8.1$ Hz), 7.67 (1H, t, $J = 7.6$ Hz).

Reference. Hey; W.; *Journal of the Chemical Society*; 1948, 2213, 2216.

**General procedure for the synthesis of sulfonamides**

The amine (1 eq) was stirred with the corresponding aromatic sulfonyl chloride (1.1 to 2 eq) in pyridine (5 mL), under nitrogen, at 20 °C for 18 hours. After this time the pyridine was removed *in vacuo* and the resulting red / pink coloured solids partitioned between hydrochloric acid (1M aqueous solution, 20 mL) and ethyl acetate (20 mL). The phases were separated and the aqueous phase extracted with ethyl acetate (3 x 20 mL). The organic phases were combined and washed with water (25 mL), brine (saturated aqueous solution, 25 mL), dried over magnesium sulfate, filtered and the solvent removed *in vacuo* to typically afford a red / brown oily solid which was purified using flash column chromatography to afford the desired compounds.

3-(Benzo[1,2,5]oxadiazole-4-sulfonylamino)-thiophene-2-carboxylic acid methyl ester(12)
The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane; 40:60) to give the title compound as a yellow solid (190 mg, 43%).
LCMS: R<sub>t</sub> 4.27 min.; purity > 90 %; MS m/z = 338 [M – 1]<sup>+</sup>.

3-(1,2-Dimethyl-1H-imidazole-4-sulfonylamino)-thiophene-2-carboxylic acid methyl ester

The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane; 25:75 increasing to ethyl acetate) to give the title compound as an off-white solid (57 mg, 28%).
TLC (ethyl acetate): R<sub>f</sub> = 0.20; LCMS: R<sub>t</sub> 3.18 min.; purity > 95 %; MS m/z = 316 [M + 1]<sup>+</sup>.

3-(4-Acetylamino-benzenesulfonylamino)-thiophene-2-carboxylic acid methyl ester

The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane 10:90 increasing to 30:70) to give the title compound as a yellow solid (265 mg, 36%).
LCMS: R<sub>t</sub> 4.40 min.; purity > 95 %; MS m/z = 355 [M + 1]<sup>+</sup>.

3-(4-Nitro-benzenesulfonylamino)-thiophene-2-carboxylic acid methyl ester
The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane; 25:75) to give the title compound as a white solid (262 mg, 46%).

TLC (ethyl acetate:iso-hexane; 50:50 v/v): Rₖ = 0.7; ¹H NMR (400 MHz; CDCl₃): δ 9.78 (1H, s), 8.32 (2H, app d, J = 9.1 Hz), 8.04 (2H, app d, J = 9.1 Hz), 7.47 (1H, d, J = 5.6 Hz), 7.43 (1H, d, J = 5.6 Hz), 3.83 (3H, s).

3-(3-Nitro-benzenesulfonylamino)-thiophene-2-carboxylic acid methyl ester

\[
\text{H}_2\text{N} \quad \text{MeO}_2\text{C} \quad \text{S} \quad \text{O} \quad \text{Cl} \\
\text{MeO}_2\text{C} \quad \text{N} \quad \text{H} \quad \text{S} \quad \text{O} \quad \text{O}_2\text{N} \\
\]

The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane; 33:67) to give the title compound as a white solid (182 mg, 62%).

TLC (ethyl acetate:iso-hexane; 50:50 v/v): Rₖ = 0.7; ¹H NMR (400 MHz; CDCl₃): δ 9.79 (1H, s), 8.69 (1H, t, J = 2.0 Hz), 8.42 (1H, ddd, J = 8.1, 2.0 and 1.0 Hz), 8.19 (1H, ddd, J = 8.1, 2.0 and 1.0 Hz), 7.71 (1H, t, J = 8.1 Hz), 7.48 (1H, d, J = 5.6 Hz), 7.43 (1H, d, J = 5.6 Hz), 3.90 (3H, s).

3-(2-Nitro-benzenesulfonylamino)-thiophene-2-carboxylic acid methyl ester

\[
\text{H}_2\text{N} \quad \text{MeO}_2\text{C} \quad \text{S} \quad \text{O} \quad \text{Cl} \\
\text{MeO}_2\text{C} \quad \text{N} \quad \text{H} \quad \text{S} \quad \text{O} \quad \text{O}_2\text{N} \\
\]

The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane; 25:75) to give the title compound as a yellow solid (935 mg, 43%).

LCMS: R₄ 4.69 min.; purity > 95 %; MS m/z – 341 [M – 1].
The general procedure described above was used and the crude residue purified by flash column chromatography (eluent: ethyl acetate:iso-hexane; 20:80) to give the title compound as a yellow solid (398 mg, 75%).

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 10.86 (1H, br s), 8.26 (2H, d, $J = 9.1$ Hz), 8.02 (2H, d, $J = 9.1$ Hz), 7.95 (1H, dd, $J = 7.9$ and 1.8 Hz), 7.72 (1H, dd, $J = 7.9$ and 1.2 Hz), 7.50 (1H, td, $J = 7.9$ and 1.8 Hz), 7.11 (1H, td, $J = 7.9$ and 1.2 Hz), 4.33 (2H, q, $J = 7.2$ Hz), 1.36 (3H, t, $J = 7.2$ Hz).

LCMS: R$_t$ 2.85 min.; purity > 99 %; MS $m/z$ – 349 [M - 1].

3-(4-tert-Butoxycarbonylamino-benzenesulfonyl)-thiophene-2-carboxylic acid methyl ester

The general procedure described above was used to give the title compound as an orange solid (500 mg, quant.) which was used without further purification.

LCMS: R$_t$ 3.13 min.; purity > 75 %; MS $m/z$ – 411 [M - 1].

3-[Methyl-(4-nitro-benzenesulfonyl)-amino]-thiophene-2-carboxylic acid methyl ester
The sulfonamide ester (190 mg, 0.55 mmol, 1eq) was dissolved in methanol/dichloromethane (10 mL, 1:1). TMS-CH$_2$N$_2$ (550 µL of 2 M solution, 1.1 mmol, 2 eq) was added and the reaction mixture was stirred for 18 hours at room temperature. A further amount of TMS-CH$_2$N$_2$ (550 µL of 2 M solution, 1.1 mmol, 2 eq) was added and the reaction mixture was stirred for a further 18 hours at room temperature. A few drops of acetic acid (20% aqueous solution) were added to the reaction mixture and the solvents were removed under reduced pressure to yield the title compound as a white solid (191 mg, 98 %).

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 8.31-8.29 (2H, d, $J$ = 9.1 Hz), 7.88-7.86 (2H, d, $J$ = 9.1), 7.50-7.48 (1H, d, $J$ = 5.5), 7.13-7.11 (1H, d, $J$ = 5.5), 3.60 (3H, s), 3.32 (3H, s).

**General procedure for the solution-phase coupling of anilines**

HATU (1 eq) was added to a stirred solution of the aniline (1 eq), the acid (1 eq) and DIPEA (3 eq) in acetonitrile, under N$_2$. The reaction mixture was heated to reflux and stirred for 20 h. After this time the reaction solvent was removed in vacuo and the residue dissolved in ethyl acetate. The organic phase was washed with 2M hydrochloric acid, saturated aqueous NaHCO$_3$ solution, water, saturated aqueous brine, dried (MgSO$_4$), filtered and the solvent removed in vacuo to afford the desired products.

**1-[[3-(6-tert-Butoxycarbonylamino-hexanoylamino)-benzyl]piperidine-4-carboxylic acid ethyl ester**

The general method described above was used to give the title compound as an off-white solid (110 mg, 80%).

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 7.52 (1H, m), 7.44 (1H, m), 7.28 (1H, m), 7.06 (1H, m), 4.60 (1H, br s), 4.14 (3H, q, $J$ = 7 Hz), 3.49 (2H, m), 3.15 (2H, m), 2.87 (2H, m), 2.37 (2H, t, $J$ = 7 Hz), 2.30 (1H, m), 2.06 (2H, t, $J$ = 11 Hz), 1.91 – 1.73 (8H, m), 1.55 (2H, m), 1.46 – 1.40 (11H, m), 1.26 (3H, t, $J$ = 7 Hz).

