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Synthesis and biological evaluation of the ascidian blood-pigment halocyamine A†

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Synthesis of the antimicrobial marine natural product halocyamine A has been achieved utilizing a combination of Sonogashira coupling, ruthenium complex/ytterbium triflate catalyzed hydroamidation and solid-phase peptide synthesis (SPPS) chemistry. The synthetic natural product exhibited only modest levels of antibacterial activities but significant antioxidant activity.

Introduction

Investigation of the natural product chemistry of blood cells of marine organisms known as ascidians over the years has led to the identification of a number of modified peptides bearing C-terminus decarboxy-enamide moieties.[1] These natural products, collectively known as tunichromes, typically incorporate either a decarboxy-(*E*)-α,β-dehydro-3,4-dihydroxyphenyl alanine (dcΔDOPA) or a decarboxy-(*E*)-α,β-dehydro-3,4,5-trihydroxyphenyl alanine (dcΔTOPA) residue at the C-terminus.[2] While no role has been ascribed with any confidence, potential ecological roles proposed for the tunichromes include iron or vanadium sequestration, cross-linking/tunic formation or as primitive wound repair or clotting agents.[3] Unusual members of the tunichrome-family are halocyamines A (**1**) and B, two DOPA-containing modified tetrapeptides, isolated from the ascidian *Halocynthia roretzi* (Fig. 1).[4]

NMR and mass spectrometry data were used to characterize the natural products, with acidic hydrolysis, labelling and HPLC analysis used to establish the configuration of the L-His and L-DOPA residues. The structures of the halocyamines were unusual additions to the tunichrome family of modified peptides in that they contained the rare *Z*-configuration 6-bromoindolic enamide moiety[5,6] at the C-terminus. Biological evaluation of halocyamine A revealed a wide range of activities, including growth inhibition of Gram-positive bacteria,[4a] Gram-negative marine bacteria and fish RNA viruses.[7] As part of our ongoing investigation of the synthesis and biological investigation of tunichromes,[8] we now report the synthesis and structural confirmation of halocyamine A (**1**) and present the results of preliminary biological evaluation.

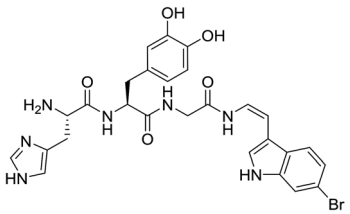

Fig. 1 Structure of halocyamine A (**1**).

Results and discussion

Prior to attempting the synthesis of halocyamine A, we chose to target the phenylenamide-containing model compound **2** (Fig. 2).

Established routes for the synthesis of enamides include dehydration[9] or elimination[10] methods, both of which favour the formation of the *E*-enamide product. In contrast, hydroamidation coupling of terminal alkynes with primary amides

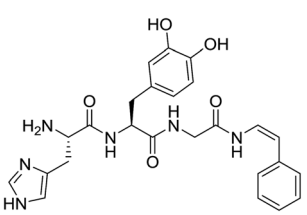

Fig. 2 Target model compound **2**.

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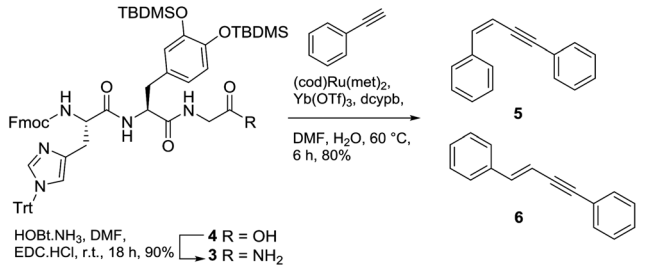
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C spectral data. See DOI: 10.1039/c7ob01122a



Scheme 1 Attempted synthesis of model enamide **2**.

