Supporting Information

Building of neomycin-nucleobase-amino acid conjugates for the inhibition of oncogenic miRNAs biogenesis.

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Figure S1. Schematic representation of possible Hoogsteen interactions formed between nucleobase S and T-A base pair.¹
Figure S2. Inhibition curves of compounds 4a-h (concentrations from 15 nM to 125 µM) in the presence of pre-miR-372 and recombinant Dicer enzyme.
Figure S3. Binding curves of compounds 4a-h (concentrations from 61 nM to 1 µM) in the presence of pre-miR-372.
Figure S4. Microscopic observation of gastric adenocarcinoma (AGS) cells without treatment (A), after 48h treatment with 50 μM of compound 4a (B), 4b (C), 4c (D), 4g (E), 4h (F) or with 25 nM of a scrambled oligonucleotide (G) or of a antimiR-372/373 oligonucleotide (H). Cell layers have been observed in phase contrast on an inverted light microscope (Zeiss) equipped with a 20X objective.
Figure S5. Relative viability of AGS cells that express high miR-372 levels in the presence of increasing concentrations of Neo-S, Neo-S-Ar and 4b. Cell growth was measured using the Cell titer reagent (Promega). Measurements were performed after a treatment of 4 days. Bars represent the mean ± standard deviation (SD) of cell-viability data relative to untreated cells.
**Figure S6.** RT-PCR quantification of LATS2 mRNA and pri-miR-371-372-373 after a 4-day treatment by 4b at the indicated concentrations. Bars represent the mean ± SD of LATS2 or pri-miR-371-373 expressions normalized to the housekeeping genes and compared to untreated cells (n = 4).
Figure S7. RT-qPCR quantification of miR-371, miR-372, miR-373, miR-17-5p, miR-200b and let-7i after a 4-day treatment of AGS cells in the presence of 50 μM of compound 4b. Bars represent the mean ± SD of miRNA expression normalized to the small nucleolar RNA RNU49 and compared to untreated cells (n = 4). *** p < 0.001, ** p < 0.01, *p < 0.05 (Student’s t test).
Figure S8. Footprinting analyses. A) Probing of the interaction of pre-miR-372 and 4b with RNase S1. Lane 1 represents intact RNA; lanes 2 and 3 represent the alkaline-hydrolysis ladder and T1-digestion ladder, respectively; lane 4 represents the cleavage pattern of uncomplexed pre-miR-372 in the presence of 2U of enzyme; lane 5 represents the cleavage pattern of uncomplexed pre-miR-372 in the presence of 1U of enzyme; lanes 6-11 represent the cleavage pattern of pre-miR-372 complexed with 0.05, 0.1, 0.5, 1, 5 and 10 mM of 4b in the presence of 1U of S1 enzyme.
**Figure S9.** Footprinting analyses. A) Sequence and secondary structure of pre-miR-372. B) Probing of the interaction of pre-miR-372 and 4a with Dicer enzyme. Lane 1 represents intact RNA; lanes 2 and 3 represent the alkaline-hydrolysis ladder and T1-digestion ladder, respectively; lane 4 represents the cleavage pattern of uncomplexed pre-miR372; lanes 5–9 represent the cleavage pattern of pre-miR-372 complexed with 0.05, 0.1, 0.5, 1, and 5 mM of 4a.
Figure S10. Quantification of Dicer footprinting analyses relative to gels showed in Figure 4 and Figure S7.
Figure S11. Docking of 4a with the pre-miR-372 hairpin loop performed by using autodock4, in which the grid boxes were fixed on the entire RNA sequence.
Synthetic Procedures.

Synthesis of amino acids-S nucleobase alkynes.

Scheme S1. Synthesis of alkynes 2a-c. Reagents: a) Boc-Lys(Boc), Boc-His(Boc) or Boc-Ala, HBTU, DIPEA, DMF, 80°C overnight; b) H₂Pd/C, DCM, MeOH, r.t., 1h; c) 4-pentynoic acid, Et₃N, chloromethylpyridinium iodide, DCM, r.t., 1h.