**5-[[4-(6-tert-Butoxycarbonylamino-hexanoylamino)-phenyl]-2-methyl-furan-3-carboxylic acid ethyl ester**

The general method described above was used to give the title compound as a pale brown solid (267 mg, 95%).

$^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 7.57 (4H, m), 7.47 (1H, br s), 6.81 (1H, s), 4.59 (1H, br s), 4.30 (2H, q, $J = 7$ Hz), 3.12 (2H, m), 2.63 (3H, s), 2.36 (2H, t, $J = 8$ Hz), 1.75 (2H, m), 1.52 (2H, m), 1.43 (11H, m), 1.36 (3H, t, $J = 7$ Hz).

**General procedure for saponification of esters**

The ester was stirred with sodium hydroxide (typically 3 – 5 eq) in ethanol (20 mL) or lithium hydroxide (3 – 5 eq) in tetrahydrofuran/water (20 mL) for 20h at 20 ºC. The solvent was removed *in vacuo* and hydrochloric acid (25 mL, 1M, aqueous solution) added. The product was then either directly isolated by filtration or by extraction. For the extraction method, the acidic solution was extracted with ethyl acetate (3 x 25 mL). The combined organic extracts were washed with water (25 mL), brine (saturated aqueous solution, 25 mL), dried over magnesium sulfate, filtered and the solvent removed *in vacuo* to afford the desired products.

3-(Benzo[1,2,5]oxadiazole-4-sulfonyl amino)-thiophene-2-carboxylic acid

The general method described above was used to give the title compound as a pale orange solid (119 mg, 65%).

TLC (ethyl acetate): $R_f$ = 0.09; LCMS: $R_t$ 4.00 min.; purity > 95 %; MS $m/z$ – 324 [M - 1]$^*$.  

3-(3-Nitro-benzenesulfonylamino)-thiophene-2-carboxylic acid
The general method described above was used to give the title compound as a yellow solid (194 mg, 83%).

TLC (ethyl acetate): $R_f = 0.1$; $^1$H NMR (400 MHz; $(CD_3)_2$SO): $\delta$ 8.61 (1H, t, $J = 2.3$ Hz), 8.49 (1H, ddd, $J = 8.2$, 2.3 and 0.9 Hz), 8.28 – 8.26 (1H, m), 7.87 (1H, t, $J = 8.2$ Hz), 7.81 (1H, t, $J = 8.2$ Hz), 7.81 (1H, d, $J = 5.5$ Hz), 7.18 (1H, d, $J = 5.5$ Hz).

3-(4-Nitro-benzenesulfonylamino)-thiophene-2-carboxylic acid

The general method described above was used to give the title compound as a yellow solid (251 mg, 97%).

TLC (ethyl acetate): $R_f = 0.1$; $^1$H NMR (400 MHz; $(CD_3)_2$SO): $\delta$ 8.38 (2H, app d, $J = 9.1$ Hz), 8.13 (2H, app d, $J = 9.1$ Hz), 7.83 (1H, d, $J = 5.6$ Hz), 7.18 (1H, d, $J = 5.6$ Hz).

3-(2-Nitro-benzenesulfonylamino)-thiophene-2-carboxylic acid

The general method described above was used to give the title compound as an off-white solid (885 mg, 99%).

TLC (ethyl acetate): $R_f = \text{baseline}$; $^1$H NMR (400 MHz; $(CD_3)_2$SO): $\delta$ 8.21 (1H, dd, $J = 1.5$ and 7.9 Hz), 8.09 (1H, dd, $J = 1.5$ and 7.9 Hz), 7.96-7.86 (2H, m), 7.87 (1H, d, $J = 5.6$ Hz), 7.36 (1H, d, $J = 5.6$ Hz).

2-(4-Nitro-benzenesulfonylamino)-thiazole-4-carboxylic acid

The general method described above was used to give the title compound as a yellow solid (145 mg, 44%).
$^1$H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 8.36-8.32 (2H, m), 8.04-8.01 (2H, m), 7.65 (1H, s); LCMS: $R_t$ 2.20 min.; MS $m/z$ – 330 [M + 1]$^+$.  

3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carboxylic acid (13)

The general method described above was used to give the title compound as a yellow solid (71 mg, 74%). TLC (ethyl acetate): $R_f$ = baseline; LCMS: $R_t$ 3.72 min.; purity > 70%; MS $m/z$ – 340 [M-1]$^-$.  

1-[3-(6-tert-Butoxycarbonylamino-hexanoylamino)-benzyl]piperidine-4-carboxylic acid

The general method described above was used to give the title compound as an off-white solid (45 mg, 50%). $^1$H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 10.11 (1H, s), 7.85 1H, s), 7.54 (1H, d, $J$ = 8 Hz), 7.36 (1H, t, $J$ = 8 Hz), 7.27 (1H, d, $J$ = 8 Hz), 6.81 (1H, br s), 4.20 (2H, m), 2.90 (4H, m), 2.31 (2H, m), 2.01 (2H, m), 1.83 (2H, m), 1.58 (2H, m), 1.36 – 1.23 (16 H, m).  

5-[4-(6-tert-Butoxycarbonylamino-hexanoylamino)-phenyl]-2-methyl-furan-3-carboxylic acid

The general method described above was used to give the title compound as an off-white solid (85 mg, 36%). $^1$H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 7.60 (4H, m), 6.87 (1H, s), 3.04 (2H, t, $J$ = 7 Hz), 2.62 (3H, s), 2.38 (2H, t, $J$ = 7 Hz), 1.72 (2H, m), 1.52 (2H, m), 1.43 (11H, m).
3-(1,2-Dimethyl-1H-imidazole-4-sulfonyl amino)-thiophene-2-carboxylic acid

![Chemical structure](image)

The general method described above was used to give the title compound as a white solid (18.5 mg, 34%). TLC (ethyl acetate:iso-hexane; 50:50 v/v): R_f = baseline; LCMS: R_t 4.46 min.; purity > 95%; MS m/z – 302 [M + 1]⁺.

3-(4-amino-benzenesulfonylamino)-thiophene-2-carboxylic acid

![Chemical structure](image)

The general method described above was used to give the title compound as a white solid (610 mg, 72%). LCMS: R_t 2.05 min.; purity > 95%; MS m/z – 297 [M - 1]⁻.

2-(4-Nitro-benzenesulfonylamino)-benzoic acid

![Chemical structure](image)

The general method described above was used to give the title compound as a yellow solid (267 mg, 77%). ¹H NMR (400 MHz; (CD₃)₂SO): δ 11.35 (1H, br s), 8.35 (2H, d, J = 9.1 Hz), 8.08 (2H, d, J = 9.1 Hz), 7.90 (1H, dd, J = 7.9, 1.5, Hz), 7.57 (1H, ddd, J = 8.4, 7.3 and 1.2 Hz), 7.49 (1H, dd, J = 8.2 and 1.2 Hz), 7.17 (1H, ddd, J = 7.9, 7.3 and 1.2 Hz); LCMS: R_t 3.03 min.; purity > 99%; MS m/z – 321 [M - 1]⁻.

1-[4-(4-tert-Butoxycarbonylamino-butylamino)-6-chloro-[1,3,5]triazin-2-yl]-piperidine-4-carboxylic acid
The general method described above was used to give the title compound as a pale beige solid (222 mg, 88%). $^1$H NMR (400 MHz; CDCl$_3$): $\delta$ 4.80-4.50 (3H, m), 3.41-3.32 (2H, m), 3.19-3.04 (4H, m), 2.66-2.57 (1H, m), 2.03-1.98 (2H, m), 1.77-1.51 (6H, m), 1.43 (9H, s); LCMS: Rt 6.09 min.; purity > 95%; MS m/z – 430 [M + 1]$^+$. 

3-[Methyl-(4-nitro-benzenesulfonyl)-amino]-thiophene-2-carboxylic acid

The general method described above was used to give the title compound as a pale beige solid (130 mg, 71%). $^1$H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 13.01 (1H, br s), 8.39 (2H, d, $J = 8.9$ Hz), 7.88 (2H, d, $J = 8.9$ Hz), 7.83 (1H, d, $J = 5.3$ Hz), 6.99 (1H, d, $J = 5.3$, Hz), 3.22 (3H, s). 

