

A new class of NO-donor pro-drugs triggered by γ -glutamyl transpeptidase with potential for reno-selective vasodilatation

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Material and methods

All reagents and solvents were of highest grade from commercial sources, unless otherwise specified. Normal phase column chromatography was performed using silica gel 60 (40–63 micron). Reverse phase column chromatography was performed using Alltech High Capacity Cartridge columns. NMR spectra were recorded on Bruker AVANCE 300, 400 or 500 instruments. ¹H and ¹³C NMR spectra were recorded using deuterated solvent as the lock and residual solvent as the internal standard. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are given in Hertz (Hz). Where necessary, resonances were assigned using two-dimensional experiments (COSY, HMBC, HSQC). Mass spectrometric (*m/z*) data was acquired by electrospray ionisation (ESI). High resolution mass analyses were recorded on a Micromass LCT TOF mass spectrometer using ESI in positive mode.

LCMS analyses were carried out on a Micromass LCT TOF coupled with a Waters 2795 HPLC using a Phenomenex Kingsorb C-18 analytical column (150 × 4.6 mm, 3 μ m) equipped with a security guard cartridge. Mobile phase consisted of 2% acetonitrile water with 0.1% TFA (A) and acetonitrile with 0.1% TFA (B). The program ran a linear gradient from 5% B in 5 min, 10% B in 10 min and 20% B in 20–25 min and 25% B in 30 min followed by isocratic conditions with 5% B for 10 min to equilibrate the column. Peaks were detected with TIC using a positive electron-spray mode at a flow rate of 0.8 mL/min.

Incubations of prodrugs – Prodrug (**2b**, **3**, **4a-b** or **14**, 100 μ M) was incubated in Krebs buffer (37°C, pH 7.4) with or without addition of with γ -GT (100 mU/mL) and the glutamyl acceptor,

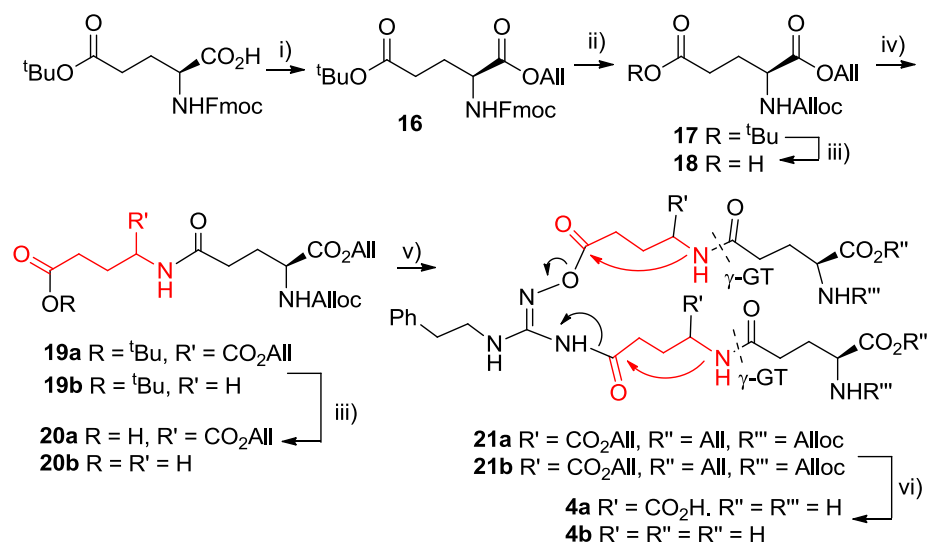
Gly-gly (5 mM). The specimen incubated with γ -GT and Gly-gly was titrated with two volume of acetonitrile and centrifuged to remove the enzyme before LCMS analysis.

The animal care and experimental procedures were in accordance with the United Kingdom Animal (Scientific Procedures) Act, 1986.

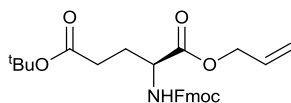
Animals used to obtain kidney homogenates were killed by a rising concentration of CO₂. Approximately 1g of kidney tissue was taken from each of three Wistar rats and homogenized with 3 mL of 0.1 M Tris buffer (pH 7.2) – the homogenates were pooled for experiments and total protein content was measured using the Lowry assay. Compound **4b** (100 μ M) was added to the homogenate and appearance of **1b** was measured at intervals for 45 min using LC-MS-MS (Micromass Quattro Micro Mass spectrometer, Micromass UK Ltd, Manchester, UK) and 2795 Alliance HT liquid chromatograph, Waters, Milford, MA, USA.

Rat isolated perfused kidney - adult male Wistar rats (250-450 g) were anaesthetized by intraperitoneal injection with sodium pentobarbital (60 mg/Kg body weight) prior to heparinisation (500 U) prior to insertion of a cannula into the renal artery via the superior mesenteric artery. Perfusion with a modified Krebs-Henseleit solution (containing 6.7% bovine serum albumin and 20 amino acids) commenced immediately. The right kidney and ureter were dissected free and transferred to a temperature-controlled moisture chamber, and perfused with 40 ml recirculating perfusate at a constant rate of 10 mL/min via a peristaltic roller pump. Perfusion pressure was measured via a side port and transducer (mean pressure ~60 mmHg). Following 20 minutes equilibration, the kidney vasculature was constricted by addition of the α_1 -adrenoceptor agonist, phenylephrine, until a perfusion pressure of 120-140 mmHg was reached prior to cumulative addition of **4b** to the perfusate (1 μ M-1 mM). Renal perfusion pressure was monitored as a measure of vasodilator activity of the compound.

Synthetic procedure

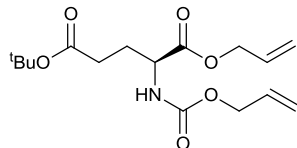


Scheme S1 Design and synthesis of Glu/GABA linked γ -glutamyl NO-donor pro-drugs of NHG: i) Cs₂CO₃, MeOH: H₂O 90 :10, then allyl bromide, DMF, °C to rt, 12 h, 81-99%; ii) Et₂NH, 20 equiv, DBU 20% equiv, THF, rt, 4h; then allyl chloroformate, dioxane, sat. aq. NaHCO₃, 77%; iii) CF₃CO₂H, DCM, rt, 2h, 90-94%; iv) *tert*-butyl γ -aminobutanoate hydrochloride, HOBT, EDC, Et₃N, DCM, rt, 24 h, 99%; v) *N*-phenethyl-*N'*-hydroxyguanidine, HOBT, EDC, NaHCO₃, DMF/DCM, rt, 24 h, 83%; vi) [Pd(PPh₃)₄], PhSiH₃, DCM, rt, 6h, 38%