4-(3-nitrophenyl)thiazole-2-N-[Nα,Nε-(di-tert-butoxycarbonyl)]-lysinamide (6a). A mixture of 4-(3-nitrophenyl)thiazole-2-amine¹ (5, 500 mg, 2.26 mmol), Boc-Lys(Boc)-OH-DCHA (1.79 g, 3.39 mmol, 1.5 eq.), HBTU (1.29 g, 3.39 mmol, 1.5 eq.), DIPEA (1.18 mL, 6.78 mmol, 3 eq.) in DMF (20 mL) was stirred at 80°C overnight. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column using a mixture DCM/acetone 95:5 as the eluent leading to desired compound 6a as a yellow solid: 443.5 mg (36%); R_f = 0.25 (DCM/Acetone 95:5); ¹H NMR (200MHz, Acetone-d₆) δ (ppm): 11.3 (br, 1H), 8.74 (t, J = 1.9 Hz, 1H), 8.32 (dt, J = 7.8, 1.2 Hz, 1H), 8.16 (ddd, J = 8.2, 2.3, 0.9 Hz, 1H), 7.78 (s, 1H), 7.72 (t, J = 8.0 Hz, 1H), 6.45 (d, J = 7.2 Hz, 1H), 6.00 (t, J = 7.2 Hz, 1H), 4.47 (q, J = 7.2 Hz, 1H), 3.09 (q, J = 6.0 Hz, 2H), 1.99-1.77 (m, 2H), 1.64-1.52 (m, 4H), 1.42 (s, 9H), 1.39 (s, 9H); ¹³C NMR (50MHz, Acetone-d₆) δ (ppm): 173.5, 160.2, 157.8, 150.7, 149.0, 138.2, 133.4, 131.9, 124.0, 122.3, 112.0, 80.6, 79.4, 56.7, 41.5, 33.3, 29.6, 24.7; mass spectrum (ESI), m/z 550.8 (M+H)⁺ (theoretical 550.2).

4-(3-nitrophenyl)thiazole-2-N-[Nα,Nε-(di-tert-butoxycarbonyl)]-histidinamide (6b). A solution of 4-(3-nitrophenyl)thiazol-2-amine¹ (5, 500 mg, 2.26 mmol), Boc-His(Boc)-
OH-DCHA (1.82 g, 3.39 mmol, 1.5 eq.), HBTU (1.29 g, 3.39 mmol, 1.5 eq.) and DIPEA (1.18 mL, 6.78 mmol, 3 eq.) in DMF (20 mL) was stirred at 80°C overnight. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column using a mixture DCM/MeOH 9:1 as the eluent leading to compound 6b as a yellow solid: 204 mg (20%); Rf = 0.73 (Cyclohexane/EtOAc 1: + 5% MeOH); 1H NMR (200 MHz, Acetone-d6) δ 11.60 (s, 1H), 7.66 (t, J = 8.0 Hz, 1H), 7.36 (s, 1H), 6.70 (d, J = 7.6 Hz, 1H), 4.82 (m, 1H), 3.34-3.08 (m, 2H), 1.58 (s, 9H), 1.40 (s, 9H); 13C NMR (50 MHz, Acetone-d6) δ 172.6, 160.1, 157.5, 150.6, 149.0, 148.7, 148.0, 138.8, 138.1, 133.4, 131.8, 123.9, 122.2, 116.7, 112.0, 87.0, 80.8, 56.4, 31.9, 29.5, 28.9; mass spectrum (ESI), m/z 459.5 (M+H-Boc)+ (theoretical 459.2).

4-(3-nitrophenyl)thiazole-2-N-[Nα,Nε-(di-tert-butoxycarbonyl)]-alaninamide (6c). A mixture of 4-(3-nitrophenyl)thiazol-2-amine1 (5, 500 mg, 2.26 mmol), Boc-Ala-OH (641.5 mg, 3.39 mmol, 1.5 eq.), HBTU (1.29 g, 3.39 mmol, 1.5 eq.) and DIPEA (1.18 mL, 6.78 mmol, 3 eq.) in DMF (20 mL) was stirred at 80°C overnight. After removal of the solvent under reduced pressure, the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 7:3 as the eluent leading to compound 6c as a yellow solid: 299 mg (33%); Rf = 0.37 (Cyclohexane/EtOAc 7:3); 1H NMR (200 MHz, Acetone-d6) δ 11.34 (s, 1H), 8.65 (t, J = 1.9 Hz, 1H), 8.30 (dt, J = 7.8, 1.2 Hz, 1H), 8.10 (ddd, J = 8.2, 2.3, 0.9 Hz, 1H), 7.71 (s, 1H), 7.69 (t, J = 8.0 Hz, 1H), 6.46 (d, J = 7.2 Hz, 1H), 4.50 (m, 1H), 1.55 (s, 3H), 1.40 (s, 9H); 13C NMR (50 MHz, Acetone-d6) δ 160.3, 150.7, 149.0, 138.2, 133.5, 131.9, 124.0, 122.3, 112.0, 80.7, 52.3, 29.5, 18.9; mass spectrum (ESI), m/z 415.6 (M+Na)+ (theoretical 415.1).