3-(2,4-Difluoro-benzenesulfonylamino)-thiophene-2-carboxylic acid

The general method described above was used to give the title compound as an off-white solid (50 mg, 52 %). TLC (ethyl acetate): $R_f$ = baseline; LCMS: $R_t$ 4.05 min.; purity > 82 %; MS m/z – 318 [M-1]$^+$. 

3-(4-tert-Butoxycarbonylamino-benzenesulfonyl)-thiophene-2-carboxylic acid
The general procedure described above was used to give the title compound as a beige solid (225 mg, 53%). $^1$H NMR (400 MHz; (CD$_3$)$_2$SO): $\delta$ 9.22 (1H, br s), 7.78 (1H, d, $J$ = 5.9 Hz), 7.28-7.25 (3H, m), 6.94 (2H, d, $J$ = 9.0 Hz), 1.41 (9H, s).

3-(4-Acetylamino-benzenesulfonylamino)-thiophene-2-carboxylic acid

The sulfonamide (0.44 mmol, 1 eq) was dissolved in pyridine (5 mL), under nitrogen, and acetyl chloride (0.48 mmol, 1.1 eq) was added as a single portion. The reaction mixture was stirred at 20 °C for 30 minutes after which time a further portion of acetyl chloride (0.48 mmol, 1.1 eq) was added. After 10 minutes stirring, water (200 $\mu$L) was added and the reaction solvents removed in vacuo. The resulting gummy oil was then purified by flash column chromatography (eluent: ethyl acetate increasing to ethyl acetate methanol; 80:20) to afford the desired product as a cream solid (143 mg, 95%). TLC (ethyl acetate:methanol, 80:20 v/v): $R_f$ 0.11; $^1$H NMR (400 MHz, (CD$_3$)$_2$SO): $\delta$ 10.29 (1H, s), 7.71 – 7.66 (4H, m), 7.51 (2H, d, $J$ = 5.4 Hz), 7.11 (2H, d, $J$ = 5.4 Hz), 2.05 (3H, s); LCMS: $R_t$ 3.28 min.; purity > 95%; MS $m/z$ – 339 [M - 1].

Synthesis of polymer supported thiophene-hydroxybenzotriazole active ester
Polymer supported hydroxybenzotriazole resin (0.9 mmol/g, 1 eq) was swollen in dichloromethane (2 mL) for 20 minutes at room temperature. 4-Dimethylaminopyridine (1.2 eq) was added followed by the acid (1.25 eq) dissolved in N, N'-dimethylformamide (1.5 mL). The resin was briefly agitated before addition of N, N'-diisopropylcarbodiimide (4.5 eq). The reaction mixture was then agitated for 3 hours at 20 °C. The orange resin was filtered, washed with dichloromethane (3 x 10 mL), N, N'-dimethylformamide (3 x 10 mL), a further portion of dichloromethane (4 x 10 mL) and dried under a stream of air. IR: 1649 cm⁻¹.

(S)-Methyl-((2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl-5-guanidino)-2-((3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl)-amino)) –pentanoate

The polymer supported thiophene (1 eq) was swollen in dichloromethane. The amine (0.75 eq) and N, N-diisopropylethylamine (2 eq) were dissolved in dichloromethane with the minimum amount of N, N-dimethylformamide and added to the pre-swollen resin. The reaction mixture was agitated at room temperature for 3 hours after which time the reaction mixture was filtered. The spent resin was washed with dichloromethane (3 x 5 mL) and N, N-dimethylformamide (3 x 1 mL) and the filtrate concentrated in vacuo. The residue was purified using flash column chromatography (eluent: ethyl acetate) to give the title compound as a yellow solid (28 mg, 34 %). LCMS: Rₚ, 6.68 min.; purity > 95 %; MS m/z – 750 [M-1]⁻.

General procedure for PyBroP coupling

Acid (1 eq) and bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (1.1 eq) were suspended in dichloromethane (5 mL) and the mixture was stirred at 20 °C for 10 minutes. N, N-Diisopropylethylamine (7 eq) was added to the mixture and stirred for a further 10 minutes. Protected amine (1.1 eq) was added and the reaction mixture was then stirred at 20 °C for 16 hours. After this time the solvent was removed in vacuo and the residue partitioned between hydrochloric acid (1M aqueous solution, 20 mL) and ethyl acetate (20 mL). The
phases were separated and the aqueous phase extracted with ethyl acetate (3 x 20 mL). The organic phases were combined and washed with brine (saturated, aqueous solution, 25 mL), dried over magnesium sulfate, filtered and the solvent removed \textit{in vacuo} to afford the desired products.

\[(S)-(\text{Thiophene-2-carbonyl-amino})-5-(2,2,4,6,7\text{-pentamethyldihydro-benzofuran-5-sulfonyl-guanidino})\text{-pentanoic acid tert-butyl ester}\]

The general method described above was used and the residue purified by column chromatography on silica gel (eluent: ethyl acetate:iso-hexane; 50:50 increasing to ethyl acetate) to give the title compound as a yellow solid (67 mg, 38 \%). TLC (ethyl acetate): \(R_f\) 0.59; LCMS: \(R_f\) 7.0 min.; purity > 95 \%; MS \(m/z\) – 593 [M + 1]\(^+\).

\[(S)-6\text{-tert-Butoxycarbonylamino-2-\{3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl-amino\}-hexanoic acid tert-butyl ester}\]

The general method described above was used and the residue purified using flash column chromatography on silica gel (eluent: ethyl acetate:iso-hexane; 10:90 increasing to 30:70) to give the title compound as a yellow solid (109 mg, 59 \%). TLC (ethyl acetate:iso-hexane; 50:50 v/v): \(R_f\) 0.50; LCMS: \(R_f\) 5.63 min.; purity > 95 \%; MS \(m/z\) – 611 [M - 1]\(^-\).
(S)-5-$N^G$-nitro-guanidino-2-[[3-(4-nitro-benzenesulfonlamino)-thiophene-2-carbonyl]-amino]-pentanoic acid methyl ester

The general method described above was used and the residue purified by (mass-directed) preparative LCMS to give the title compound as a white solid (9.6 mg, 3%). LCMS: $R_t$ 6.53 min.; purity > 95 %; MS $m/z$ – 542 [M - 1].

5-tert-butyl ester, 1-methyl ester ((S)-2-[[3-(4-Nitro-benzenesulfonlamino)-thiophene-2-carbonyl]-amino])-pentanedioic acid

The general method described above was used and the residue purified using flash column chromatography on silica gel (eluent: ethyl acetate:iso-hexane; 20:80 increasing to 40:60) to give the title compound as a yellow solid (93 mg, 59 %). TLC (ethyl acetate:iso-hexane; 50:50 v/v). $R_f$ = 0.26; $^1$H-NMR (400 MHz, (CD$_3$)$_2$SO): $\delta$ 8.32 (2H, d, $J$ = 8.9 Hz), 8.01 (2H, d, $J$ = 8.9 Hz), 7.76 – 7.72 (1H, m), 7.14 (2H, d, $J$ = 7.14 Hz), 4.40 – 4.36 (1H, m), 3.73 (3H, s), 2.25 – 2.22 (2H, m), 2.02 – 2.00 (1H, m), 1.89 – 1.83 (1H, m), 1.35 (9H, s); LCMS: $R_t$ 5.63 min.; purity > 95 %; MS $m/z$ – 526 [M - 1].
(S)-5-tert-butoxycarbonyl-guanidino-2-[[3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-oxo-pentanoic acid methyl ester

The general method above was used and the residue purified using flash column chromatography on silica gel (eluent: ethyl acetate:iso-hexane; 25:75 increasing to 75:25) to give the title compound as a yellow solid (21 mg, 38 %). TLC (ethyl acetate): Rf 0.36; LCMS: Rf 2.91 min.; purity > 80 %; MS m/z – 613 [M + 1]+.