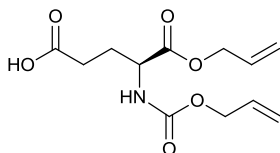


Compound 16.¹ A solution of Fmoc-Glu(O^tBu)-OH (12.75 g, 30 mmol) in aqueous methanol (90%, 100 mL) was titrated with a solution of Cs₂CO₃ (5.17 g, 15.7 mmol, 25% in water) to pH 7. The solvents were removed under vacuum. The residue was co-evaporated successively with absolute ethanol (4 × 100 mL), toluene (2 × 100 mL) and then dried under vacuum overnight. The cesium salt thus obtained was suspended in anhydrous DMF (100 mL), cooled to 0°C, and treated with allyl bromide (5.2 mL, 60 mmol) by dropwise addition over 20 min. After 1 h stirring the solution was allowed to warm to room temperature and stirring was continued for a further 10 h before it was vacuumed to dryness. The residue was taken up with water (100 mL) and then extracted with EtOAc (3 × 100 mL) and the combined organic layers were dried over MgSO₄ and concentrated. Flash column chromatography of the residue (EtOAc/Pet Ether 1:1) gave the product as a white solid which was recrystallised from Et₂O to afford needle crystals. Yield: 13.8 g, 99%. δ_H (CDCl₃, 400 MHz): 7.78 (d, 2H, *J* 7.6 Hz, Fm H), 7.76 (dd, 2H, *J* 7.1, 3.6 Hz, Fm H), 7.41 (t, 2H, *J* 7.4 Hz, Fm H), 7.33 (t, 2H, *J* 7.4 Hz, Fm H), 5.92 (ddd, 1H, *J* 17.0, 10.6, 5.8 Hz, Allyl =CH), 5.51 (d, 1H, *J* 8.2 Hz, NHFmoc), 5.35 (dd, 1H, *J* 17.1, 1.0 Hz, ½ Allyl =CH₂), 5.28 (dd, 1H, *J* 10.4, 1.1 Hz, ½ Allyl =CH₂), 4.67 (d, 2H, *J* 5.6 Hz, Allyl CH₂), 4.46-4.35 (m, 3H, Glu- α + Fmoc CH₂), 4.24 (t, 1H, *J* 7.0 Hz, Fmoc CH), 2.35 (m, 2H, Glu- γ), 2.20 (m, 1H,

$\frac{1}{2}$ Glu- β), 1.99 (m, 1H, $\frac{1}{2}$ Glu- β). δ_C (CDCl₃, 100 MHz) 172.1, 171.8, 156.0, 143.9, 143.7, 141.3, 131.5, 127.7, 127.1, 125.2, 125.1, 120.0, 119.0, 80.9, 67.1, 66.2, 53.6, 47.2, 31.5, 28.1, 27.6; Found: C, 69.91; H, 6.82; N, 3.03%; Calc for C₂₇H₃₁NO₆ C, 69.66; H, 6.71; N, 3.01%. m/z (ESI⁺) 488 [M + Na]⁺.

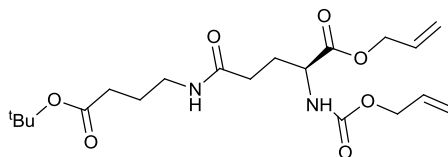


Compound 17.^{1,2} Diethylamine (freshly distilled, 24 mL, 232 mmol) and DBU (0.36 mL, 2.4 mmol) were added to a solution of **16** QZNO232 (5.3 g, 11.6 mmol) in THF (30 mL). The mixture was stirred under an argon atmosphere in darkness for 12 hr at room temperature before the solvents were removed under rotary evaporation. The residue was further co-evaporated with EtOAc (2 × 50 mL) and then taken up by dioxane (20 mL) and aqueous Na₂CO₃ (20%, 20 mL). The mixture was cooled to 0°C on an ice-bath and allyl chloroformate (1.86 mL, 17.4 mmol) was added dropwise over 10 min with stirring. Stirring was continued at 0°C for another 2 h and the resultant was diluted with water (200 mL) and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with water and brine successively and dried over anhydrous MgSO₄. Removal of the solvent and column chromatography (SiO₂, DCM : Et₂O 10 : 1) of the residue gave the product as a pale yellow oil (2.9 g, 77%). δ_H (CDCl₃, 300 MHz) 5.88 (m, 2H, 2 × Allyl =CH), 5.45 (d, 1H, J 8.4 Hz, NHAlloc), 5.26 (m, 4H, 2 × Allyl =CH₂), 4.62 (dt, 2H, J 5.7, 1.2 Hz, Allyl CH₂), 4.55 (d, 2H, J 5.4 Hz, Allyl CH₂), 4.37 (m, 1H, Glu- α), 2.32 (m, 2H, Glu- γ), 2.16 (m, 1H, $\frac{1}{2}$ Glu- β), 1.93 (m, 1H, $\frac{1}{2}$ Glu- β), 1.42 (s, 9H, ^tBu); δ_C (CDCl₃, 75.5 MHz) 172.4, 172.2, 156.2, 133.0, 131.9, 119.3, 118.2, 81.2, 66.5, 66.2, 53.9, 31.8, 28.4, 27.9; Found: C 58.87, H 7.42, N 4.43; Calc for C₁₆H₂₅NO₆ C, 58.72; H, 7.70; N, 4.28%. m/z (ESI⁺) 350 [M + Na]⁺, 294 [M – C₄H₈ + Na]⁺. HRESI⁺: 350.1569 Calc for C₁₆H₂₅NO₆Na 350.1580.

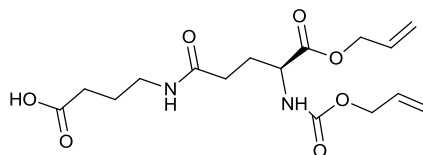


Compound 18.^{1,2} Compound **17** (9.7 g, 29.6 mmol) was treated with trifluoroacetic acid in DCM (50 : 50, 40 mL) at room temperature overnight. After removal of solvents, the residue was co-evaporated with toluene (3 × 50 mL), EtOAc (50 mL) and then diethyl ether (50 mL) to give the product as a yellowish oil. Yield: 7.56 g, 94%; δ_H (CDCl₃, 300 MHz): 5.70 (m, 2H, 2 × Allyl =CH), 5.27 (d, 1H, J 7.7 Hz, NHAlloc), 5.08 (m, 4H, 2 × Allyl =CH₂), 4.48 (d, 2H, J 5.8 Hz, Allyl CH₂), 4.42 (m, 2H, J 5.3 Hz, Allyl CH₂), 4.28 (m, 1H, Glu- α), 2.34 (m, 2H, Glu- γ), 2.11 (m, 1H, $\frac{1}{2}$ Glu- β), 1.89 (m, 1H, $\frac{1}{2}$ Glu- β); δ_C (CDCl₃) 178.2, 172.0, 156.3, 132.8, 131.7, 119.7, 118.4, 66.7, 66.4, 53.6, 30.3, 27.9. Found: C, 51.74; H, 6.48; N, 4.58%. Calcd. for

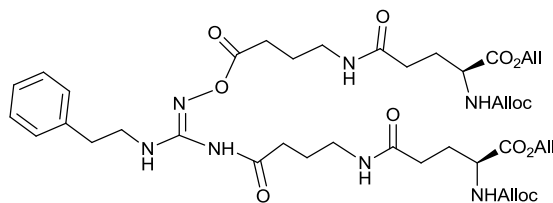
$C_{12}H_{17}NO_6 \cdot 0.5H_2O$ C, 51.43; H, 6.47; N, 4.99%. m/z (ESI⁻) 270 [M - H⁺]; m/z (ESI⁺) 294 [M + Na]⁺.