using a ruthenium complex/ytterbium triflate catalyst has been shown to give exclusively the *Z*-enamide product.[11] Thus we envisaged that **2** could be prepared by reaction of the appropriately protected tripeptide L-His-L-DOPA-Gly-NH_2 (**3**) with phenylacetylene (Scheme 1). Tripeptide **3** was prepared by standard Fmoc solid-phase peptide synthesis procedures using 2-chlorotrityl resin, protected amino acids Fmoc-His(Trt)-OH, Fmoc-DOPA(TBDMS)$_2$-OH[12] and Fmoc-Gly with HATU as the coupling agent. Cleavage from the resin using 2,2,2-trifluoroethanol afforded the protected tripeptide carboxylic acid **4** in 79% yield over seven steps. Subsequent reaction of **4** with HOBt·NH_3 resulted in smooth conversion to the required tripeptide amide **3** (90% yield). Attempted hydroamidation of **3** and phenylacetylene using 5 mol% bis(2-methylallyl)(1,5-cyctooctadiene)ruthenium(II) heated at 60 °C for 6 h failed to afford the expected enamide-containing product. Unexpectedly, the only products detected were 1,4-disubstituted enynes **5** and **6**, isolated as a mixture in a ratio of 1 : 0.8 in 80% yield. Comparison with literature NMR data previously reported for **5** and **6** confirmed their identities.[13]

Based on the outcome of this reaction, we speculated that the steric bulk of tripeptide-amide **3** resulted in crowding at the ruthenium catalytic centre, preventing the progress of the expected hydroamidation reaction.

We next explored an alternative route to model analogue **2**: disconnection at the DOPA-Gly amide bond which would require dipeptide **7** and enamide **8** (Fig. 3). Protected dipeptide Fmoc-His(Trt)-DOPA(TBDMS)$_2$-OH **7** was prepared by SPPS, in a similar manner to that described for **4**, in 85% yield over five steps.

The synthesis of styryl enamide **8** was achieved in two steps, whereby hydroamidation of Fmoc-glycinamide (**9**) with phenylacetylene gave protected enamide **10** (96% yield, based upon

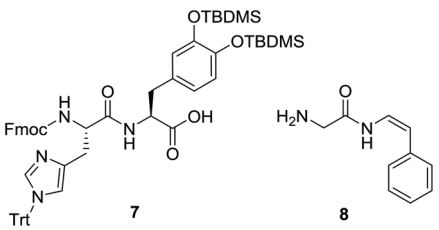
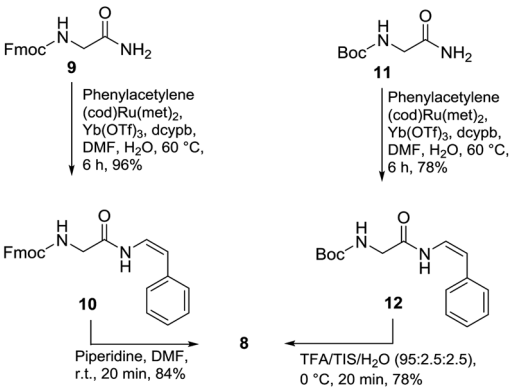


Fig. 3 Fragments **7** and **8**.

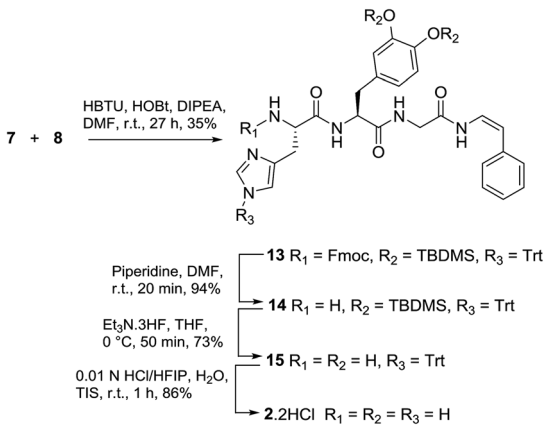


Scheme 2 Synthesis of styryl enamide **8**.