1-methyl ((S)-2-[[3-(4-Nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino])-pentanedioic acid 5-tert-butyl ester, 1-methyl ester ((S)-2-[[3-(4-Nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino])-pentanedioic acid (93 mg, 0.18 mmol) was stirred with hydrochloric acid (4M solution in 1,4-dioxane) for 18 hours at room temperature. After this time the solvent was removed in vacuo to afford a yellow foam (85 mg, quant.) which was used without
further purification. TLC (methanol:ethyl acetate; 20:80 v/v) R\text{f} = 0.01; LCMS: R\text{t} 2.91 min.; purity > 95%; MS m/z - 470 [M - 1].

**4-tert-Butoxycarbonylamino-butylamino)-acetic acid ethyl ester**

\[
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\text{O} \\
\text{N} \\
\text{H}_2
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\text{O} \\
\text{O}
\end{array} \rightarrow \begin{array}{c}
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The protected diamine (1 eq), potassium iodide (1 eq) and triethylamine (2.6 eq) were combined in dichloromethane (20 mL) under nitrogen. Ethyl bromoacetate (1 eq) was added drop-wise. The reaction mixture was stirred at 40 °C for 24 hours and then the solvent was removed \textit{in vacuo}. The residue was taken up in water (20 mL) and extracted with methyl tert-butyl ether (1x 20 mL and 2 x 10 mL). The combined organic phases were washed with brine (saturated, aqueous solution, 10 mL) dried over magnesium sulfate and the solvent removed \textit{in vacuo} to afford a brown oil which was purified using flash column chromatography (eluent: iso-hexane:ethyl acetate; 9:1) to afford the desired compound as a pale yellow oil (427 mg, 28%). TLC (ethyl acetate): R\text{f} = 0.2; LCMS: R\text{t} 1.41 min; purity >95%; MS m/z - 275 [M + 1].

[[3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carbonyl]-(4-tert-butoxycarbonyl amino-butyl)-amino]-acetic acid ethyl ester

\[
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The acid (1eq) and (2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (1 eq) were stirred in N, N-dimethylformamide (5 mL) at 20 °C for 10 minutes, N, N-diisopropylethylamine (3 eq) was added and the reaction mixture was stirred for a further 5 minutes. The secondary amine (1.2 eq) was added to the reaction mixture and
stirred at 40 °C to 80 °C for 16 hours. The reaction mixture was partitioned between brine (saturated, aqueous solution, 25 mL) and ethyl acetate (25 mL). The phases were separated and the organic phase was washed with brine (saturated, aqueous solution, 4 x 25 mL) and hydrochloric acid (1M aqueous solution, 25 mL). The hydrochloric acid was back extracted with ethyl acetate (25 mL) and the organic phases were combined and washed with brine (saturated, aqueous solution, 25 mL), dried over magnesium sulfate, filtered and the solvent removed in vacuo to afford the desired products. The crude residue was purified using flash column chromatography (eluent: iso-hexane/ethyl acetate, 100:0 increasing to 30:70) to afford the desired compound as a pale yellow gum (150 mg, 43%). TLC (iso-hexane:ethyl acetate, 1:1 v/v): Rf 0.38; LCMS: Rt 3.25 min, purity > 95%; MS m/z -620 [M + Na]+, 598 [M + 1]+, 498 [M-Boc]+.

(S)-5-guanidino-2-[[3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-oxo-pentanoic acid (HCl salt) (51)

Methyl-(S)-5-tert-butoxycarbonyl-guanidino-2-[[3-(4-nitro-benzene sulfonylamino)-thiophene-2-carbonyl]-amino]-5-oxo-pentanoate (10 mg, 0.016 mmol) was stirred with hydrochloric acid (2M solution, water:tetrahydrofuran, 1:1) at 80 °C for 4 hours. After this time the reaction solvent was removed in vacuo and the crude residue was purified by elution through a 2 g C-18 column (eluent: water, followed by acetonitrile:water (20:80) to afford the desired compound as a yellow solid (2.2 mg, 28%). 1H NMR (400MHz, (CD3)2SO/D2O): 8.34 (2H, d, J = 8.8 Hz), 8.03 (2H, d, J = 8.8 Hz), 7.70 (1H, d, J = 5.6 Hz), 7.12 (1H, d, J = 5.6 Hz), 4.31 (1H, dd, J = 9.8 Hz and 5.0 Hz), 3.58 (2H, t, J = 6.6 Hz), 2.10 – 2.10 (1H, m), 1.98 – 1.89 (1H, m); 13C NMR (600MHz, d6-DMSO): δ 174.5, 172.8, 163.6, 154.4, 150.6,
144.3, 140.0, 131.0, 128.8, 125.2, 121.7, 117.0, 51.7, 33.2, 25.5; LCMS: Rf 4.3 min.; purity > 95%; MS m/z = 499.3 [M + 1]⁺; HRMS (m/z): [M]⁺ calcd. for C₁₇H₁₉N₆O₈S₂, 499.0706; found, 499.0705.

(S)-5-N⁶-nitro-guanidino-2-[[3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-pentanoic acid (HCl salt) (53)

\[
\text{HCl}
\]

The ester was stirred with hydrochloric acid (2M solution, water:tetrahydrofuran; 1:1) at 80 °C for 4 hours. After this time the reaction solvent was removed in vacuo to afford the desired compound. The crude residue was purified by elution through a 2 g C-18 column (eluent: water, followed by acetonitrile:water; 20:80) to afford the desired compound as a yellow solid (7 mg, 77%). LCMS: Rf 5.4 min.; purity > 95%; MS m/z = 530.2 [M + 1]⁺.

[[3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carbonyl]-(4-tert-butoxycarbonylamino-butyl)-amino]-acetic acid

The starting ester (20 mg, 0.027 mmol) was stirred with sodium hydroxide (5 mg, 5 eq) in tetrahydrofuran: water (4:1; 125 μL) at 50 °C for 5 hours. The solvent was removed in vacuo and the residue treated with 2 N hydrochloric acid. A precipitate formed, which was
collected by filtration to give the title compound as a yellow solid (13 mg, 68 %). The material was used crude without further purification or analysis.

**General procedure for the formation of bis-(tert-butoxycarbonyl) guanidines in solution**

The amine (1 eq) was stirred with \( N, N \)-diisopropylethylamine (10 eq) in \( N, N \)-dimethylformamide (2 mL) at 20 °C. \( N, N \)-bis-Boc-1H-pyrazole-1-carboxamidine (5 eq) was then added in one portion and the reaction mixture stirred for a further one hour at room temperature. After this time the solvent was removed *in vacuo* and the residue was purified using flash column chromatography.

\[(S)-6-\text{N, N'}-\text{di-tert-butoxyl- guanidino-2-}\{3-(4-nitro-benzenesulfonyl amino)-thiophene-2-carbonyl\}-\text{amino}\}-\text{hexanoic acid}\]

![Chemical structure](image)

The general method described above was used to give the title compound as a yellow solid (33 mg, 47 %). TLC (methanol:ethyl acetate; 20:80 v/v): \( R_f \) 0.12; LCMS: \( R_t \) 8.2 min.; purity > 95 %; MS \( m/z \) - 699 \([M + 1]^+\).

\{[3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carbonyl]-[4-(N',N''-di-tert-butoxycarbonyl-guanidino)-butyl]-amino\}-acetic acid ethyl ester
The general method described above was used to give the title compound as a colourless oil (16.4 mg, 58%). TLC (iso-hexane:ethyl acetate; 50:50 v/v): Rₖ 0.39; LCMS: Rₖ 9.0 min, purity >95%, MS m/z – 740 [M + 1]+.

(S)-6-Amino-2-[[3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-hexanoic acid (47)

The nitro-phenyl sulfonamide was stirred with hydrochloric acid (4M solution in 1,4-dioxane) for 18 hours at 20 °C. After this time the solvent was removed in vacuo to afford a yellow solid (49 mg, quant.) which was used without further purification. LCMS: Rₖ 2.91 min.; purity > 95 %; MS m/z – 457 [M + 1]+.

General procedure for the removal of tert-butoxy-carbonyl or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl groups using trifluoroacetic acid / dichloromethane

The protected amine was stirred in trifluoroacetic acid: dichloromethane (2.5 mL, 1:1) for 4 hours at 20 °C. After this time the solvent was removed in vacuo.
(S)-2-[[3-(4-Amino-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-6-guanidino-hexanoic acid (TFA salt) (50)

The general method above was used. The crude residue purified by elution through a 2 g C-18 column (eluent: water, followed by acetonitrile:water; 20:80) to afford the desired compound as a white solid (5.6 mg, 96%). LCMS: Rt 3.9 min.; purity > 95 %; MS m/z – 469 [M + 1]+.