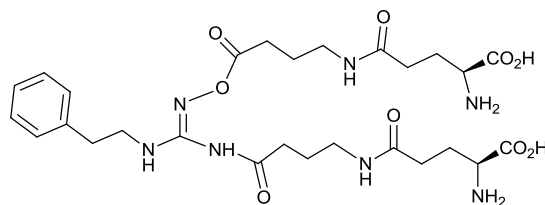
Compound 19b. *tert*-Butyl γ -aminobutanoate hydrochloride (1.02 g, 5.2 mmol), Alloc-Glu-OAll **18** (1.5 g, 5.5 mmol), EDC (1.2 g, 6.28 mmol), HOBt (0.74 g, 5.4 mmol) and triethylamine (1.5 mL, 10.78 mmol) were mixed in DCM (20 mL). The mixture was stirred at RT for 24 h. The mixture was diluted with DCM (100 mL) and washed with water. The organic layer was dried (MgSO₄) and solvent removed in rotary evaporator and the residue was flashed column chromatographed (silica gel, Pet ether/EtOAc 5 : 1 to 3 : 1) to give the product as colourless oil (4.69 g, 99%); δ_H (CDCl₃, 300 MHz) 6.13 (br s, 1H, NH), 5.92 (m, 2H, 2 \times Allyl =CH), 5.69 (d, 1H, J 7.8 Hz, NHAlloc), 5.29 (m, 4H, 2 \times Allyl =CH₂), 4.66 (dt, 2H, J 5.8, 1.3 Hz, Allyl CH₂), 4.57 (dt, 2H, J 5.6, 1.3 Hz, Allyl CH₂), 4.36 (m, 1H, Glu- α), 3.28 (t, 2H, J 7.2 Hz, CH₂N), 2.28 (t, 4H, J 7.2 Hz, CH₂CO + Glu- γ), 2.23 (m, 1H, $\frac{1}{2}$ Glu- β), 2.00 (m, 1H, $\frac{1}{2}$ Glu- β), 1.80 (quintet, 2H, J 7.2 Hz, Gaba CH₂), 1.45 (s, 9H); δ_C (CDCl₃, 100 MHz) 172.8, 171.9, 171.7, 156.2, 132.5, 131.4, 119.1, 117.9, 80.6, 66.2, 65.9, 53.6, 39.2, 33.0, 32.5, 28.5, 28.1, 24.6; Found: C 58.00, H 7.83, N 6.84%; $C_{20}H_{32}N_2O_7$ requires: C 58.24, H 7.82, N 6.79%; m/z (ESI⁺) 413 [M + H⁺]; 435 [M + Na⁺]; 451 [M + K⁺].



Compound 20b. Compound **19b** (4.72 g, 11.5 mmol) was treated with trifluoroacetic acid in DCM (50 : 50, 40 mL) at room temperature overnight. After removal of solvents, the residue was co-evaporated with toluene (3 \times 50 mL) and then ether followed by column chromatography (DCM : EtOAc 4 : 1 to 1 : 1) to give the product as a colourless oil which gradually solidified as wax (3.68 g, 90%). δ_H (CDCl₃, 400 MHz) 7.25 (br s, COOH), 6.68 (br s, 1H, Gaba NH), 5.95-5.84 (m, 3H, Glu NH + 2 \times Allyl =CH), 5.35-5.19 (m, 4H, 2 \times Allyl =CH₂), 4.63 (d, 2H, Allyl CH₂, J 5.6), 4.56 (br s, 2H, Allyl CH₂), 4.32 (m, 1H, Glu- α), 2.39 (t, 2H, GABA- α), 2.31 (t, 2H, Glu- γ), 2.21 (m, 1H, Glu- β), 2.00 (m, 1H, Glu- β), 1.83 (quintet, 2H, GABA- β , J 6.80); δ_C (CDCl₃, 100 MHz) 177.1, 173.2, 172.0, 156.7, 132.6, 131.6, 119.3, 118.2, 66.4, 66.3, 53.8, 39.2, 32.6, 31.6, 28.6, 24.7; m/z (ESI⁻) 355 [M-H⁺]; m/z (ESI⁺) 379 [M + Na]⁺; HRESI⁺ 379.1482; $C_{16}H_{24}N_2O_7Na$ requires 379.1481.

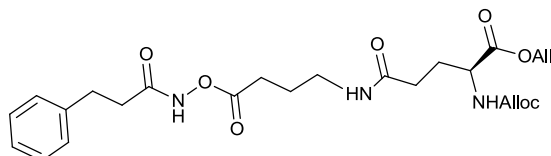


Compound 21b. A mixture of compound *N*-phenethyl-*N'*-hydroxyguandine hydrochloride (0.303 g, 1.38 mmol), the Glu-GABA dipeptide **20b** (1.18 g, 3.3 mmol), HOBt (0.535 g, 2.87 mmol), EDC (0.759 g, 2.87 mmol) and sodium bicarbonate (0.75 g, 8.93 mmol) DMF/DCM (1/3, 20 mL) was stirred at RT for 24 h. The mixture was further diluted with DCM (100 mL) and washed with water (3 times) and brine. The organic layers were combined and dried (MgSO₄). After solvent removal, the residue was purified by column chromatography (silica gel, DCM/EtOAc 4:1) to afford the product as white wax (1.0 g, 83%). δ_{H} (CDCl₃, 400 MHz) 9.39 (br s, 1H, NHCO), 7.53 (br, 1H, NHCN), 7.25-7.11 (m, 5H, PhH), 6.35 (br s, 2H, 2 \times NH of GABA), 5.98 (d, 1H, NH of Glu, *J* 7.6), 5.91 (d, 1H, NH of Glu, *J* 7.5 Hz), 5.88-5.75 (m, 4H, 4 \times Allyl =CH), 5.28-5.12 (m, 8H, 4 \times Allyl =CH₂), 4.56 (d, 4H, 2 \times Allyl CH₂, *J* 5.7 Hz), 4.48 (d, 4H, 2 \times Allyl CH₂, *J* 5.4 Hz), 4.25 (m, 2H, 2 \times Glu- α), 3.40 (dt, 2H, CH₂N of NHG, *J* 5.6, 7.0 Hz), 3.22 (m, 4H, 2 \times GABA- γ CH₂), 2.84 (t, 2H, PhCH₂, *J* 7.0 Hz), 2.38 (m, 4H, 2 \times GABA- α CH₂), 2.22 (m, 4H, 2 \times Glu- γ CH₂), 2.12 (m, 2H, 2 \times Glu- β , CH), 1.92 (m, 2H, 2 \times Glu- β), 1.77 (m, 4H, 2 \times GABA- β CH₂); δ_{C} (CDCl₃, 100 MHz) 174.2, 172.6, 171.7, 171.3 (overlapped), 156.4, 152.6, 138.9, 132.5 (overlapped), 131.4, 131.4, 128.8, 128.5, 126.4, 119.2, 118.0 (overlapped), 66.2 (overlapped), 66.0 (overlapped), 53.6 (overlapped), 43.1, 42.4, 38.5, 38.4, 35.0, 34.5, 32.7, 32.4, 30.1, 28.5, 28.2, 25.6; Found: C 57.62, H 6.73, N 11.45%; C₄₁ H₅₇ N₇ O₁₃ requires C 57.53, H 6.71, N 11.45%; *m/z* (ESI⁺) 856 [M + H]⁺; 878 [M + Na]⁺.

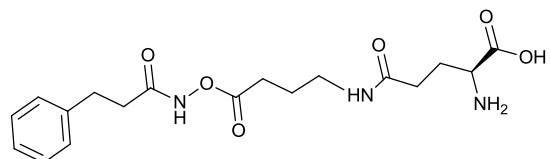


Compound 4b. [Pd(PPh₃)₄] (61 mg, 0.053 mmol) and PhSiH₃ (0.45 mL, 0.877 mmol) were added to a degassed solution of **21b** (0.45 g, 0.53 mmol) in DCM (20 mL). The mixture was stirred at room temperature for 6 h with the vessel covered in foil to exclude light from the reaction. MeOH (2 mL) was added to quench the excess of phenylsilane and the mixture was stirred for another 20 min before the solvents were replaced with diethyl ether. The resulting solid was filtered off and washed with diethyl ether and then taken up in distilled water (20 mL). The aqueous solution was filtered through a celite pad and then a C-18 cartridge (eluted with CH₃CN/H₂O 10 : 90 to 40 : 60). The ninhydrin-active fraction was collected and freeze dried and further purified with preparative HPLC as a white fluffy solid (122 mg, 38%). (δ_{H} (D₂O, 300 MHz) 7.30-7.07 (m, 5H, PhH), 3.66-3.60 (m, 2H, 2 \times Glu- γ CH), 3.29 (dt, 2H, *J* 9.4, 6.6 Hz