4-(3-aminophenyl)thiazole-2-N-[Nα,Nε-(di-tert-butoxycarbonyl)]-lysinamide (7a). Compound 6a (243.5 mg, 0.44 mmol) was dissolved in a mixture of CH2Cl2 and MeOH (1:1, v/v, 20 mL) and was stirred under an hydrogen atmosphere in the presence of 10% palladium on activated carbon (54.2 mg) for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure leading the desired compound 7a as a yellow solid: 109.4 mg (47%); Rf = 0.5 (1:1 Cyclohexane/EtOAc); 1H NMR (200MHz, Acetone-d6) δ (ppm): 7.31 (s, 1H), 7.20-7.00 (m, 3H), 6.60-6.55 (m, 1H), 4.46-4.39 (m, 1H), 3.10-3.00 (m, 2H), 1.60-1.50 (m, 4H), 1.41 (s, 9H), 1.39 (s, 9H), 1.25-1.30 (m, 2H); 13C NMR (50MHz, Acetone-d6) δ (ppm): 158.1, 156.8, 153.2, 151.3, 150.6, 149.4, 136.2, 130.0, 121.2, 119.6, 117.7, 114.8, 107.8, 79.6, 78.4, 55.6, 40.5, 32.3, 27.5, 23.7; mass spectrum (ESI), m/z 520.8 (M+H)+ (theoretical 520.2).
4-(3-aminophenyl)thiazole-2-N-[Na,Na-(di-tert-butoxycarbonyl)]-histidinamide (7b). A solution of compound 6b (79 mg, 0.14 mmol) in a mixture of CH₂Cl₂ and methanol (1:1, v/v, 20 mL) was stirred under an hydrogen atmosphere in the presence of 10% palladium on activated carbon (34.5 mg) for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure to give compound 7b as a yellow solid: 73.6 mg (98%); R_f = 0.1 (Cyclohexane/EtOAc 1:1); ^1H NMR (500MHz, CD₃OD) δ (ppm): 8.14 (s, 1H), 7.37-7.18 (m, 4H), 7.13 (t, J = 7.8 Hz, 1H), 6.68 (d, J = 7.3 Hz, 1H), 4.65-4.39 (m, 1H), 3.16-2.92 (m, 2H), 1.59 (s, 9H), 1.42 (s, 9H); ^13C NMR (125MHz, CD₃OD) δ (ppm): 172.1, 158.9, 157.8, 151.8, 149.0, 148.1, 139.6, 138.5, 136.6, 117.3, 116.4, 114.2, 108.5, 87.2, 81.0, 55.6, 45.1, 28.7, 28.0, 27.3, 20.9. mass spectrum (ESI), m/z 529.1 (M+H)^+ (theoretical 529.2).

4-(3-aminophenyl)thiazole-2-N-[Na,Na-(di-tert-butoxycarbonyl)]-alaninamide (7c). A solution of compound 6c (151 mg, 0.385 mmol) in a mixture of DCM and MeOH (1:1, v/v, 20 mL) was stirred under an hydrogen atmosphere in the presence of 10% palladium on activated carbon (47 mg) for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated under reduced pressure to give the product as a yellow solid: 130 mg (93%); R_f = 0.5 (Cyclohexane/EtOAc 1:1); ^1H NMR (200MHz, Acetone-d₆) δ (ppm): 7.55-7.50 (m, 1H), 7.25-7.00 (m, 3H), 6.70-6.60 (m, 1H), 4.55-4.50 (m, 1H), 1.47 (d, J = 6.0 Hz, 3H), 1.41 (s, 9H); ^13C NMR (50MHz, Acetone-d₆) δ (ppm): 172.5, 158.1, 156.4, 149.4, 145.3, 135.4, 130.0, 121.7, 115.6, 114.9, 107.8, 79.7, 51.0, 25.7, 18.0; mass spectrum (ESI), m/z 385.5 (M+Na)^+ (theoretical 385.1).