[[3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carbonyl]-(4-guanidinobutyl)-amino]-acetic acid (TFA salt) (55)

The general method above was used. The crude residue was triturated in diethyl ether to give a white solid (6.4 mg, 68%). LCMS: Rt 4.0 min.; purity > 90 %; MS m/z – 512 [M + 1]+.

(S)-Methyl-(5-Guanidino-2-((3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl)-amino))-pentanoate (TFA salt) (S13)
The general method above was used. The crude residue purified by elution through a 2 g C-18 column (eluent: water, followed by acetonitrile:water; 20:80) to afford the desired compound as a yellow solid (13.6 mg, 64%). LCMS: R, 6.9 min.; purity > 94 %; MS \( m/z = 499 \) [M + 1].

**General procedure for the removal of tert-butyl, tert-butoxycarbonyl or 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl groups using trifluoroacetic acid**

The protected compound was dissolved in trifluoroacetic acid / triisopropylsilane / water (10 mL, 95:2.5:2.5) and the reaction mixture was stirred at 20 °C for 16-48 hours. The solvents were removed *in vacuo* and the compound was purified by preparative LCMS.

(S)-5-Guanidino-2-[(thiophene-2-carbonyl)-amino]-pentanoic acid (33)

The general procedure described above was used to give the title compound as a white solid (21 mg, 70 %). LCMS: R, 0.5 min.; purity > 95 %; MS \( m/z = 285 \) [M + 1].

(S)-2-[[3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid methyl ester (48)
Amberlyst 15 (Aldrich, 300 mg) was soaked in methanol for 24 hours. The Amberlyst was then filtered and neutralized with 4M NH₃ in methanol. The resin was then reactivated by washing it with 3M hydrochloric acid in methanol:water (1:1), methanol, tetrahydrofuran and dichloromethane successively. The resin, prepared in this way, was added to a suspension of the acid (82 μmol, 1eq) in methanol (5 mL). As soon as the resin was added the solution became clear. The solution was stirred at 45 °C for 2 hours and then filtered. The resin was washed several times with 4M NH₃ in MeOH (solution turned yellow). The filtered solution was evaporated in vacuo to give the title compound as a dark yellow solid (34mg, 81%).

LCMS: R_t 3.31 min.; purity > 95 %; MS m/z 512 [M + 1]^+. 

3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carboxylic acid ((S)-4 guanidino-1-hydroxymethyl-butyl)-amide (54) 

Methyl ester (66.5 μmol, 1 eq) was suspended in dry tetrahydrofuran (4 mL). N, N-Dimethylformamide (0.5 mL) was added to aid the dissolution of the starting material, but the solution remained cloudy. This mixture was cooled at -10 °C and lithium dimethylamino borohydride (1.0M in THF, 80 μmol, 1.2 eq) was added to the solution and stirring continued for 2 hours. Then the mixture was allowed to reach 20 °C and stirring continued for 16 hours. The reaction was quenched with water (190 μl) and the solvent removed in vacuo. The crude
material was purified by preparative LCMS to obtain the title compound as a yellow solid (3.7 mg, 10%). LCMS: R; 3.93 min.; purity > 98 %; MS m/z 484.2 [M + 1]+.

**Procedure for coupling of Fmoc-Arg(Me2)-OH to 2-Chlorotrityl chloride resin**

2-Chlorotrityl chloride resin (commercially available, 100 mg, 140 µmol) was swollen with dichloromethane (2 mL) for 30 minutes, the dichloromethane was then removed. Fmoc-Arg(Me2)-OH.HCl (89 mg, 210 µmol, 1.5 eq) in dichloromethane (2 mL) and N, N-diisopropylethylamine (146 µL, 840 µmol, 6 eq) were added to the resin and the reaction mixture was agitated for 2 hours. The reagents were removed from the resin and the resin was washed with dichloromethane (10 mL), methanol (10 mL), N, N,dimethylformamide (10 mL), dichloromethane (10 mL), and diethyl ether (20 mL). The resin was dried under vacuum and infra red spectroscopy was carried out. IR: 1709 and 1628 cm⁻¹.

**General procedure for removal of the resin-bound N-terminal Fmoc group**

The Fmoc-Arg-Wang resin (commercially available) or Fmoc-Arg(Me)2-Clt resin was swollen with N, N-dimethylformamide (2 mL) for 30 minutes. The N, N-dimethylformamide was removed, piperidine in N, N-dimethylformamide (2 mL, 1:5) was added and the resin agitated for 5 minutes. The solvent was removed, and further piperidine in N, N-dimethylformamide (2 mL, 1:5) was added and agitated for a further 15 minutes. The solvents were removed and the resin was washed with dichloromethane (5 mL), methanol (5 mL), and N, N-dimethylformamide (5 mL). A Kaiser test was performed and, if positive (i.e., free amine present), the resin was deemed suitable for further transformation.

**Procedure for coupling to resin-bound Arg(Me2)-2-chlorotrityl chloride resin**

The scaffold (3 eq), 1-hydroxybenzotriazole hydrate (3 eq) and N, N'-diisopropylcarbodiimide (3 eq) were dissolved in N, N-dimethylformamide (2 mL) and added to the resin. The reaction mixture was agitated for 3 hours at room temperature. The reagents were removed from the resin and the resin was washed with dichloromethane (5 mL),
methanol (5 mL), and \(N, N\)-dimethylformamide (5 mL). A Kaiser test was performed and was negative (i.e., no free amine present), therefore the resin was deemed suitable for further transformation.

**General procedure for coupling to resin-bound Arg-Wang or 4-(Di-Boc-guanidino)-Phe-Wang**

**Method A:** (HOBt, DIC)
The scaffold (3 eq), 1-hydroxybenzotriazole hydrate (3 eq) and \(N, N'\)-diisopropylcarbodiimide (3 eq) were dissolved in \(N, N\)-dimethylformamide (2 mL) and added to the resin. The reaction mixture was agitated for 3-18 hours at room temperature. The reagents were removed from the resin and the resin was washed with dichloromethane (5 mL), methanol (5 mL), and \(N, N\)-dimethylformamide (5 mL). A Kaiser test was performed and, if negative (i.e., no free amine present), the resin was deemed suitable for further transformation.

**Method B:** (PyBrop, DIPEA)
The scaffold (3 eq), bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (4 eq) were dissolved in \(N, N\)-dimethylformamide (2 mL) and added to the resin. \(N, N\)-diisopropylethylamine (9 eq) was added and the reaction mixture was agitated for 3-18 hours at room temperature. The reagents were removed from the resin and the resin was washed with dichloromethane (5 mL), methanol (5 mL), and \(N, N\)-dimethylformamide (5 mL). A Kaiser test was performed and, if negative (i.e., no free amine present), the resin was deemed suitable for further transformation.

**Method C:** (PyBroP, DIPEA, DCM, NMP)
The scaffold (3 eq) and bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (4 eq) were dissolved in dichloromethane (1.9 mL) and \(N\)-Methyl-2-pyrrolidone (0.1 mL). \(N, N\)-Diisopropylethylamine (9 eq) was added and the mixture was stirred for 10 minutes. The mixture was added to the resin (100 mg, 65 \(\mu\)mol) and agitated for 18 hours at room temperature. The reagents were removed from the resin and the resin was washed with dichloromethane (5 mL), methanol (5 mL), and \(N, N\)-dimethylformamide (5 mL). A Kaiser test was performed and, if negative (i.e., no free amine present), the resin was deemed suitable for further transformation.

**General procedure for the reduction of resin-bound aromatic nitro compounds**
The nitro-containing resin (65 µmol) was swollen with \( N, N \)-dimethylformamide (2 mL) for 30 minutes. The \( N, N \)-dimethylformamide was removed, tin chloride dihydrate (20 eq) was added to the resin in \( N, N \)-dimethylformamide (2 mL) and the reaction mixture was agitated for 3 hours followed by 18 hours, with fresh reagents at 20 °C. The reagents were removed and the resin was washed with \( N, N \)-dimethylformamide (5 mL), 20% pyridine in \( N, N \)-dimethylformamide (5 mL), dichloromethane (5 mL), methanol (5 mL) and \( N, N \)-dimethylformamide (5 mL). A chloranil test for anilines was carried out and, if positive (i.e., free aniline present), the resin was deemed suitable for further transformations.