CH₂N); 3.15 -3.06 (m, 4H, 2 × GABA-γ CH₂), 2.81 (t, 1H, *J* 6.7 Hz, ½ PhCH₂), 2.69 (t, 1H, *J* 6.4 Hz, ½ PhCH₂), 2.45-2.22 (m, 8H, 2 × GABA-α CH₂ + 2 × Glu-γ CH₂), 2.05-1.94 (m, 4H, 2 × Glu-β CH₂), 1.79-1.64 (m, 4H, 2 × GABA-β CH₂); δ_C (D₂O, 400 MHz) 175.3, 174.7, 174.4, 173.9 (overlapped), 157.8, 138.2, 129.2, 128.5, 126.6, 54.1(overlapped), 48.6, 38.4 (overlapped), 38.2, 33.8, 31.7, 31.6, 31.5, 26.5, 24.9, 24.0, 23.5; *m/z* (ESI⁺) 608 [M + H]⁺; 630 [M + Na]⁺; 646 [M + K]⁺; HRESI⁺ 630.2849 [M + Na]⁺; C₂₇H₄₁N₇O₉ Na requires 630.2823; ESI⁻ 606 [M-H]⁻; HRESI⁻ 606.2869 [M - H]⁻, Calc for C₂₇H₄₀N₇O₉ 606.2888.

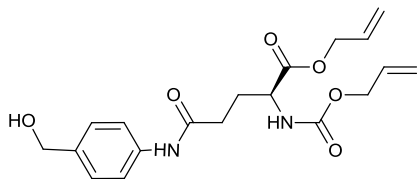


Compound 22. 3-Phenylpropionohydroxamic acid (0.33 g, 2 mmol) was mixed with Glu-GABA dipeptide **20b** (0.356 g, 1.0 mmol) and ByBop (0.52 g, 1.0 mmol) in DMF (5 mL) with stirring. The solution was cooled to -10°C and diisopropylethylamine (0.175 mL, 1.0 mmol) was added dropwise. The mixture was allowed to warm up to room temperature and stirred for 2 h before being diluted with water (50 mL) and extracted with DCM. The combined extracts were washed with water and brine successively and dried (MgSO₄). After removal of solvent, the residue was purified by flash column chromatography (silica gel, Pet ether/EtOAc 5 : 1 to 3 : 1) to give the product as white solid (0.377 g, 75%). δ_H (300 MHz, CDCl₃) 9.91(1H, NHO), 7.27 (m, 5H), 6.72 (br s, 1H, Gaba NH), 5.92 (m, 3H, 2 × Allyl =CH + NH), 5.29 (m, 4H, 2 × Allyl =CH₂), 4.65 (d, 2H, *J* 5.7 Hz, Allyl CH₂), 4.56 (d, 2H, *J* 5.6 Hz, Allyl CH₂), 4.33 (m, 1H, Glu-α), 3.35(q, 2H, *J* 6.4 Hz, Gaba CH₂N), 3.02 (t, 2H, *J* 7.4Hz, CH₂CO), 2.60 (t, 2H, *J* 7.4Hz, PhCH₂), 2.52 (t, 2H, *J* 6.7Hz, Gaba CH₂CO₂), 2.30 (t, 2H, *J* 6.4 Hz, Glu-γ), 2.20 (m, 1H, Glu-β), 1.99 (m, 1H, Glu-β), 1.94 (quintet, 2H, Gaba CH₂); δ_C (75.5 MHz, CDCl₃) 173.1, 172.2, 171.9, 156.7, 140.6, 132.9, 131.9, 129.0, 128.7, 126.8, 119.4, 118.3, 66.5, 66.3, 54.1, 39.0, 34.9, 32.6, 31.4, 29.8, 28.4, 24.7; Found: C 57.77, H 6.23, N 7.82%; C₂₅H₃₃N₃O₈ + H₂O requires C 57.58, H 6.76, N 8.05; *m/z* (ESI⁺) 526 [M + Na]⁺.

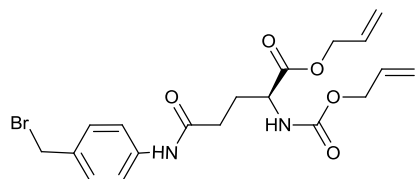


Compound 3. [Pd(PPh₃)₄] (92 mg, 0.08 mmol) and PhSiH₃ (0.45 mL, 0.877 mmol) were added to a degassed solution of **22** (0.403 g, 0.80 mmol) in DCM (20 mL). The mixture was stirred at room temperature for 6 h with the vessel covered in foil to exclude light from the reaction. MeOH (2 mL) was added to quench the excess of phenylsilane and the mixture was stirred for another 20 min before the solvents were replaced with diethyl ether. The resulting solid was filtered off and washed with diethyl ether and then taken up in distilled water (20 mL). The

aqueous solution was filtered through a celite pad and then a C-18 cartridge (eluted with CH₃CN/H₂O 10 : 90 to 40 : 60). The ninhydrin-active fraction was collected and freeze dried to give the product as white solid (0.130 g, 43%). δ_{H} (300 MHz, DMSO-d₆) 8.11 (t, 1H, *J* 5.5 Hz, NH of Gaba), 7.22 (m, 5H, Ar H), 3.21 (t, 1H, *J* 6.4, Glu- α CH), 3.01 (dt, 2H, *J* 7.0, 5.5 Hz, Glu- β CH₂), 2.84 (t, 2H, *J* 7.4 Hz, CH₂), 2.43 (t, 2H, *J* 7.4 Hz, CH₂), 2.42 (t, 2H, *J* 7.3 Hz, CH₂), 2.25 (t, 2H, *J* 7.4 Hz, CH₂), 1.87 (m, 2H, CH₂), 1.71 (quintet, 2H, CH₂); δ_{C} (DMSO-d₆, 75.5 MHz) 172.0, 171.0, 169.9, 168.9, 140.6, 128.2, 128.6, 125.9, 53.7, 37.7, 33.5, 31.8, 30.5, 28.6, 27.0, 24.3. Found: C 56.62, H 6.72, N 10.91%. C₁₈H₂₅N₃O₆ requires C 56.98, H 6.64, N 11.08%; *m/z* (ESI⁺) 380 [M + H]⁺, 402 [M + Na]⁺; *m/z* (ESI⁻), 378 [M-H]⁻; *m/z* (HRESI⁺) 380.1823, C₁₈H₂₆N₃O₆ requires 380.1322.

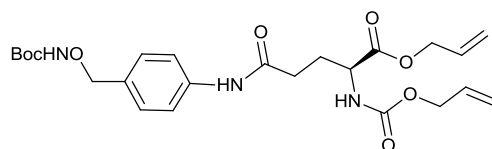


Compound 5. EEDQ (2.20 g, 8.90 mmol) was added to a solution of Alloc-Glu-OAll **18** (2.0 g, 7.37 mmol) and *para*-aminobenzylalcohol (1.09 g, 8.86 mmol) in DCM (50 mL). The mixture was stirred under an atmosphere of argon at ambient temperature overnight and poured into a 1N HCl solution (50 mL). The layers were separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with water, brine and dried (MgSO₄). The solvent was removed by rotary evaporation. The residue was purified *via* flash chromatography (silica gel, Et₂O) to give the product as a white solid (2.37 g, 85%). δ_{H} (CDCl₃, 400 MHz) 8.18 (s, NH), 7.56 (d, 2H, *J* 8.2 Hz, Ar H), 7.33 (d, 2H, *J* 8.4 Hz, Ar H), 5.95-5.85 (m, 2H, 2 × Allyl =CH), 5.62 (d, 1H, *J* 7.9 Hz, NHAlloc), 5.36-5.22 (m, 4H, 2 × Allyl =CH₂), 4.66-4.64 (m, 4H, benzyl CH₂ + Allyl CH₂), 4.59 (2H, *J* 5.6, 1.2 Hz, Allyl CH₂), 4.44 (m, 1H Glu- α), 2.48 (m, 2H, Glu- γ), 2.36 (m, 1H, ½ Glu- β), 2.02 (m, 1H, ½ Glu- β). δ_{C} (CDCl₃, 75.5 MHz) 172.2, 171.3, 157.0, 137.7, 137.3, 132.8, 131.7, 128.1, 120.6, 119.5, 118.4, 66.6, 66.4, 64.9, 54.1, 33.8, 28.8; Found: C 60.65, H 6.58, N 7.39%; Calc. for C₁₉H₂₄N₂O₆ C 60.63, H 6.43, N, 7.74%. *m/z* [ESI⁺] 399 [M + Na]⁺.