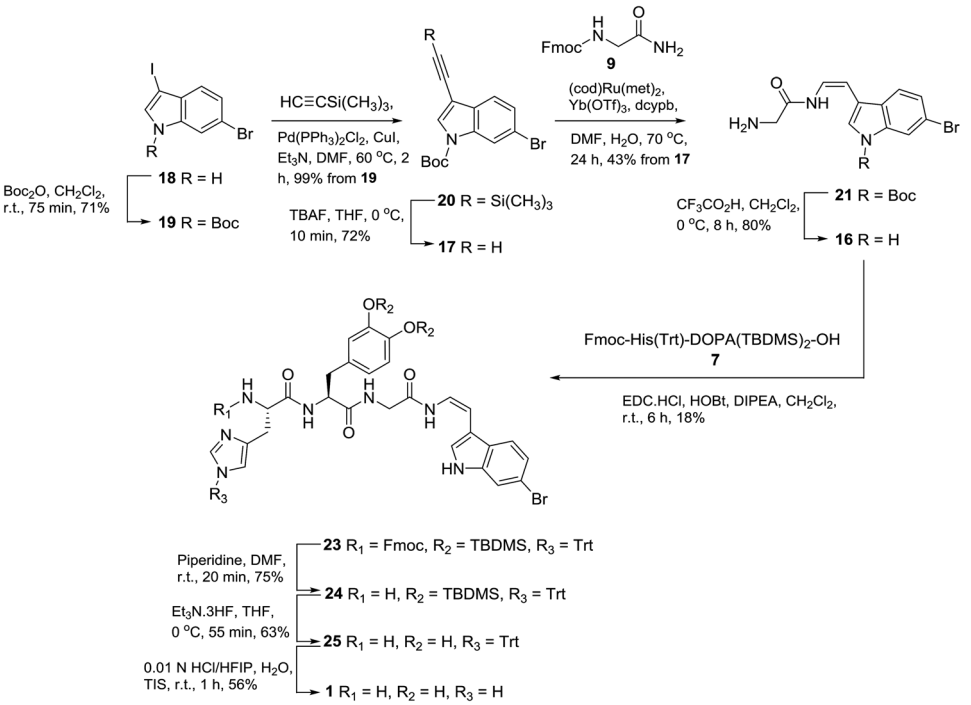
stoichiometry of **9**),[14] which upon reaction with 20% piperidine/DMF gave **8** (84% yield) (Scheme 2). Alternatively, **8** could be prepared *via* hydroamidation of Boc-Gly-NH_2 (**11**) with phenylacetylene to give **12** (78% yield) which was deprotected cleanly in TFA/TIS/H_2O (95 : 2.5 : 2.5) to give **8** (78% yield).

With **7** and **8** in-hand, coupling using HBTU and HOBt in DMF gave **13** in 35% yield (Scheme 3). Stepwise deprotection of the N-terminus using piperidine/DMF (20 min) gave **14** (94% yield), followed by deprotection of the catechol group (triethylamine-trihydrogen fluoride, THF, 55 min) to give **15** (73% yield) and finally, removal of the trityl protecting group (0.01 N HCl/HFIP, 1 h) gave phenyl enamide model compound **2** as the dihydrochloride salt.

With the successful synthesis of phenylenamide model **2**, we chose to employ a similar disconnection methodology for the synthesis of halocyamine A (**1**), requiring protected dipeptide L-His-L-DOPA (**7**) and glycyl-indolic enamide **16** (Scheme 4). We have recently demonstrated that the ruthenium catalysed hydroamidation methodology allows rapid synthetic entry to indolic *Z*-enamides by coupling an appropriately substituted indole-3-alkyne (*i.e.* **17**) with a primary amide.[15]



Scheme 3 Synthesis of phenyl enamide model **2**.



Scheme 4 Synthesis of halocyamine A (**1**).

Entry to **17** was achieved by Boc-protection of the known dihaloindole **18** [16,17] to give *N*-Boc-6-bromo-3-iodo indole (**19**), which, followed by Sonogashira alkynation, gave TMS-protected acetylene **20** in 99% yield (Scheme 4). Subsequent desilylation using TBAF in THF gave terminal acetylene **17** in 72% yield. Hydroamidation of **17** and Fmoc-glycinamide (**9**) using 5 mol% bis(2-methylallyl)(1,5-cyctooctadiene)ruthenium(II) heated at 70 °C for 24 h afforded glycyl enamide **21** (43%), exclusively as the *Z*-enamide. Of note was that the reaction product was deprotected at the N-terminus.

A minor (6%) by-product, *E*-enyne **22** (Fig. 4) was also purified from the product mixture. Detection of a sodiated molecular ion corresponding to $C_{30}H_{28}{}^{79}Br_2N_2O_4Na$, (observed $[M + Na]^+$ 661.0302, calcd 661.0308) as well as two nearly identical sets of ^1H NMR resonances attributable to a 3-substituted *N*-Boc-6-bromoindole fragment suggested **22** to be a dimer related to the starting material **17**. Observation of *E*-alkene (δ_H 7.10, d, J = 16.4 Hz; δ_H 6.44, d, J = 16.4 Hz) and disubstituted alkyne resonances (δ_C 92.9, 83.0), combined with 2D NMR data analysis identified the minor product as the (*E*)-1,4-disubstituted enyne **22**. Repeating the hydroamidation reaction in the absence of Fmoc-glycinamide afforded **22** in 28% yield. Of note, a number of transition metals are known to promote alkyne dimerization,[18] including ruthenium,[19] though with somewhat variable regio- (head-to-head *vs*. head-to-tail) and stereoselectivity.