4-(3-pentynoylamidophenyl)thiazole-2-N-[Na,Na-(di-tert-butoxycarbonyl)]-lysinamide (2a). 4-Pentyonoic acid (20.8 mg, 0.21 mmol, 1.1 eq.) was dissolved in dry dichloromethane (20 mL) and then triethylamine (80 μL, 0.38 mmol, 2 eq.), chloromethylpyridinium iodide (98.6 mg, 0.38 mmol, 2 eq.) and compound 7a (100 mg, 0.19 mmol) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture Cyclohexane/EtOAc 1:1 as the eluent leading to desired compound 2a as a slightly yellow solid: yield 57 mg (50%); R_f = 0.29 (Cyclohexane/EtOAc 1:1); ^1H NMR (200 MHz, Acetone-d₆) δ 11.22 (br, 1H), 9.26 (br, 1H), 8.38 (s, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.42 (s, 1H), 7.31 (t, J = 7.9 Hz, 1H), 6.40 (d, J = 7.4 Hz, 1H), 5.99 (br, 1H), 4.44 (m, 1H), 3.09 (m, 2H), 2.71-2.46 (m, 4H), 2.39 (t, J = 2.3 Hz, 1H), 1.98-1.70 (m, 2H), 1.60-1.38 (m, 22H); ^13C NMR (50 MHz, Acetone-d₆) δ 173.3, 171.1, 170.2, 159.5, 157.8, 151.5,
141.6, 137.2, 130.8, 122.8, 120.4, 118.9, 109.6, 85.0, 80.6, 79.4, 71.3, 56.7, 41.6, 37.6, 33.4, 29.6, 28.5, 24.7, 16.0; mass spectrum (ESI), \(m/z\) 601.0 (M+H)^+ (theoretical 601.3).

4-(3-pentynoylamidophenyl)thiazole-2-N-[\(\text{N}^\alpha,\text{N}^\varepsilon\)-(di-tert-butoxycarbonyl)]-histidinamide 2b. 4-Pentynoic acid (10.7 mg, 0.11 mmol, 1.1 eq.) was dissolved in dry DCM (15 mL) and then triethylamine (46 \(\mu L\), 0.20 mmol, 2 eq.), chloromethylpyridinium iodide (56.2 mg, 0.20 mmol, 2 eq.) and compound 7b (63.6 mg, 0.10 mmol) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 1:1 as the eluent leading to desired compound 2b as a slightly yellow solid: yield 31.6 mg (47%); \(R_f = 0.24\) (Cyclohexane/EtOAc 1:1); \(^1\text{H} \text{NMR}\) (500 MHz, CD\(_3\)OD) \(\delta 8.24\) - 8.09 (m, 2H), 7.63 (d, \(J = 7.8\) Hz, 1H), 7.47 (t, \(J = 7.8\) Hz, 1H), 7.39-7.31 (m, 3H), 4.64-4.52 (m, 1H), 3.13-2.89 (m, 2H), 2.64-2.42 (m, 4H), 2.29 (t, \(J = 2.5\) Hz, 1H), 1.59 (s, 9H), 1.42 (s, 9H); \(^{13}\text{C} \text{NMR}\) (125 MHz, CD\(_3\)OD) \(\delta 173.4, 172.8, 172.4, 159.2, 157.8, 151.8, 148.6, 140.2, 136.6, 130.1, 123.0, 120.8, 119.0, 109.2, 87.2, 83.6, 70.0, 36.9, 28.7, 28.0, 27.3, 15.6; mass spectrum (ESI), \(m/z\) 610.0 (M+H)^+ (theoretical 610.2).