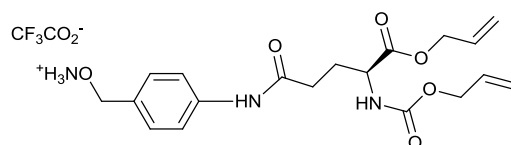


Compound 6. Phosphorus tribromide (1.36 mL, 14.47 mmol) was added to an ice-cooled solution of **5** (3.62 g, 9.62 mmol) in THF (50 mL). The mixture was stirred at 0°C for 2 h before it was neutralized with ice-cold saturated aqueous NaHCO₃ (20 mL) and further diluted with water (200 mL). The resulting opaque solution was extracted with EtOAc (3 × 100 mL). The combined extracts were dried (MgSO₄) and the solvent was removed under reduced pressure. The residue

was passed through a short silica gel column eluted with Et₂O to give the product as a white wax (3.66 g, 87%). δ_{H} (CDCl₃, 300 MHz) 8.48 (s, 1H, NH), 7.53 (d, 2H, *J* 8.3 Hz, Ar H), 7.32 (d, 2H, *J* 8.3 Hz, Ar H), 5.87 (m, 2H, 2 × Allyl =CH), 5.77 (d, 1H, *J* 8.2 Hz, NH), 5.27 (m, 4H, 2 × Allyl =CH₂), 4.62 (d, 2H, *J* 5.8 Hz, Allyl CH₂), 4.56 (d, 2H, *J* 5.9 Hz, Allyl CH₂), 4.47 (s, 2H, BrCH₂), 4.42 (m, 1H, Glu- α), 2.46 (m, 2H, Glu- γ), 2.32 (m, 1H, ½ Glu- β), 2.02 (m, 1H, ½ Glu- β); δ_{C} (75.5 MHz, CDCl₃) 172.0, 170.9, 157.1, 138.6, 133.8, 132.7, 131.6, 130.2, 120.3, 119.8, 118.6, 66.8, 66.6, 53.8, 34.2, 33.9, 29.7. Found: C 52.04, H 5.28, N, 6.38 %; Calcd. for C₁₉H₂₃BrN₂O₅ C 51.95, H 5.28, N 6.38%; *m/z* (ESI⁺) 439/441 [M + H]⁺, 461/463 [M + Na]⁺.

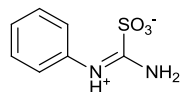


Compound 7. Sodium hydride (0.21 g, 5.26 mmol, 60% in mineral oil) was added to an ice-cold solution of BocNHOH (0.70 g, 5.26 mmol) in THF (30 mL). The mixture was allowed to warm up to rt and stirring was continued for two hours before it was cooled to 0°C again. Compound **6** (2.00 g, 5.14 mmol) in THF (20 mL) was then added dropwise to the resultant suspension cooled in ice bath. The resulting mixture was stirred at 0°C for 4 h and then poured into ice water (200 mL). The mixture was extracted with EtOAc (3 times). The combined extracts were washed with water and brine and dried over anhydrous MgSO₄. Removal of solvent followed by column chromatography (silica gel, Et₂O) gave the product as colorless oil which gradually solidified as colorless wax (1.85 g, 83%). δ_{H} (300 MHz, CDCl₃) 8.29 (s, 1H, NH), 7.58 (d, 2H, *J* 8.3 Hz, Ar H), 7.35 (d, 2H, *J* 8.3 Hz, Ar H), 7.14 (s, 1H, NH), 5.90 (m, 2H, 2 × Allyl =CH), 5.67 (d, 1H, *J* 8.0 Hz, NHAlloc), 5.29 (m, 4H, 2 × Allyl =CH₂), 4.81 (s, 2H, benzyl CH₂), 4.62 (d, 2H, *J* 5.9 Hz, Allyl CH₂), 4.59 (d, 2H, *J* 4.4 Hz, Allyl CH₂), 4.44 (m, 1H, Glu- α), 2.48 (m, 2H, Glu- γ), 2.35 (m, 1H, ½ Glu- β), 2.02 (m, 1H, ½ Glu- β), 1.49 (s, 9H, ^tBu CH₃). δ_{C} (75.5 MHz, CDCl₃) 171.5, 170.3, 156.8, 138.3, 132.3, 131.4, 131.3, 130.0, 119.7, 119.4, 118.2, 81.8, 77.0, 66.4, 66.2, 53.4, 33.8, 29.6, 28.2; Found: C 56.93, H 6.58, N 8.05%; C₂₄H₃₃N₃O₈ • H₂O requires C 56.57, H 6.92, N 8.25%; C₂₄H₃₃N₃O₈ requires C 58.64, H 6.77, N 8.55%; *m/z* (ESI⁺) 514 [M + Na]⁺.

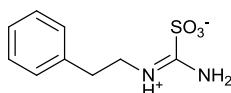


Compound 8. Trifluoroacetic acid (10 mL) was added to a solution of compound **7** (3.75 g, 7.6 mmol) in DCM (10 mL). The mixture was stirred at room temperature overnight. After solvent removal under vacuum, the residue was sonicated with Et₂O. The solid was then filtered and washed with ether to give the product as trifluoroacetate salt (3.86 g, 92%). δ_{H} (300 MHz, DMSO-*d*₆) 10.10 (s, 1H, NH), 7.77 (d, 1H, *J* 7.9 Hz, NHAlloc), 7.62 (d, 2H, *J* 8.5 Hz, Ar H),

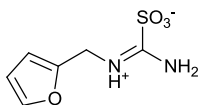
7.32 (d, 2H, J 8.5 Hz, Ar H), 5.95 (m, 2H, $2 \times$ Allyl =CH), 5.22 (m, 4H, $2 \times$ Allyl =CH₂), 4.87 (s, 2H, benzyl CH₂), 4.59 (dt, 2H, J 5.2, 1.9 Hz, Allyl CH₂), 4.47 (d, 2H, J 5.3 Hz, Allyl CH₂), 4.11 (m, 1H, Glu- α), 2.45 (t, 2H, J 7.4 Hz, Glu- γ), 2.10 (m, 1H, $\frac{1}{2}$ Glu- β), 1.85 (m, 1H, $\frac{1}{2}$ Glu- β). Found: C 50.05, H 4.96, N 8.29%; C₂₁H₂₆F₃N₃O₈ requires C 49.90, H 5.18, N 8.31; m/z (ESIMS⁺) 392 [M + H]⁺, 414 [M + Na]⁺.