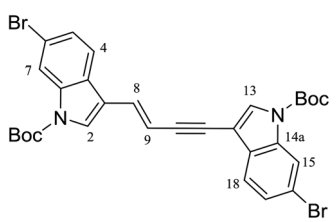


Fig. 4 Structure of *E*-enyne **22**.

Removal of the Boc protecting group of enamide **21** using TFA/CH_2Cl_2 afforded **16** in 80% yield. Peptide coupling (EDC, HOBt, DIPEA, 6 h) of enamide **16** and dipeptide acid **7** gave protected halocyamine A **23** in a disappointing yield of 18%. Efforts to increase the yield of this reaction by altering the coupling agent (HATU or HBTU), reaction time (9 h or 24 h), and the ratio of reactants **7**/**16** (2 : 1, 1 : 1 or 1 : 2) met with no success (data not shown). Sequential Fmoc deprotection (piperidine, DMF, 20 min) gave **24** (75% yield), followed by desilylation (triethylamine trihydrofluoride, THF, 55 min) to give **25** (63% yield) and removal of the trityl group (HCl/HFIP, H_2O, TIS, 1 h) gave the crude peptide that was purified by reversed-phase C_8 column chromatography [H_2O/MeOH] to afford halocyamine A (**1**) dihydrochloride salt in 56% yield. NMR (Table S1†) and optical rotation [+3.4 (*c* 1.07); lit.[4a] +5.2 (*c* 0.50)] data observed for synthetic **1** were in good agreement with those reported for the natural product.[4a]

The original reports of the halocyamines noted their abilities to inhibit the growth of Gram-positive bacteria, Gram-negative marine bacteria and fish RNA viruses.[4a,7] Halocyamine A was evaluated against a panel of Gram-positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus intermedius* 1051997), Gram-negative (*Pseudomonas aeruginosa* ATCC

27853, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212), and marine Gram-negative bacteria (*Vibrio harveyi* ATCC 14126, *Vibrio alginolyticus* ATCC 17749 and *Listonella anguillarum* ATCC 19264) and for antioxidant activity in the DPPH radical scavenging and oxygen radical absorbance (ORAC) assays. Somewhat at odds with the original isolation report,[4a,7] only modest antibacterial activity was observed for halocyamine A towards *P. aeruginosa* and *E. faecalis*, (both MIC 100 μM) and *V. harveyi* (IC_{50} 129 μM); no antibacterial activity was observed against the other organisms (MIC > 200 μM). A significant antioxidant activity was observed in the DPPH assay (IC_{50} of 26.6 ± 2.9 μM; positive control ascorbic acid IC_{50} 101 ± 8 μM) while in the ORAC assay, halocyamine A (**1**) was more active (relative ORAC value 1.29 ± 0.09) than Trolox, a water-soluble vitamin E analogue (ORAC value 1) and ascorbic acid (ORAC value 0.61 + 0.06).[20]

Conclusions

In summary, we have described a total synthesis of the marine natural product halocyamine A (**1**), making use of a ruthenium-catalysed hydroamidation of an indole acetylene with Fmoc-glycinamide to form the critical *Z*-enamide moiety. The natural product exhibits only mild levels of antibacterial activity. The relative ease of synthesis of halocyamine A now opens the door for future investigation of the potential ecological roles played by this unusual member of the tunichrome family of marine natural products.