4-(3-pentynoylamidophenyl)thiazole-2-N-[\(\text{N}^\alpha,\text{N}^\varepsilon\)-(di-tert-butoxycarbonyl)]-alaninamide (2c). 4-Pentynoic acid (29.4 mg, 0.36 mmol, 1.1 eq.) was dissolved in dry DCM (30 mL) and then triethylamine (0.12 mL, 0.66 mmol, 2 eq.), chloromethylpyridinium iodide (153.5 mg, 0.66 mmol, 2 eq.) and compound 7c (120 mg, 0.33 mmol) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 5:5 as the eluent leading to desired compound 2c as a slightly yellow solid: yield 85 mg (58%); \(R_f = 0.6\) (Cyclohexane/EtOAc 1:1); \(^1\text{H} \text{NMR}\) (500 MHz, Acetone-\(d_6\)) \(\delta 11.14\) (br, 1H), 9.27 (br, 1H), 8.35 (s, 1H), 7.58 (d, \(J = 7.7\) Hz, 1H), 7.49 (d, \(J = 7.6\) Hz, 1H), 7.40 (s, 1H), 7.31 (d, \(J = 7.9\) Hz, 1H), 6.45 (d, \(J = 5.5\) Hz, 1H), 4.52 (t, \(J = 6.4\) Hz, 1H), 2.67-2.56 (m, 4H), 2.37 (t, \(J = 2.6\) Hz, 1H), 1.48 (d, \(J = 7.1\) Hz, 3H), 1.41 (s, 9H); \(^{13}\text{C} \text{NMR}\) (125 MHz, Acetone-\(d_6\)) \(\delta 173.6, 171.2, 159.5, 157.4, 151.4, 141.5, 137.1, 130.7, 122.8, 120.5, 118.9, 109.6, 84.5, 80.7, 71.3, 52.2, 37.6, 28.4, 19.0, 16.0; mass spectrum (ESI), \(m/z\) 443.6 (M+H)^+ (theoretical 443.2).
Scheme S2. Synthesis of alkynes 2d-f. Reagents: a) Chloromethylpyridinium iodide, Et3N, DCM Δ, 1h; b) TFA, DCM, r.t., overnight; c) Boc-Lys(Boc), Boc-His(Boc) or , HBTU, DIPEA, DCM, r.t., overnight.

**Nα-[tert-butoxycarbonyl]-[N-(2-N-acetylamino-4-(3-aminophenyl)-thiazole)]-propargylglycinamide (8).** Boc-L-propargylglycine (220 mg, 1.03 mmol) was dissolved in dry dichloromethane (55 mL) and then triethylamine (0.43 mL, 2.06 mmol, 2 eq.), chloromethylpyridinium iodide (528 mg, 2.06 mmol, 2 eq.) and compound S1 (265 mg, 1.13 mmol, 1.1 eq.) were added successively. The reaction mixture was stirred under reflux for 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 1:1 leading to desired compound 8 as a slightly yellow solid: yield 353.5 mg (80%); Rf = 0.5 (Cyclohexane/EtOAc 1:1); 1H NMR (200 MHz, Acetone-d6) δ 11.16 (br, 1H), 9.39 (br, 1H), 8.36 (s, 1H), 7.62 (dt, J = 7.8, 1.2 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.39 (s, 1H), 7.33 (t, J = 7.9 Hz, 1H), 6.38 (d, J = 7.9 Hz, 1H), 4.46 (q, J = 7.0 Hz, 1H), 2.77 (td, J = 6.1, 2.6 Hz, 2H), 2.45 (t, J = 2.6 Hz, 1H), 2.29 (s, 3H), 1.43 (s, 9H); 13C NMR (50 MHz, Acetone-d6) δ 170.8, 170.1, 159.8, 151.1, 141.0, 137.3, 130.8, 123.2, 120.8, 119.3, 109.4, 81.7, 80.9, 73.4, 56.0, 29.5, 25.5, 23.8; mass spectrum (ESI), m/z 451.6 (M+Na)⁺ (theoretical 451.2).

**N-[2-N-acetylamino-4-(3-aminophenyl)-thiazole]-propargylglycinamide (9).** To a solution of 8 (313.5 mg, 0.73 mmol) in DCM (5 mL) was added TFA (0.56 mL, 7.3 mmol, 10 eq.). The reaction was stirred at room temperature overnight. The solvent was then removed under
reduced pressure and the crude product was employed in the next step without further purification; mass spectrum (ESI) m/z 329.3 (M+H)^+ (theoretical 329.1).