Compound 9a.³ Hydrogen peroxide (3.35 mL, 29.9 mmol) was added dropwise to a cooled (0°C) and stirred suspension of 1-phenylthiourea (1.52 g, 10.0 mmol) and Na₂MoO₄·H₂O (38 mg, 0.15 mmol) in H₂O (20 mL). The mixture was gradually warmed to room temperature and stirring was continued for another 4 h before it was cooled in ice bath again. The solid precipitated out was collected by suction filtration followed by washing with ice-cooled water (3 times) to give the product as a white amorphous solid which was recrystallised by layering ether and petroleum ether over its MeOH solution to afford colourless crystals (1.54 g, 77%). δ_H (DMSO-d₆, 100.6 MHz) 11.39 (v br s, 1H, NH), 9.59 (s, 1H, NH, H-bonded), 9.28 (v br s, 1H, NH), 7.49 (t, 2H, J 7.6 Hz, H-2,6), 7.39 (t, 1H, J 7.6 Hz, H-4), 7.28 (d, J 7.6 Hz, H-3,5); δ_C (DMSO-d₆, 100.6 MHz) 165.5, 134.1, 129.9, 128.4, 125.4. Found: C 42.20, H 3.85, N 14.23, S 16.02%; Calcd. for C₇H₈N₂SO₃ C 41.99, H 4.03, N 13.99, S 16.01; m/z (ESI) 199 [M-H]⁻.

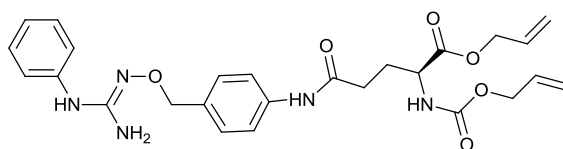


Compound 9b. This is prepared in the same way as **9a** from phenethylthiourea (0.862 g, 4.78 mmol). Colorless crystals (0.82 g, 75%). δ_H (DMSO-d₆, 300 MHz) 9.65 (br s, 1H, NH), 9.26 (br s, 2H, NH₂), 7.27 (m, 5H, Ar H), 3.47 (br s, 2H, CH₂N), 2.84 (t, 2H, J 7.5 Hz, CH₂); δ_C (DMSO-d₆, 75.5 MHz): 165.3, 137.9, 128.8, 128.3, 126.4, 43.3, 33.0; Found C 47.69, H 5.40, N 12.10; Calcd. for C₉H₁₂N₂SO₃ C 47.36, H 5.30, N 12.27; m/z (ESI) 227 [M-H]⁻.

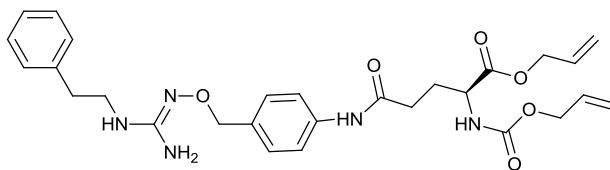


Compound 9c. This was prepared in the same way as **9a** from furfurylthiourea (2.314 g, 14.8 mmol). White solid (2.72 g, 90%). δ_H (DMSO-d₆, 400 MHz) 10.06 (br s, 1H, NH⁺), 9.48 (s, $\frac{1}{2}$ NH₂, intramolecular hydrogen bound with SO₃⁻), 9.39 (br s, $\frac{1}{2}$ NH₂, not intramolecular hydrogen bonded), 7.66 (dd, 1H, J 1.8, 0.8 Hz, furfuryl H-5), 6.43 (dd, J 3.2, 1.9 Hz, furfuryl H-4), 6.40 (d, J 3.2 Hz, furfuryl H-3), 4.48 (s, 2H, CH₂). δ_C (DMSO-d₆, 100.6 MHz): 166.2, 148.7,

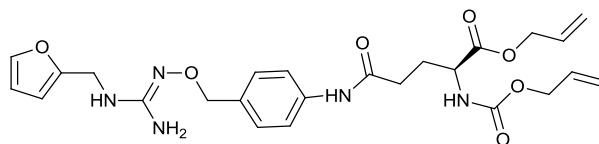
143.7, 111.0, 109.3, 38.7; Found C 35.50, H 3.88, N 13.40%; Calcd. for C₆H₈N₂SO₄ C 35.29, H 3.95, N 13.72%; m/z (ESI⁺) 227 [M+ H]⁺, m/z (ESI⁻) 203 [M-H]⁻.



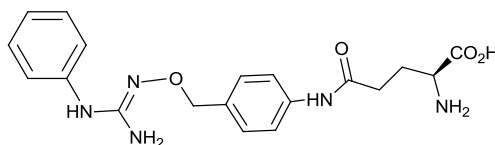
Compound 10a. DMAP (0.122 g, 1.00 mmol) and triethylamine (0.56 mL, 3.97 mmol) were added to a suspension of **9a** (0.200 g, 1.0 mmol) and **8** (0.505 g, 1.00 mmol) in DCM (10 mL). The mixture was stirred at room temperature for 24 h before the solvent was removed under reduced pressure. The residue was passed through a silica column eluted with a gradient from ethyl acetate to ethyl acetate/MeOH 100:10 to give the product as a white solid (0.24 g, 47%). δ_H (DMSO-d₆, 400 HMz) 9.92 (s, 1H, NH), 7.76 (d, 1H, J 7.9 Hz, NH), 7.71 (br s, 1H, NH), 7.54 (d, 2H, J 8.4 Hz, Ar H), 7.32 (d, 2H, J 8.4 Hz, Ar H), 7.27 (2H, d, J 7.6 Hz, Ph H), 7.15 (t, 2H, J 7.9 Hz, Ph H), 6.77 (t, 1H, J 7.2 Hz, Ph H), 5.90 (m, 2H, 2 × Allyl =CH), 5.30 (m, 4H, NH₂ + Allyl =CH₂), 5.20 (m, 2H, Allyl =CH₂), 4.76 (s, 2H, ArCH₂), 4.59 (d, 2H, J 5.3 Hz, Allyl CH₂), 4.48 (m, 2H, Allyl CH₂), 4.12 (m, 1H, Glu- α), 2.44 (t, 2H, J 5.6 Hz, Glu- γ), 2.10 (m, 1H, ½ Glu- β), 1.86 (m, 1H, ½ Glu- β); δ_C (DMSO-d₆, 100 MHz): 172.3, 170.7, 156.5, 151.9, 141.6, 138.5, 133.7, 132.6, 129.1, 129.0, 120.3, 119.2, 118.2, 117.7, 117.6, 74.5, 65.4, 65.1, 53.7, 32.8, 26.6; Found C 61.05, H 6.38, N 13.21%; Calcd. for C₂₆H₃₁N₅O₆ C 61.28, H 6.13, N 13.74%; m/z (ESI⁺) 510 [M + H]⁺, 532 [M + Na]⁺; HRESI⁺ 510.2337, Calc. for C₂₆H₃₂N₅O₆ 510.2353.



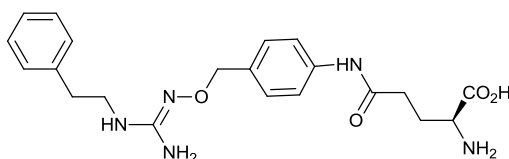
Compound 10b. This is prepared in the same way as **10a** from **9b** (0.689 g, 3.02 mmol) and **8** (1.53 g, 3.02 mmol) to give the product as a white solid (1.0 g, 53%). δ_H (DMSO-d₆) 9.91 (s, 1H, NHCO), 7.75 (d, 1H, J 7.8 Hz, NH), 7.52 (d, 2H, J 8.5 Hz, Ar H), 7.29-7.15 (m, 7H, Ar H), 5.90 (m, 2H, 2 × Allyl =CH), 5.30 (m, 2H, Allyl =CH₂), 5.19 (m, 2H, Allyl =CH₂), 5.02 (br s, 2H, NH₂), 4.87 (br s, 1H, NH), 4.63 (s, 2H, OCH₂Ar), 4.58 (dt, 2H, J 5.3, 1.4 Hz, Allyl CH₂), 4.47 (d, 2H, J 4.7 Hz, Allyl CH₂), 4.10 (m, 1H, Glu- α), 3.08 (dt, 2H, J 7.0, 6.3 Hz, NHCH₂), 2.69 (t, J 7.0 Hz, CH₂CH₂NH), 2.43 (t, 2H, J 7.3 Hz, Glu- γ), 2.10 (m, 1H, ½ Glu- β), 1.86 (m, 1H, ½ Glu- β); ¹³C NMR (DMSO-d₆) 172.2, 170.4, 156.3, 155.1, 140.2, 138.7, 134.1, 133.8, 132.7, 129.0, 128.8, 128.6, 126.3, 119.0, 118.1, 117.5, 74.3, 65.2, 64.9, 53.8, 42.7, 35.6, 32.8, 26.6; Found C 62.70, H 6.59, N 13.26%; Calcd. for C₂₈H₃₅N₅O₆ C 62.56, H 6.56, N 13.03%; m/z (ESI⁺) 538 [M + H]⁺, HRESI⁺ 538.2666, Calc. for C₂₈H₃₆N₅O₆ 538.2666.