Experimental

General information and materials

Optical rotations were recorded using a 0.1 dm cell in methanol or dichloromethane. NMR spectra were recorded at either 500 or 400 MHz for ^{1}H nuclei and 125 or 100 MHz for ^{13}C nuclei. Residual solvent signals were used as reference (CD_3OD: δ_H 3.31, δ_C 49.0; $CDCl_3$: δ_H TMS 0, δ_C 77.16; DMSO-d_6: δ_H 2.50, δ_C 39.52). ^{1}H NMR data are reported as position (δ), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, br = broad, obs = obscured), coupling constant (J, Hz), and the assignment of the atom. ^{13}C NMR data are reported as position (δ) and assignment of the atom. Assignments were based on 2D NMR data acquired using standard pulse sequences. (+)-ESI-MS data were acquired on a micrOTOF Q II mass spectrometer. Column chromatography was carried out with either C_8 reversed-phase or silica gel. All solvents used were distilled analytical grade or better. Chemical reagents were purchased from a commercial supplier and used without purification. 6-Bromo-3-iodo-indole **18** was prepared by a literature method.[16,17]

Fmoc-His(Trt)-DOPA(TBDMS)$_2$-Gly-OH (4). Fmoc-glycine (0.595 g, 2.00 mmol) dissolved in CH_2Cl_2 (15 mL) was added to 2-chlorotrityl chloride resin (loading 0.5 mmol g^{-1}, 4.00 g) followed by DIPEA (0.35 mL, 2.0 mmol). The mixture was agitated for 10 min and DIPEA (0.52 mL, 3.0 mmol) was added and the mixture was further shaken for 1 h. The solution was drained and the resin was washed with DMF (10 mL). A solution of CH_2Cl_2/MeOH/DIPEA (80 : 15 : 5, 25 mL) was added and the mixture agitated for 20 min. The solution was drained and the procedure repeated. The Fmoc-glycine-loaded resin was rinsed with DMF before a solution of piperidine/DMF (10 mL, 1 : 4) was added to the resin and agitated for 10 min. The solution was drained and the procedure was repeated for a further 20 min. The solution was drained off while the remaining resin was washed with DMF (15 mL), isopropanol (15 mL), followed by *n*-hexane (15 mL). The resin was then extensively dried before storing in the desiccator for 16 h. CH_2Cl_2 (25 mL) was used to swell the resin, which was then drained, before a solution of Fmoc-DOPA(TBDMS)$_2$-OH (2.59 g, 4.00 mmol), HOBt (1.01 g, 7.50 mmol), HATU (2.85 g, 7.50 mmol) and DIPEA (1.74 mL, 10.0 mmol) in DMF (7.50 mL) was added to the resin and shaken for 2 h. The solution was then drained and the resin was washed with DMF (10 mL). A solution of piperidine in DMF (10 mL, 1 : 4) was added to the Fmoc-DOPA (TBDMS)$_2$-Gly-loaded resin and agitated. After 10 min, the solution was drained and the procedure was repeated for a further 20 min. The solution was drained and the DOPA (TBDMS)$_2$-Gly-loaded resin was washed with DMF (10 mL) before a solution of Fmoc-His(Trt)-OH (3.1 g, 5.0 mmol), HOBt (1.01 g, 7.50 mmol), HATU (2.85 g, 7.50 mmol) and DIPEA (1.74 mL, 10.0 mmol) in DMF (7.50 mL) was added. The mixture was agitated for 90 min before the solution was drained. The Fmoc-His(Trt)-DOPA(TBDMS)$_2$-Gly-loaded resin was washed with DMF (15 mL), isopropanol (15 mL), followed by *n*-hexane (15 mL) and was extensively dried before storing in the desiccator for 16 h. The protected peptide was cleaved from the resin using a solution of 2,2,2-trifluoroethanol in CH_2Cl_2 (25 mL, 1 : 4) to give the desired product **4** as a yellow solid (1.71 g, 79%). M.p. 130–131 °C; $[\alpha]_D^{22.7}$ −21.5 (*c* 0.14, CH_2Cl_2); R_f 0.52 (CH_2Cl_2/MeOH 9 : 1); IR (ATR) ν_{max} 3278, 3036, 2930, 1662, 1508, 1446, 1251, 1128 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75–7.69 (3H, m, NH-10, 2H-FmocAr), 7.54–7.44 (3H, m, H-6 and 2H-FmocAr), 7.32–7.24 (12H, m, NH-20, 2H-FmocAr, 9H-TrtAr), 7.20–7.18 (2H, m, 2H-FmocAr), 7.01–7.00 (6H, m, 6H-TrtAr), 6.71–6.64 (4H, m, H-8, H-14, H-17 and H-18), 6.40 (1H, br s, NH-1), 4.75 (1H, d, J = 4.4 Hz, H-11), 4.43 (1H, br s, H-2), 4.25–4.13 (3H, m, H_2-21a and CO_2CH_2CH), 4.03 (1H, br s, CO_2CH_2CH), 3.69 (1H, br d, J = 17.4 Hz, H_2-21b), 3.19 (1H, br d, J = 10.3 Hz, H_2-12a), 3.12–3.10 (1H, m, H_2-3a), 2.90–2.86 (1H, m, H_2-12b), 2.80 (1H, br s, H_2-3b), 0.93–0.91 (18H, m, 2SiC(CH_3)$_3$), 0.14–0.09 (12H, m, 2Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.0 (C-22), 171.7 (C-19), 170.8 (C-9), 156.2 (CO_2CH_2CH), 146.8 (C-15), 145.7 (C-16), 144.0 (C-FmocAr), 143.9 (C-FmocAr), 141.5 (2C-FmocAr or 3C-TrtAr), 141.3 (2C-FmocAr or 3C-TrtAr), 137.5 (C-6), 134.8 (C-4), 129.9 (C-13), 129.7 (6C-TrtAr), 128.6 (3C-TrtAr), 128.4 (6C-TrtAr), 127.8 (2C-FmocAr), 127.2 (2C-FmocAr), 125.5 (C-FmocAr), 125.3 (C-FmocAr), 122.5 (C-14 or C-18), 122.0 (C-14 or C-18), 121.1 (C-17), 120.4 (C-8), 120.0 (2C-FmocAr), 76.5 (CAr_3), 67.2 ($COCH_2CH$), 55.5 (C-2), 55.1 (C-11), 47.2 (CO_2CH_2CH), 42.5