N-[2-N-acetylamino-4-(3-aminophenyl)-thiazole]-[Nα,Nε-(di-tert-butoxycarbonyl)]-lysyl-glycinamide (2d). A mixture of 9 (50 mg, 0.113 mmol), Boc-Lys(Boc)-OH-DCHA (59.6 mg, 0.113 mmol, 1 eq.), HBTU (42.9 mg, 0.113 mmol, 1 eq.) and DIPEA (59 μL, 0.339 mmol, 3 eq.) in DCM (13 mL) was stirred at room temperature overnight. Solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to desired compound 2d as a yellow solid: 64 mg (86%); Rf = 0.15 (Cyclohexane/EA 1/1); 1H NMR (500 MHz, Acetone-d6) δ 11.14 (br, 1H), 9.24 (br, 1H), 8.38 (s, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.62 (d, J = 7.9 Hz, 1H), 7.37 (s, 1H), 7.33 (t, J = 7.9 Hz, 1H), 6.58 (br, 1H), 5.99 (br, 1H), 4.67 (q, J = 6.4 Hz, 1H), 4.11-4.02 (m, 1H), 3.04-2.97 (m, 2H), 2.90-2.80 (m, 2H), 2.53 (t, J = 2.6 Hz, 1H), 2.28 (s, 3H), 1.90-1.72 (m, 2H), 1.57-1.33 (m, 22H); 13C NMR (125 MHz, Acetone-d6) δ 174.6, 170.4, 170.2, 157.2, 150.6, 148.3, 140.4, 140.2, 130.2, 123.3, 121.0, 119.3, 109.3, 81.6, 81.3, 79.6, 73.4, 57.9, 54.5, 41.4, 39.7, 29.7, 29.6, 28.5, 24.5, 23.8, 22.9; mass spectrum (ESI), m/z 658.1 (M+H)^+ (theoretical 658.3).

N-[2-N-acetylamino-4-(3-aminophenyl)-thiazole]-[Nα,Nε-(imidazole)-(di-tert-butoxycarbonyl)]-histidyl-glycinamide (2e). A mixture of 9 (50 mg, 0.113 mmol), Boc-His(Boc)-OH-DCHA (60.6 mg, 0.113 mmol, 1 eq.), HBTU (42.9 mg, 0.113 mmol, 1 eq.) and DIPEA (59 μL, 0.339 mmol, 3 eq.) in DCM (13 mL) was stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound 2e as a yellow solid: 71 mg (94%); Rf = 0.18 (Cyclohexane/EtOAc 4:6); 1H NMR (500 MHz, Acetone-d6) δ 11.22; 11.17 (br, 1H), 9.83; 9.57 (br, 1H), 8.36; 8.32 (s, 1H), 8.13 (s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.40-7.06 (m, 3H), 6.65; 6.49 (br, 1H), 4.70 (m, 1H), 4.50-4.34 (m, 1H), 3.18-3.01 (m, 2H), 2.81 (m, 2H), 2.47; 2.44 (t, J = 2.6 Hz, 1H), 2.28 (s, 3H), 1.59; 1.40-1.33 (s, 18H); 13C NMR (125 MHz, Acetone-d6) δ 172.9, 169.9, 169.8, 169.6, 159.3, 157.2, 150.6, 150.5, 148.3, 140.4, 140.2, 130.2, 130.1, 122.7, 120.8, 119.2, 108.7, 86.7, 80.9, 80.6, 72.9, 56.2, 53.9, 39.2, 28.9, 28.3, 23.3, 22.5; mass spectrum (ESI), m/z 667.1 (M+H)^+ (theoretical 667.3).

N-[2-N-acetylamino-4-(3-aminophenyl)-thiazole]-[Nα-(tert-butoxycarbonyl)-N,N-(di-benzyloxy carbonylguanidino)]-arginyl-glycinamide (2f). A mixture of 9 (50 mg, 0.113 mmol), di-Z-Arg(Boc)-OH (61.3 mg, 0.113 mmol, 1 eq.), HBTU (42.9 mg, 0.113 mmol, 1 eq.), DIPEA (59 μL, 0.339 mmol, 3 eq.) in DCM (13 mL) was stirred at room temperature overnight.
Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound 2f as a yellow solid: 87.5 mg (91%); R_f = 0.29 (Cyclohexane/EtOAc 1:1); 1H NMR (500 MHz, Acetone-d_6) δ 11.15 (br, 1H), 9.46 (br, 2H), 9.26 (br, 1H), 8.37 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.65-7.57 (m, 2H), 7.50-7.22 (m, 13H), 6.50 (d, J = 5.1 Hz, 1H), 5.28 (s, 2H), 5.12 (s, 2H), 4.66 (q, J = 6.6 Hz, 1H), 4.21-3.97 (m, 3H), 2.81-2.77 (m, 2H), 2.45 (t, J = 2.6 Hz, 1H), 2.28 (s, 3H), 1.86-1.68 (m, 4H), 1.38 (m, 9H); 13C NMR (125 MHz, Acetone-d_6) δ 174.1, 170.2, 170.1, 165.5, 162.5, 159.8, 158.2, 157.6, 151.2, 140.9, 139.4, 130.7, 130.5, 130.3, 130.2, 130.1, 129.7, 129.5, 123.2, 120.9, 119.3, 109.3, 81.6, 81.0, 73.6, 70.4, 68.2, 57.3, 54.6, 46.0, 39.7, 29.6, 27.1, 23.8, 23.0; mass spectrum (ESI), m/z 854.6 (M+H)⁺ (theoretical 854.3).