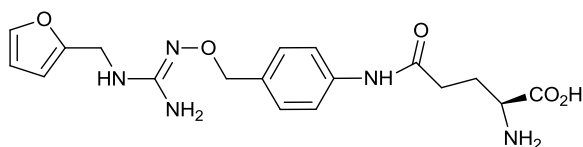
Compound 10c. This was prepared in the same way as **10a** from **9c** (0.106 g, 0.52 mmol) and **8** (0.262 g, 0.52 mmol). Off-white semi-solid (0.100 g, 38%). δ_{H} (DMSO- d_6 , ppm) 9.90 (s, 1H, NHCO), 7.76 (d, 1H, J 7.9 Hz, NH), 7.74 (dd, 1H, J 1.8, 0.8 Hz, furfuryl H-5), 7.52 (d, 2H, J 8.2 Hz, Ar H), 7.27 (d, 2H, J 8.6 Hz), 6.36 (dd, 1H, J 3.16, 1.9 Hz, furfuryl H-4), 6.19 (dd, 1H, J 3.2, 0.7 Hz, furfuryl H-3, Ar H), 5.90 (m, 2H, Allyl =CH), 5.34-5.17 (m, 4H, Allyl =CH₂), 5.13 (br s, 1H, NH), 4.99 (br s, 2H, NH₂), 4.62 (s, 2H, CH₂), 4.59 (dt, 2H, J 5.3, 1.4 Hz, Allyl CH₂), 4.49 (m, 2H, J 4.7 Hz, Allyl CH₂), 4.10 (m, 1H, Glu- α), 4.03 (d, 2H, J 4.9 Hz, furfuryl CH₂), 2.44 (t, 2H, J 7.4 Hz, Glu- γ), 2.11 (m, 1H, $\frac{1}{2}$ Glu- β), 1.87 (m, 1H, $\frac{1}{2}$ Glu- β). δ_{C} (DMSO- d_6 , 100 MHz): 171.9, 170.1, 156.0, 154.3, 153.3, 141.7, 138.3, 133.7, 133.5, 132.4, 128.5, 118.6, 117.7, 117.1, 110.4, 106.6, 73.8, 64.9, 64.6, 53.5, 37.7, 32.5, 26.3; Found C 58.88, H 6.42, N 13.14%; C₂₅H₃₁N₅O₇ requires C 58.47, H 6.08, N 13.64%; m/z (ESI⁺) 514 [M + H]⁺; m/z (HRESI⁺) 514.2300, Calcd. for C₂₅H₃₁N₅O₇ 514.2302.



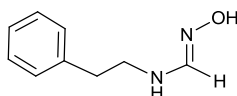
Compound 4a. [Pd(PPh₃)₄] (0.046 g, 0.04 mmol) and phenylsilane (0.6 mL, 3.33 mmol) were added to a degassed solution of **10a** (0.203 g, 0.40 mmol) in DCM (10 mL). The mixture was stirred at room temperature for 6h with the vessel covered in foil to exclude light from the reaction. MeOH (1 mL) was added to quench the excess of phenylsilane and the mixture was stirred for another 20 min before the solvents were replaced with diethyl ether. The resulting solid was filtered off and washed with diethyl ether and then taken up in distilled water (200 mL). The aqueous solution was filtered through a celite pad and then a C-18 cartridge (eluted with acetonitrile and water = 1 : 1). The ninhydrin-active fraction was collected and freeze dried to give the product as a white solid (0.137 g, 89%). δ_{H} (DMSO- d_6 , 400 MHz) 10.38 (s, 1H, NHC=O), 8.02 (br s, 1H, NH of guanidine), 7.58 (d, 2H, J 8.4 Hz, ArH), 7.31 (d, 4H, J 8.4 Hz, Ar H), 7.13 (2H, t, J 7.9 Hz, Ph H), 6.74 (t, 1H, J 7.3 Hz, Ph H), 5.41 (br s, 2H, NH₂), 4.75 (s, 2H, ArCH₂), 3.42 (br s, H₂O + NH₃⁺), 3.32 (t, 1H, J 6.38 Hz, Glu- α), 2.50 (m, Glu- γ + DMSO- d_6), 1.98 (m, 2H, Glu- β); δ_{C} (DMSO- d_6 , 100.6 MHz): 170.8, 170.5, 151.5, 141.7, 138.5, 133.5, 128.5, 128.4, 119.2, 118.0, 117.0, 74.0, 53.5, 32.8, 27.0; Found C 58.93, H 5.48, N 17.99%; Calcd. for C₁₉H₂₃N₅O₄ C 59.19, H 6.01, N 18.16%; m/z (ESI⁺) 386 [M + H]⁺; HRESI⁺, 386.1830, Calcd. for C₁₉H₂₄N₅O₄ 386.1830.



Compound 4b. This is prepared in the same as **4a** from **10b** (0.263 g, 0.489 mmol), [Pd(PPh₃)₄] (0.056 g, 0.049 mmol) and phenylsilane (0.6 mL, 4.0 mmol). White solid (0.150 g, 74%). δ_{H} (MeOD, 300 MHz) 7.48-7.24 (m, 7H, Ar H), 7.11 (d, 2H, *J* 8.2 Hz, Ar H), 4.75 (s, 2H, CH₂), 3.74 (t, 1H, *J* 6.2 Hz, Glu- α), 3.32 (t, 2H, *J* 6.6 Hz, NHCH₂), 2.69 (t, 2H, *J* 6.6 Hz, NHCH₂CH₂), 2.60-2.54 (m, 2H, Glu- γ), 2.16 (q, 2H, *J* 7.4 Hz, Glu- β); δ_{C} (62.5 MHz, DMSO-*d*₆) 27.6, 33.5, 35.8, 42.9, 54.1, 74.4, 119.2, 126.5, 128.8, 129.0, 129.2, 133.7, 138.5, 140.0, 154.8, 170.0, 170.8; ν_{max} (cm⁻¹, KBr disc) 3417, 3297, 3104, 3060 (NH, NH₂, NH₃⁺), 1654, 1604 (C=O and C=C), 1522 (C=C and C=N); *m/z* (ESI⁺) 414 [M + H]⁺, HRESI⁺ 414.2138, Calcd. for C₂₁H₂₈N₅O₄ 414.2141.