**General Procedure for coupling of acids to resin bound aniline compounds**

The aniline resin (1 eq) was swollen in the minimum quantity of dichloromethane for 20 minutes, meanwhile the acid (4 eq), bromotripyrrolidinophosphonium hexafluorophosphate (4.8 eq) and 2,6-lutidine (15 eq) were stirred at 20 °C and added to the pre-swollen resin. The reaction mixture was then agitated at room temperature for 24 hours to effect coupling and then washed with \( N, N \)-dimethylformamide (3 x 10 ml), \( N, N \)-dimethylformamide: \( N, N \)-diisopropylethylamine (1:1, 3 x 10 ml), further \( N, N \)-dimethylformamide (3 x 10 ml), dichloromethane (3 x 10 ml), methanol (3 x 10 ml) and Et₂O (3 x 10 ml). A chloranil test for anilines was carried out and, if negative (i.e., no free aniline present), the resin was deemed suitable for cleavage.

**General procedure for reductive aminations on the solid phase**

The resin (100 mg, 65 µmol) was washed with dichloromethane (2 x 5 mL). The aldehyde (10 eq) was added to the washed resin in a solution of acetic acid in 1,2-dichloroethane (2 mL, 2:98), the reaction mixture was agitated for 3 hours at 20 °C. Sodium triacetoxyborohydride (20 eq) was added and the reaction mixture was agitated for 2 days at 20 °C. The reagents were removed and the resin was washed with dichloromethane (2 x 5 mL), methanol, (2 x 5 mL), \( N, N \)-dimethylformamide (2 x 5 mL), and dichloromethane (2 x 5 mL).
General procedure for cleaving from resin
The resin (approx. 100 mg) was washed thoroughly with dichloromethane (3 x 5 mL) and
dried with nitrogen, then a solution of trifluoroacetic acid (1.9 mL), triisopropyl silane (50
µL) and water (50 µL) was added and the cleavage mixture was agitated for 90 minutes. The
cleavage mixture was removed and the resin was further washed with dichloromethane (1
mL). The cleavage/dichloromethane mixtures were combined, further agitated for 90 minutes
and added drop-wise to cold diethyl ether (30 mL, -78 °C). Typically, a white solid
precipitated and was pelleted by centrifugation, the diethyl ether was decanted and another
portion of cold diethyl ether (20 mL) was added, thoroughly mixed, centrifuged, and
decanted. This process was repeated once more. The crude compound was dried under
vacuum and purified either by elution through a 2 g C-18 column (eluent: acetonitrile/water)
or by (mass-directed) preparative LCMS. The purified peptides were then lyophilized (-54
°C, 0.08 mbar) to give the purified final products.

(S)-2-{{3’-(6-Amino-hexanoylamino)-biphenyl-3-carbonyl}-amino}-5-guanidino-
pentanoic acid (TFA salt) (9)

The general procedure described above was used and the crude compound was purified by
elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a
white solid (5.0 mg). LCMS: R_t 7.67 min.; purity > 90%; MS m/z – 484 [M + 1]^+.

(S)-2-{{3’-(4-Aminomethyl-cyclohexanecarbonyl)-amino}-biphenyl-4-carbonyl}-
amino)-5-guanidino-pentanoic acid (TFA salt) (25)
The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (10.5 mg). LCMS: R_t 3.85 min.; purity > 95%; MS m/z = 252 [M + 2]^+.

(S)-2-\{5-[4-(6-Amino-hexanoylamino)-phenyl]-2-methyl-furan-3-carbonyl\}-amino)-5-guanidino-pentanoic acid (TFA salt) (26)

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (15.9 mg). LCMS: R_t 2.19 min.; purity > 90%; MS m/z = 487 [M + 1]^+.

(S)-2-\{1-[3-(6-Amino-hexanoylamino)-benzyl]-piperidine-4-carbonyl\}-amino)-5-guanidino-pentanoic acid (TFA salt) (27)
The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (10.5 mg). LCMS: R<sub>t</sub> 3.85 min.; purity > 95%; MS <i>m/z</i> 252 [M + 2]<sup>+</sup>.

(S)-2-({1-[4-(4-Amino-butylamino)-6-hydroxy-[1,3,5]triazin-2-yl]-piperidine-4-carbonyl}-amino)-5-guanidino-pentanoic acid (TFA salt) (28)

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (10.2 mg). LCMS: R<sub>t</sub> 3.88 min.; purity > 92 %; MS <i>m/z</i> 467 [M + 1]<sup>+</sup>.

(S)-5-Guanidino-2-[2-(piperidine-4-sulfonyl)-benzoylamino]-pentanoic acid (TFA salt) (29)
The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (17.4 mg).

LCMS: Rₜ 3.53 min.; purity > 95%; MS m/z – 213 [M + 2]⁺.

(S)-2-[(3-(4-Amino-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (TFA salt) (30)

The general procedure described above was used. The crude final compound was purified by preparative LCMS where eluent A was 0.1% trifluoroacetic acid/water and eluent B was 0.1% trifluoroacetic acid/acetonitrile to give the title compound as a yellow solid (8.3 mg).

¹H NMR (400MHz, d₆-DMSO/D₂O): 7.66 (1H, d, J = 5.5 Hz), 7.40 (2H, d, J = 8.8 Hz), 7.08 (1H, d, J = 5.5 Hz), 6.55 (2H, d, J = 8.8 Hz), 4.28 (1H, dd, J = 9.7 Hz and 4.9 Hz), 3.09 (2H, t, J = 7.0 Hz), 1.88 – 1.77 (1H, m), 1.77 - 1.66 (1H, m), 1.55 – 1.47 (2H, m); ¹³C NMR (600MHz, d₆-DMSO): δ 172.9, 163.5, 156.3, 141.6, 130.1, 128.8, 123.1, 120.7, 114.0, 113.2, 112.7, 51.9, 40.1, 27.2, 25.3; LCMS: Rₜ 2.85 min.; purity > 85 %; MS m/z – 455 [M + 1]⁺; HRMS (m/z): [M]⁺ calcd. for C₁₇H₂₃N₆O₅S₂, 455.1171; found, 455.1170
(S)-2-[(4-Amino-benzenesulfonylamino)-benzoylamino]-5-guanidino-pentanoic acid (TFA salt) (32)

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (20.8 mg).

(S)-2-[[3-(3-Amino-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (TFA salt) (34)

The general procedure described above was used. The crude final compound was purified by preparative LCMS where eluent A was 0.1% trifluoroacetic acid/water and eluent B was 0.1% trifluoroacetic acid/acetonitrile to give the title compound as a white solid (15.9 mg).

$^1$H NMR (400MHz, (CD$_3$)$_2$SO): $\delta$ 10.85 (1H, s), 8.57 (1H, d, $J = 7.6$ Hz), 7.74 (1H, d, $J = 5.3$ Hz), 7.61 (1H, t, $J = 5.6$ Hz), 7.49-6.79 (4H, br m), 7.17 (1H, t, $J = 8.0$ Hz), 7.11 (1H, d, $J = 5.3$ Hz), 7.01 (1H, t, $J = 2.0$ Hz), 6.89 (1H, ddd, $J = 8.0$, 2.0 and 1.0 Hz), 6.77 (1H, ddd, $J = 8.0$, 2.0, 1.0 Hz), 4.35-4.30 (1H, m), 3.13-3.08 (2H, m), 1.90 – 1.81 (1H, m), 1.78 - 1.68 (1H, m), 1.56 – 1.48 (2H, m); $^{13}$C NMR (400MHz, (CD$_3$)$_2$SO): $\delta$ 172.9, 163.5, 156.7, 149.3, 141.3, 139.4, 130.3, 129.9, 120.3, 118.3, 114.1, 113.2, 111.0, 52.0, 40.3, 27.4, 25.4; LCMS:
Rt 3.48 min.; purity > 85 %; MS m/z – 456.3 [M + 1]⁺; HRMS (m/z): [M]⁺ calcd. for C₁₇H₂₃N₆O₅S₂, 455.1171; found, 455.1178.