Scheme S3. Synthesis of alkynes 2g-h. Reagents: a) Chloromethylpyridinium iodide, Et_3N, DCM, Δ, 1h; b) TFA, DCM, r.t., overnight; c) Boc-Lys(Boc) or Boc-His(Boc), HBTU, DIPEA, DCM, r.t., overnight.

Na-(tert-butoxycarbonyl)-[N-(2-N-toluoylamino-4-(3-aminophenyl)-thiazole)]-propargylglycinamide (11). Boc-L-propargylglycine (100 mg, 0.47 mmol) was dissolved in dry DCM (25 mL) and triethylamine (0.2 mL, 0.94 mmol, 2 eq.), chloromethylpyridinium iodide (240 mg, 0.94 mmol, 2 eq.) and compound 10^2 (160 mg, 0.52 mmol, 1.1 eq.) were added successively. The reaction mixture was stirred under reflux 1 h, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane-EtOAc 7:3 as the eluent leading to desired compound 11 as a slightly yellow solid: yield 177.6 mg (75%); R_f = 0.34 (cyclohexane/ethyl acetate 7/3); 1H
NMR (200 MHz, Acetone-\textit{d}6) \(\delta\) 11.54 (br, 1H), 9.41 (br, 1H), 8.42 (s, 1H), 8.12 (d, \(J = 8.3\) Hz, 2H), 7.66 (dt, \(J = 7.7, 1.2\) Hz, 1H), 7.54-7.48 (m, 1H), 7.48 (s, 1H), 7.41-7.31 (m, 3H), 6.40 (d, \(J = 7.9\) Hz, 1H), 4.46 (q, \(J = 7.0\) Hz, 1H), 2.77 (td, \(J = 6.1, 2.6\) Hz, 2H), 2.51 (t, \(J = 2.6\) Hz, 1H), 2.44 (s, 3H), 1.44 (s, 9H); \(^{13}\)C NMR (50 MHz, Acetone-\textit{d}6) \(\delta\) 170.9, 166.8, 160.3, 157.3, 151.5, 145.1, 141.0, 137.4, 131.6, 131.2, 130.8, 129.9, 123.2, 120.8, 119.4, 109.9, 81.7, 73.4, 73.3, 56.1, 29.5, 23.8, 22.5; mass spectrum (ESI), \(m/\zeta\) 505.7 (M+H\(^{+}\)) (theoretical 505.3).

\[\text{N-(2-N-toluoylamino-4-(3-aminophenyl)-thiazole)-propargylglycinamide (12).}\]

To a solution of 11 (167.6 mg, 0.33 mmol) in DCM (3 mL) was added TFA (0.26 mL, 3.3 mmol, 10 eq.) and stirred at room temperature overnight. The solvent was removed under reduced pressure and the crude product was used in the next step without further purification: 118 mg (83%); \(^{1}\)H NMR (200 MHz, CD\textit{OD}) \(\delta\) 8.24 (t, \(J = 1.5\) Hz, 1H), 7.93 (d, \(J = 8.2\) Hz, 2H), 7.75 (dt, \(J = 7.6, 1.5\) Hz, 1H), 7.53-7.34 (m, 5H), 4.20 (t, \(J = 6.6\) Hz, 1H), 3.02-2.90 (m, 2H), 2.72 (t, \(J = 2.6\) Hz, 1H), 2.43 (s, 3H); \(^{13}\)C NMR (50 MHz, CD\textit{OD}) \(\delta\) 167.6, 166.9, 160.2, 150.9, 144.9, 139.3, 137.0, 130.9, 130.5, 130.4, 129.1, 123.8, 120.7, 118.9, 109.6, 77.2, 75.2, 53.6, 22.5, 21.6; mass spectrum (ESI), \(m/\zeta\) 405.4 (M+H\(^{+}\)) (theoretical 405.1).

\[\text{N-[2-N-toluoylamino-4-(3-aminophenyl)-thiazole]-[N\text{\textalpha},N\text{\textepsilon}-(di-\text{tert-butoxycarbonyl})]-lysyl-glycinamide (2g).}\]