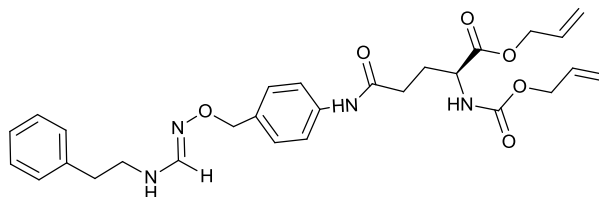


Compound 4c. This was prepared in the same way as **4a** from **10c** (0.300 g, 0.584 mmol). Pale yellow solid (0.083 g, 37%). δ_{H} (DMSO-*d*₆) 10.38 (s, 1H, NH), 7.56 (dd, 2H, 8.5 Hz, Ar H), 7.54 (d, furfuryl H-5), 7.25 (d, 2H, *J* 8.5 Hz, Ar H), 6.36 (dd, 1H, *J* 3.2, 1.9 Hz, furfuryl H-4), 6.20 (d, 1H, *J* 3.1, 0.6 Hz, furfuryl H-3), 5.42 (br s, 1H, NH), 5.19 (br s, 2H, NH₂), 4.63 (s, 2H, ArCH₂), 4.04 (s, 2H, furfuryl CH₂), 3.40 (br s, NH₃⁺ + H₂O from DMSO-*d*₆), 3.32 (t, *J* 6.4 Hz, Glu- α), 2.50 (m, Glu- γ + DMSO-*d*₆), 1.98 (m, 2H, Glu- β); δ_{C} (DMSO-*d*₆, 100.6 MHz) 170.8, 170.5, 154.5, 153.2, 141.8, 138.5, 133.4, 128.6, 118.7, 110.4, 106.7, 74.1, 53.5, 37.7, 32.8, 27.0; *m/z* (ESI⁺) 390 [M + H]⁺, *m/z* (HRESI⁺) 390.1786; Calcd. for C₁₈H₂₄N₅O₅ 390.1777.

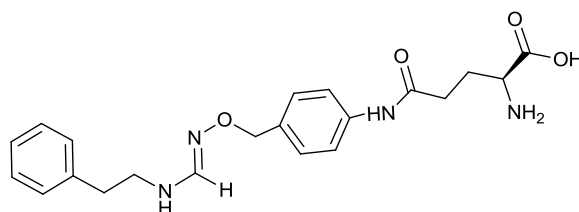


Compound 12.⁴ This is prepared by adopting literature method for similar compounds.⁴ Phenylethylamine hydrochloride (2.0 g, 12.7 mmol) was heated at reflux in dimethylformamide dimethylacetal (34 mL, 256 mmol). After the excess of DMF dimethylacetal was removed in vacuum, the intermediate **11** was taken up in MeOH. Hydroxylamine hydrochloride (3.2 g, 31.7 mmol) and sodium acetate (5.6 g, 68.3 mmol) was added and the solution was stirred at RT overnight. The mixture was filtered off to remove the solid and the filtrate was concentrated under vacuum. The residue was taken up in water and sonicated. The solid precipitated out was collected by filtration followed by washing with water to give the product as white flake solid.

(1.3 g, 63%). δ_{H} (DMSO- d_6 , 300 MHz) 8.99 (s, 1H, OH), 7.31-7.16 (m, 5H, Ph H), 6.56 (d, 1H, J 11.0 Hz, CH), 5.71 (dt, 1H, J 11.0, 6.0 Hz, NH), 3.19 (dt, 2H, J 6.3, 8.3 Hz, CH_2NH), 2.71 (t, 2H, J 7.4 Hz, PhCH_2); δ_{C} (DMSO- d_6 , 75.5 MHz) 145.1, 139.3, 128.7, 128.3, 126.0, 45.9, 37.7; Found: C 65.39, H 7.63, N 17.38%; Calcd. for $\text{C}_9\text{H}_{12}\text{N}_2\text{O}$ C 65.83, H 7.36, N 17.06%; m/z (ESI^+) 165 $[\text{M} + \text{H}]^+$.



Compound 13. Phenylethylamine (1.3 ml, 10.0 mmol) was added to dimethylformamide dimethylacetal (2.66 ml, 20.0 mmol). The mixture was heated at 120°C for 4 h before the excess dimethylacetal was removed in vacuum. The intermediate **11** was dissolved in THF (10 mL). Compound **8** (0.505 g, 1 mmol) and DBU (0.16 ml, 1.07 mmol) was added to the solution and the mixture was heated at reflux in an argon atmosphere overnight and then cooled to RT. After solvent removal, the residue was purified by column chromatography (EtOAc to EtOAc/MeOH 95 : 5) to give the product as colourless gum which solidified as wax upon treatment with diethyl ether (0.15 g, 29%). δ_{H} (CDCl_3) 8.18 (s, 1H, NH), 7.46 (d, J 8.2 Hz, Bn H), 7.31-7.26 (m, 5H, Ph H), 7.08 (d, 2H, J 8.2 Hz Bn H), 6.47 (d, 1H, J 11.3 Hz, CH), 5.81 (2H, Allyl =CH), 5.61 (d, 1H, J 8.1 Hz, NHAlloc), 5.28-5.12 (m, 4H, Allyl =CH₂), 4.94 (dt, 1H, J 6.72, 10.8 Hz, NHCN), 4.56 (2H, dt, J 5.9, 1.2 Hz, Allyl CH₂), 4.50 (dd, J 5.6, 1.3 Hz, Allyl CH₂), 4.35 (m, 1H, Glu- α), 3.23 (q, 2H, J 6.8 Hz, CH₂N), 2.70 (t, 2H, J 7.02 Hz, PhCH₂), 2.38 (m, 2H, Glu- γ), 2.26 (m, 1H, $\frac{1}{2}$ Glu- β), 1.95 (m, 1H, $\frac{1}{2}$ Glu- β). δ_{C} (CDCl_3) 171.8, 10.4, 156.8, 145.9, 138.5, 137.8, 134.3, 132.6, 131.5, 129.1, 129.0, 128.9, 128.8, 126.8, 119.9, 119.5, 118.3, 75.1, 66.6, 66.3, 53.6, 47.0, 38.4, 34.0, 29.6; Found C 63.17, H 6.50, 10.23%; Calcd. for $\text{C}_{28}\text{H}_{34}\text{N}_4\text{O}_6 \cdot 0.5 \text{H}_2\text{O}$ C 63.26, H 6.64, N 10.23%; m/z (ESI^+) 523 $[\text{M} + \text{H}]^+$, 545 $[\text{M} + \text{Na}]^+$.



Compound 14. This was prepared in the same way as for **4a** from **13** (0.212 g, 0.406 mmol) as white solid (0.210 g, 53%). Mp. >184°C (decom). δ_{H} (DMSO- d_6 , 300 MHz) 10.38 (s, 1H, NH), 7.55 (dd, 2H, J 8.5 Hz, Ar H), 7.30-7.16 (m, 7H, Ar H), 6.57 (d, 1H, J 11.37, 6.6 Hz, CH=N), 6.02 (dt, 1H, J 11.3, 7.3 Hz, NHC=N), 4.75 (s, 2H, Bn CH₂), 3.56-3.12 (br s + m, Glu- α + CH₂N + NH_3^+ + H_2O from DMSO- d_6), 2.71 (t, 2H, J 7.4 Hz, PhCH₂), 2.50 (m, Glu- γ + DMSO- d_6), 1.94 (m, 2H, Glu- β). δ_{C} (DMSO- d_6 , 75.5 MHz) 170.8 (overlapped), 145.4, 139.1, 138.5, 133.4, 128.7, 128.2, 128.1, 126.0, 118.7, 73.7, 53.5, 46.2, 37.3, 32.9, 27.1; m/z (ESI^+) 399 $[\text{M} + \text{H}]^+$, 421 $[\text{M} + \text{Na}]^+$; m/z (HRESI⁺) 399.2035, Calcd. For $\text{C}_{21}\text{H}_{27}\text{N}_4\text{O}_4$ 399.2032.

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^1H and ^{13}C NMR spectra of the synthesised compounds