(C-21), 37.7 (C-12), 31.0 (C-3), 26.1 ($2SiC(CH_3)_3$), 18.5 ($2SiC(CH_3)_3$), −4.0 ($2Si(CH_3)_2$); (+)-HRESIMS $[M + H]^+$ 1084.5052 (calcd for $C_{63}H_{74}N_5O_8Si$, 1084.5070).

Fmoc-His(Trt)-DOPA(TBDMS)$_2$-Gly-NH$_2$ (3). DMF (1 mL) was added to **4** (1.0 g, 0.92 mmol) and HOBt·NH_3 (0.280 g, 1.85 mmol), followed by EDC·HCl (0.18, 0.92 mmol). The solution was stirred at r.t. for 18 h under nitrogen. EtOAc (15 mL) was then added and the mixture washed with water (20 mL) followed by brine (20 mL), dried ($MgSO_4$) and solvent removed *in vacuo*. Purification by silica gel column chromatography (eluting with *n*-hexane/EtOAc 1 : 9 to EtOAc) afforded the desired product **3** as a white solid (0.90 g, 90%). M.p. 148–149 °C; $[\alpha]_D^{22.7}$ −8.1 (*c* 0.36, CH_2Cl_2); R_f 0.63 (CH_2Cl_2/MeOH 9 : 1); IR (ATR) ν_{max} 3298, 1721, 1640, 1507, 1250 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.88 (1H, br s, NH-20), 7.76–7.73 (2H, m, 2H-FmocAr), 7.55–7.52 (2H, m, 2H-FmocAr), 7.38–7.36 (2H, m, 2H-FmocAr), 7.34–7.32 (10H, m, H-6 and 9H-TrtAr), 7.23–7.20 (2H, m, 2H-FmocAr), 7.09–7.07 (6H, m, 6H-TrtAr), 6.74 (1H, d, *J* = 7.6 Hz, H-17), 6.67 (1H, d, *J* = 1.7 Hz, H-14), 6.65 (1H, br s, H-8), 6.64 (1H, br s, NH_2-23a), 6.56 (1H, d, *J* = 7.6 Hz, H-18), 6.44 (1H, d, *J* = 5.9 Hz, NH-10), 6.22 (1H, d, *J* = 5.0 Hz, NH-1), 5.16 (1H, br s, NH_2-23b), 4.49–4.48 (1H, m, H-11), 4.33–4.28 (2H, m, CO_2CH_2CH), 4.23–4.22 (1H, m, H-2), 4.16–4.12 (2H, m, H_2-21a and CO_2CH_2CH), 3.56 (1H, dd, *J* = 17.0, 5.1 Hz, H_2-21b), 3.09 (1H, dd, *J* = 14.3, 5.2 Hz, H_2-12a), 3.05–3.01 (2H, m, H_2-3a and H_2-12b), 2.82 (1H, dd, *J* = 15.4, 5.0 Hz, H_2-3b), 0.95–0.93 (18H, m, $2SiC(CH_3)_3$), 0.15–0.14 (12H, m, $2Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.2 (C-9 and C-22), 171.3 (C-19), 156.3 (CO_2CH_2CH), 147.6 (C-15), 146.5 (C-16), 143.8 (2C-FmocAr), 142.2 (3C-TrtAr), 141.4 (2C-FmocAr), 138.8 (C-6), 135.5 (C-4), 129.8 (6C-TrtAr), 129.1 (C-13), 128.5 (3C-TrtAr), 128.4 (6C-TrtAr), 127.9 (2C-FmocAr), 127.2 (2C-FmocAr), 125.2 (2C-FmocAr), 121.9 (C-14 or C-18), 121.8 (C-14 or C-18), 121.5 (C-17), 120.5 (C-8), 120.2 (2C-FmocAr), 75.8 (CAr_3), 67.4 (CO_2CH_2CH), 55.5 (C-2 and C-11), 47.2 (CO_2CH_2CH), 43.1 (C-21), 36.2 (C-12), 30.8 (C-3), 26.0 ($2SiC(CH_3)_3$), 18.6 ($SiC(CH_3)_3$), 18.5 ($SiC(CH_3)_3$), −3.9 ($2Si(CH_3)_2$); (+)-HRESIMS $[M + H]^+$ 1083.5202 (calcd for $C_{63}H_{75}N_6O_7Si$, 1083.5230).