(R)-2-[[3-(4-Amino-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (45)

The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a yellow solid (14.4 mg).

LCMS: Rt 8.6 min.; purity > 95 %; MS m/z – 455 [M + 1]⁺.

(S)-2-[[3-(4-Amino-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-3-(4-guanidino-phenyl)-propionic acid (49)

The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a yellow solid (1.8 mg).

LCMS: Rt 5.84 min.; purity > 85 %; MS m/z –503 [M + 1]⁺.

(S)-2-[[3-(4-Acetylamino-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (37)
The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a white solid (12.9 mg).

LCMS: Rt 3.88 min.; purity > 94 %; MS m/z – 497 [M + 1]+.

(S)-2-[[3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (TFA salt) (38)

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a yellow solid (3.8 mg).

LCMS: Rf 6.34 min.; purity > 95 %; MS m/z –485 [M + 1]+; HRMS: (m/z) [M]+ calcd. for C17H21N6O7S2, 485.0913; found, 485.0910.

(S)-2-[[3-(2-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (TFA salt) (39)
The general procedure described above was used. The crude final compound was purified by preparative LCMS where eluent A was 0.1% trifluoroacetic acid/water and eluent B was 0.1% trifluoroacetic acid/acetonitrile to give the title compound as a yellow solid (7.0 mg).

$^1$H NMR (400MHz, (CD$_3$)$_2$SO): δ 8.14 (1H, dd, $J$ = 7.5 and 1.6 Hz), 7.99-7.97 (1H, m), 7.89-7.80 (2H, m), 7.70 (1H, d, $J$ = 5.6 Hz), 7.70 (1H, d, $J$ = 5.6 Hz), 4.30 (1H, dd, $J$ = 9.9 and 4.8 Hz), 3.07 (2H, t, $J$ = 7.1 Hz), 1.87 – 1.78 (1H, m), 1.75 - 1.65 (1H, m), 1.56 – 1.43 (2H, m);

$^{13}$C NMR (600MHz, (CD$_3$)$_2$SO): δ 173.0, 163.3, 156.3, 147.4, 134.8, 132.9, 132.0, 130.2, 125.0, 120.5, 115.7, 114.5, 51.8, 40.1, 27.3, 25.2; LCMS: R, 6.36 min.; purity > 98%; MS m/z – 486 [M+1]$^+$; HRMS (m/z): [M]$^+$ calcd. for C$_{17}$H$_{21}$N$_6$O$_7$S$_2$, 485.0913; found, 485.0922.

(S)-5-Guanidino-2-([3-[methyl-(4-nitro-benzenesulfonyl)-amino]-thiophene-2-carbonyl]-amino)-pentanoic acid (TFA salt) (40)

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: acetonitrile/water) to give the title compound as a white solid (8.6 mg).
LCMS: $R_t 6.05$ min.; purity $> 99\%$; MS $m/z -499\ [M + 1]^+$. 

$$(S)-2-\{[3-(2\text{-Amino-benzenesulfonylamino})\text{-thiophene-2-carbonyl}]\text{-amino}\} -5\text{-guanidino-pentanoic acid (TFA salt) (35)}$$

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (eluent: methanol/water) to give the title compound as an off-white solid (10.8 mg).

LCMS: $R_t 6.19$ min.; purity $> 95\%$; MS $m/z -455\ [M + 1]^+$. 

$$(S)-2-\{[3-(\text{Benzo}[1,2,5]\text{-thiadiazole-4-sulfonylamino})\text{-thiophene-2-carbonyl}]\text{-amino}\} -5\text{-guanidino-pentanoic acid (TFA salt) (2, EG00229)}$$

The general procedure described above was used. The crude final compound was purified by (mass-directed) preparative LCMS where eluent A was 0.1% trifluoroacetic acid/water and eluent B was 0.1% trifluoroacetic acid/acetonitrile to give the title compound as a yellow solid (8.6 mg). Data as for the large scale preparation.
(S)-5-(N’,N’’-Dimethyl-guanidino)-2-{[3-(4-nitro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino}-pentanoic acid (52)

![Chemical structure](image)

The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a yellow solid (1.2 mg). LCMS: R_t 4.5 min.; purity > 83 %; MS m/z – 513 [M + 1]^+.

(S)-2-{[3-(1,2-Dimethyl-1H-imidazole-4-sulfonylamino)-thiophene-2-carbonyl]-amino}-5-guanidino-pentanoic acid (43)

![Chemical structure](image)

The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a white solid (3.7 mg). LCMS: R_t 3.3 min.; purity > 99 %; MS m/z –458.2 [M + 1]^+.

(S)-2-{[3-(Benzo[1,2,5]oxadiazole-4-sulfonylamino)-thiophene-2-carbonyl]-amino}-5-guanidino-pentanoic acid (42)

![Chemical structure](image)
The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a yellow solid (12.3 mg). LCMS: R<sub>t</sub> 4.2 min.; purity > 99%; MS <i>m/z</i> 482.2 [M + 1]<sup>+</sup>.

(5)-2-[(3-[(5-Chloro-1,3-dimethyl-1H-pyrazol-4-ylmethyl)-amino]-benzenesulfonylamino]-thiophene-2-carbonyl)-amino]-5-guanidino-pentanoic acid (44)

The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a yellow gum (3.6 mg). <sup>1</sup>H NMR (400MHz, (CD<sub>3</sub>)<sub>2</sub>SO /D<sub>2</sub>O): δ 7.37 (1H, d, J = 5.5 Hz), 7.11 (1H, t, J = 7.8 Hz), 6.98 (1H, d, J = 5.5 Hz), 6.96-6.95 (1H, m), 6.89 (1H, d, J = 7.8 Hz), 6.66 (1H, dd, J = 7.8 Hz and 1.9 Hz), 4.28 (1H, dd, J = 8.5 and 5.0 Hz), 3.91 (2H, s), 3.63 (3H, s), 3.10 (2H, t, J = 6.9 Hz), 2.09 (3H, s), 1.84 – 1.74 (1H, m), 1.73 - 1.65 (1H, m), 1.58 – 1.51 (2H, m); <sup>13</sup>C NMR (600MHz, (CD<sub>3</sub>)<sub>2</sub>SO): δ 174.0, 163.9, 156.8, 148.7, 147.0, 129.6, 128.6, 126.2, 122.5, 115.1, 114.1, 114.0, 112.8, 109.7, 52.1, 40.7, 36.3, 36.0, 28.8, 25.5, 12.6; LCMS: R<sub>t</sub> 4.8 min.; purity > 96%; MS <i>m/z</i> 597.2 [M + 1]<sup>+</sup>; HRMS (<i>m/z</i>): [M]<sup>−</sup> calcd. for C<sub>23</sub>H<sub>30</sub>N<sub>8</sub>O<sub>5</sub>S<sub>2</sub>Cl, 597.1469; found, 597.1462.
(S)-2-[[3-(2,4-Difluoro-benzenesulfonylamino)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (TFA salt) (41)

The general procedure described above was used and the crude compound was purified by elution through a 2 g C-18 column (elucent: acetonitrile/water) to give the title compound as a white solid (7.5 mg). LCMS: Rt 4.74 min.; purity > 97 %; MS m/z – 476 [M+1]+.

(S)-2-[[3-(4-Amino-phenylsulfamoyl)-thiophene-2-carbonyl]-amino]-5-guanidino-pentanoic acid (31)

The general procedure described above was used and the crude compound purified by preparative LCMS to give the title compound as a yellow solid (1.0 mg). LCMS: Rt 2.99 min.; purity > 89 %; MS m/z – 455 [M+1]+.