A mixture of 12 (50 mg, 0.096 mmol), Boc-Lys(Boc)-OH-DCHA (50.9 mg, 0.096 mmol, 1 eq.), HBTU (36.6 mg, 0.096 mmol, 1 eq.), DIPEA (50 \(\mu\)L, 0.29 mmol, 3 eq.) in DCM (10 mL) was stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound 2g as a yellow solid that was employed in the following step without further purification: 55.5 mg (78%); \(R_f = 0.39\) (Cyclohexane/EA 1/1); mass spectrum (ESI), \(m/\zeta\) 734.3 (M+H\(^{+}\)) (theoretical 734.3).

\[\text{N-[2-N-toluoylamino-4-(3-aminophenyl)-thiazole]-[N\text{\textalpha},N\text{\textepsilon}midazole-(di-\text{tert-butoxycarbonyl})]-histidyl-glycinamide (2h).}\]

A mixture of 12 (50 mg, 0.096 mmol), Boc-His(Boc)-OH-DCHA (51.7 mg, 0.096 mmol, 1 eq.), HBTU (36.6 mg, 0.096 mmol, 1 eq.), DIPEA (50 \(\mu\)L, 0.29 mmol, 3 eq.) in DCM (10 mL) was stirred at room temperature overnight. Solvent was then removed under reduced pressure and the crude product was purified by flash chromatography on a silica gel column using a mixture cyclohexane/EtOAc 1:1 as the eluent leading to compound 2h as a yellow solid: 59.6 mg (83%); \(R_f = 0.24\) (Cyclohexane/EtOAc 1:1);

\(^{1}\)H NMR (500 MHz, CD\textit{OD}) \(\delta\) 8.18 (s, 1H), 8.07 (s, 1H), 7.91 (d, \(J = 8.0\) Hz, 2H), 7.67 (d, \(J = 7.7\) Hz, 1H), 7.49 (d, \(J = 7.8\) Hz, 1H), 7.36 (m, 1H), 7.34-7.31 (m, 4H), 4.67 (t, \(J = 6.2\) Hz, 1H), 4.42 (t, \(J = 6.2\) Hz, 1H), 3.07 (dd, \(J = 14.7, 4.7\) Hz, 1H), 2.94 (dd, \(J = 14.7, 4.7\) Hz, 1H), 2.44 (s, 1H), 1.44 (s, 9H).
2.83-2.74 (m, 3H), 2.40 (s, 3H), 1.54 (s, 9H), 1.39 (s, 9H); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$

174.0, 170.3, 167.5, 160.0, 151.0, 148.1, 144.8, 139.6, 138.5, 136.6, 130.8, 130.5, 130.1, 129.0, 123.4, 121.2, 119.6, 116.5, 109.4, 87.1, 81.1, 80.2, 72.7, 56.0, 54.2, 38.9, 28.7, 28.0, 22.5, 21.6;

mass spectrum (ESI), $m/z$ 743.2 (M+H)$^+$ (theoretical 743.3).
Figure S12. $^1$H, $^{13}$C and HRMS spectra for compound 3a.
Figure S13. $^1$H, $^{13}$C and HRMS spectra for compound 3b.
Figure S14. $^1$H, $^{13}$C and HRMS spectra for compound 3c.
Figure S15. $^1$H, $^{13}$C and HRMS spectra for compound 3d.
Figure S16. $^1$H, $^{13}$C and HRMS spectra for compound 3e.
Figure S17. $^1$H, $^{13}$C and HRMS spectra for compound 3f.
Figure S18. $^1$H, $^{13}$C and HRMS spectra for compound 3g.
Figure S19. $^1$H, $^{13}$C and HRMS spectra for compound 3h.
Figure S20. $^1$H, $^{13}$C and HRMS spectra for compound 4a.
Figure S21. $^1$H, $^{13}$C and HRMS spectra for compound 4b.
Figure S22. $^1$H, $^{13}$C and HRMS spectra for compound 4c.
Figure S23. $^1$H, $^{13}$C and HRMS spectra for compound 4d.
Figure S24. $^1$H, $^{13}$C and HRMS spectra for compound 4e.
Figure S25. $^1$H, $^{13}$C and HRMS spectra for compound 4f.
Figure S26. $^1$H, $^{13}$C and HRMS spectra for compound 4g.
Figure S27. $^1$H, $^{13}$C and HRMS spectra for compound 4h.