A mixture of (*Z*)-but-1-en-3-yne-1,4-diyldibenzene (5) and (*E*)-but-1-en-3-yne-1,4-diyldibenzene (6). An oven-dried flask was charged with **3** (51.0 mg, 47.1 μmol), bis(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (0.75 mg, 2.36 μmol), 1,4-bis(dicyclohexylphosphino)butane (1.27 mg, 2.83 μmol) and ytterbium triflate (1.17 mg, 1.88 μmol) under an atmosphere of nitrogen. Degassed DMF (0.2 mL) and phenylacetylene (0.01 mL, 94.2 μmol) were added to the flask followed by degassed water (5 μL, 0.28 mmol). The solution was stirred at 60 °C for 6 h, then poured into sat. aqueous $NaHCO_3$ (10 mL). The resulting mixture was extracted with EtOAc (3 × 20 mL), the combined organic layers were then washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by silica gel column chromatography (eluting with *n*-hexane) gave a mixture of **5** and **6** as an orange oil (7.7 mg, 80%). Data for a mixture of **5** and **6**: R_f 0.34 (*n*-hexane); IR (ATR) ν_{max} 3059, 3025, 1596, 1489, 1447, 1263, 1176 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.92 (2H, d, *J* = 7.5 Hz, 2H-Ar), 7.50–7.46 (4H, m, 4H-Ar), 7.43–7.28 (14H, m, 14H-Ar), 7.04 (1H, d, *J* = 16.2 Hz, H_E-1), 6.70 (1H, d, *J* = 12.0 Hz, H_Z-1), 6.39 (1H, d, *J* = 16.2 Hz, H_E-2), 5.92 (1H, d, *J* = 12.0 Hz, H_Z-2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 141.4 (C_E-1), 138.8 (C_Z-1), 136.7 (C_Z–Ar), 136.5 (C_E–Ar), 131.7 (C–Ar), 131.6 (C–Ar), 128.92 (C–Ar), 128.89 (C–Ar), 128.8 (C–Ar), 128.7 (C–Ar), 128.6 (C–Ar), 128.51 (C–Ar), 128.49 (C–Ar), 128.4 (C–Ar), 128.3 (C–Ar), 126.5 (C–Ar), 123.62 (C–Ar), 123.57 (C–Ar), 108.3 (C_E-2), 107.6 (C_Z-2), 96.0 (C_Z-4), 91.9 (C_E-4), 89.0 (C_E-3), 88.4 (C_Z-3); (+)-HRESIMS $[M + H]^+$ 205.1001 (calcd for $C_{16}H_{13}$, 205.1012).