3-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-thiophene-2-carboxylic acid ((S)-4-guanidino-1-hydroxycarbamoyl-butyl)-amide (56)
The acid (1 eq), N, N-diisopropylethylamine (9 eq) and (2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) (1.5 eq) were stirred in N, N-dimethylformamide (2 mL) at 40 °C for 30 minutes. Hydroxylamine hydrochloride (1.5 eq) was added and the reaction mixture stirred at 40 °C for 16 hours. After this time, the solvent was removed in vacuo and the residue purified by preparative LCMS to give the title compound as a yellow solid (21 mg, 52 %). ¹H NMR (400MHz, (CD₃)₂SO): δ 10.53 (1H, s), 9.92 (1H, br s), 8.86 (1H, br s), 8.16-8.10 (1H, m), 7.72 (1H, dd, J = 8.6 and 7.1 Hz), 7.46 (1H, t, J = 5.6 Hz), 4.27-4.20 (1H, m), 3.25-3.20 (2H, m), 1.83-1.58 (4H, m); ¹³C NMR (400MHz, (CD₃)₂SO): δ 168.4, 163.0, 156.7, 155.1, 149.6, 128.7, 128.2, 127.1, 123.5, 122.5, 114.4, 50.1, 40.5, 29.0, 25.1; LCMS: Rₜ 4.02 min.; purity > 87 %; MS m/z – 513 [M + 1]⁺; HRMS (m/z): [M]⁺ calcd. for C₁₇H₂₁N₈O₅S₃, 513.0797; found, 513.0789.

**General Procedure for the reduction of aromatic nitro compounds using Raney Nickel/hydrazine**

The aromatic-nitro starting material (1 eq) was dissolved in methanol (1 mL) and a small quantity of Raney Nickel (approx. 5 - 10 mg) was added, followed by hydrazine monohydrate (10 eq). Effervescence was observed and the reaction mixture was sealed and stirred for 18 hours at room temperature. The reaction mixture was filtered through Celite, washed with methanol/water (4:1) and the solvents removed in vacuo to provide the crude product. The residue was loaded onto a 2 g C-18 column (eluent: acetonitrile/water, 5:95) and purified by reverse-phase chromatography.

(S)-2-(3-(4-amino-N-methylphenylsulfonamido)thiophene-2-carboxamido)-5-guanidinopentanoic acid (36)
The general method described above was used to give the title compounds as a green gum (12.0 mg). LCMS: Rt 1.53 min.; purity > 85 %; MS m/z – 469 [M - 1].

(S)-6-N, N’-di-tert-butoxyl carbonyl guanidino-2-[[3-(4-amino-benzenesulfonyl amino)-thiophene-2-carbonyl]-amino]-hexanoic acid

The general method described above was used to give the title compounds as a white solid (6.0 mg, 37%). LCMS: R, 6.69 min.; purity > 95 %; MS m/z – 669 [M + 1].
**Supplementary information**

**Figure legends**

**Figure S1.** Mutational analysis of $^{125}$I-VEGF-A binding to NRP1 expressing cells.

a) Mutated residues mapped onto NRP1 surface, rotated 180 deg from Fig. 2a.
b) Equal protein expression was assessed by Western Blotting for NRP-1 and GAPDH as indicated.

**Figure S2.** NRP1 residues showing chemical shift perturbations on ligand binding.

Solvent accessible surface representations of the structure NRP1 b1 with colour highlighting reflecting those residues whose backbone amide NH cross peak exhibits a significant chemical shift perturbation (compound $\Delta \delta > 0.15$, see Methods) upon addition of a saturating concentration of tuftsin (A), EG3287 (B), Ac-DKPRR-OH (C), and EG00229 (D). Panels A-D: tuftsin-bound NRP1 b1 coordinates (PDB code 2ORZ) where used; panel The intensity of the colour is scaled to the residue-by-residue value of the compound $\Delta \delta$. For each ligand the two views of the NRP1 b1 domain are related by a 180-degree rotation of the structure about the longitudinal axis in the plane of the paper.

**Figure S3.** Ligand-dependent backbone amide chemical shift perturbations for NRP1 ligands. s plotted as a function of residue number, with representation of the NRP1 b1 secondary structure elements denoted according to the nomenclature of Spraggon *et al*. The compound $\Delta \delta = \sqrt{(\Delta \delta_N/6.5)^2 + \Delta \delta_H^2}$, where $\Delta \delta_N$ and $\Delta \delta_H$ are measured in ppm, is plotted for saturating concentrations of tuftsin (A), EG3287 (B), Ac-DKPRR-OH (C), and EG00229 (D).

**Figure S4.** Maximal ligand dependent chemical shift perturbations for NRP1 ligands

Depiction of the maximal ligand-dependent $^1$H (x-axis) and $^{15}$N (y-axis) chemical shift perturbations for NRP1 b1 backbone NH cross peaks for residues W301 (black triangle), G318 (grey square), Y322 (grey circle), A344 (open triangle), I345 (grey triangle’), K347 (open circle), T349 (open diamond), K351 (black circle), G414 (back diamond), M417 (open square). Small symbols are used for ligands tuftsin, EG3287 and Ac-DKPRR-OH, large symbols for EG00229.

**Figure S5.** Crystal structure of NRP1 b1 domain complexed with the ligand EG00229 EG00229 (green stick models). Tuftsin (H-TKPR-OH, only KPR-OH visible, magenta sticks, PDB: 2ORZ) is shown for comparison. Key binding residues lining the pocket are shown as yellow stick models.

**Figure S6.** Inhibition of $^{125}$I or biotin labeled VEGF-A binding to NRP1 by EG00229.

a) Confluent PAE/NRP1 or c) A549 cells were incubated for 2 h at 4°C with 0.1 nM $^{125}$I-VEGF in the presence of the indicated concentrations of EG00229 and specific VEGF binding determined as described in Materials and Methods. b) Confluent HUVECs or d) DU145 cells were infected with adenovirus encoding wild-type full-length NRP1 (Ad.NRP1), and 2 days later, cells were incubated for 2 hours at room temperature with 2 nM biotinylated VEGF in the presence of the indicated concentrations of EG00229. Specific VEGF binding determined as described in Materials and Methods. Values presented are means±s.e.m obtained from three independent experiments each performed in triplicate. $R^2$ values ranged from 0.9911 to 0.9996.

**Figure S7.** EG00229 inhibits VEGF-induced VEGFR2 activation.

Confluent HUVECs were pre-treated for 30 minutes with the indicated concentrations of EG00229 or with an equivalent volume of solvent (DMSO), and were then treated with or
without 25 ng/ml VEGF-A165 for 5 minutes at 37°C. Total VEGFR2 and VEGFR2 phosphorylated at tyrosine 1175 were then determined in treated cell lysates using a specific ELISA.

**Figure S8.** Sensitization of carcinoma cells to chemotherapeutic agents by EG00229. A549 cells were incubated in serum-free medium containing 5-FU at the indicated concentrations in the absence or presence of 100 μM EG00229. Cell viability was measured after 48 h treatment. **, p < 0.01 for the chemotherapeutic drug alone versus drug plus EG00229, two-way ANOVA with Bonferroni’s tests.
Mutated residues mapped onto NRP1 surface, rotated 180 deg from Fig. 2a.
Figure S2
Supplementary Fig. S3
Supplementary Fig. S4

Fig. Y
Supplementary Fig. S5
Supplementary Fig. S6

Effects of EG00229 on VEGF binding to NRP1

a) PÆ/NRP1, IC50 = 8 μM

b) HUVEC/Ad.NRP1, IC50 = 23 μM

c) A549, IC50 = 7 μM

d) DU145/Ad.NRP1, IC50 = 3 μM
Effects of EG00229 on VEGF-induced VEGFR2 phosphorylation in HUVECs

**Supplementary Fig. S7**

**pY1175-VEGFR2**

|                  | Control | 0 µM EG229 | 30 µM EG229 | 100 µM EG229 |
|------------------|---------|------------|-------------|--------------|
| Absorbance       | 0.0     | 1.0        | 0.8         | 0.7          |

**Total VEGFR2**

|                  | Control | 0 µM EG229 | 30 µM EG229 | 100 µM EG229 |
|------------------|---------|------------|-------------|--------------|
| Absorbance       | 1.5     | 1.5        | 1.5         | 1.5          |
Effects of EG00229 on lung carcinoma A549 cell response to the chemotherapeutic agents

- 5-FU, IC$_{50}$ = 78 μM
- 5-FU + 100 μM EG00229, IC$_{50}$ = 18 μM