(9*H*-Fluoren-9-yl)methyl (*Z*)-(2-oxo-2-(styrylamino)ethyl) carbamate (10). Fmoc-Gly-NH_2 (**9**) (0.296 g, 1.00 mmol), bis(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (0.016 g, 0.050 mmol), 1,4-bis(dicyclohexylphosphino)butane (0.027 g, 0.060 mmol) and ytterbium triflate (0.025 g, 0.040 mmol) were placed in a two neck flask and the air evacuated. The system was then flushed with nitrogen. Degassed DMF (3 mL), phenylacetylene (0.22 mL, 2.0 mmol) and degassed water (0.108 mL, 6.00 mmol) were added. The mixture was stirred under nitrogen at 60 °C for 6 h. The reaction mixture was added into sat. aqueous $NaHCO_3$ solution (30 mL) and was extracted with EtOAc (4 × 20 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried ($MgSO_4$), filtered, and the solvent removed *in vacuo*. Purification using silica gel column chromatography (eluting with *n*-hexane/EtOAc 9 : 1 to *n*-hexane/EtOAc 7 : 3) gave the desired product **10** as a yellow foam (0.38 g, 96%). M.p. 54–55 °C; R_f 0.68 (*n*-hexane/EtOAc 1 : 1); IR (ATR) ν_{max} 3305, 1686, 1647, 1514, 1481, 1448, 1334, 1253 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.31 (1H, br d, *J* = 9.9 Hz, NH-4), 7.74 (2H, d, *J* = 7.2 Hz, 2H-FmocAr), 7.53 (2H, d, *J* = 7.2 Hz, 2H-FmocAr), 7.38 (2H, t, *J* = 7.2 Hz, 2H-FmocAr), 7.27 (4H, t, *J* = 7.2 Hz, 2H-FmocAr and 2H-9), 7.22 (2H, d, *J* = 7.2 Hz, 2H-8), 7.15 (1H, t, *J* = 7.2 Hz, H-10), 6.89 (1H, dd, *J* = 9.9, 9.7 Hz, H-5), 5.77 (1H, d, *J* = 9.7 Hz, H-6), 5.60 (1H, br s, NH-1), 4.36 (2H, d, *J* = 6.9 Hz, CO_2CH_2CH), 4.15 (1H, t, *J* = 6.9 Hz, CO_2CH_2CH), 3.87 (2H, br s, H_2-2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 167.0 (C-3), 156.9 (CO_2CH_2CH), 143.7 (2C-FmocAr), 141.4 (2C-FmocAr), 135.3 (C-7), 129.1 (2C-9), 128.0 (2C-8), 127.9 (2C-FmocAr), 127.2 (2C-FmocAr and C-10), 125.1 (2C-FmocAr), 121.2 (C-5), 120.1 (2C-FmocAr), 111.5 (C-6), 67.5 (CO_2CH_2CH), 47.1 (CO_2CH_2CH), 44.9 (C-2); (+)-HRESIMS $[M + Na]^+$ 421.1529 (calcd for $C_{25}H_{22}N_2NaO_3$, 421.1523).

***tert*-Butyl (*Z*)-(2-oxo-2-(styrylamino)ethyl)carbamate (12).** Boc-Gly-NH_2 (**11**) (0.17 g, 1.0 mmol), bis(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (0.016 g, 0.050 mmol), 1,4-bis(dicyclohexylphosphino)butane (0.027 g, 0.060 mmol) and ytterbium triflate (0.024 g, 0.040 mmol) were placed under vacuum and then flushed with nitrogen (four times). Subsequently, degassed DMF (3 mL), phenylacetylene (0.22 mL, 2.0 mmol) and degassed water (0.108 mL, 6.00 mmol) were added. The mixture was stirred under nitrogen at 60 °C for 6 h, then poured into sat. aqueous $NaHCO_3$ (30 mL). The resulting mixture was extracted with EtOAc (4 × 20 mL), the combined organic layers were washed with water (30 mL) and brine (30 mL), dried ($MgSO_4$) and the solvent removed *in vacuo*. Purification by silica gel column

chromatography (eluting with *n*-hexane/EtOAc 9 : 1 to *n*-hexane/EtOAc 7.5 : 2.5) gave the desired product **12** as a yellow oil (0.21 g, 78%). R_f 0.48 (*n*-hexane/EtOAc 7 : 3); IR (ATR) ν_{max} 3333, 2977, 1674, 1512, 1453, 1368, 1252 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.42 (1H, d, J = 10.4 Hz, NH-4), 7.37 (2H, t, J = 7.7 Hz, 2H-9), 7.27 (2H, d, J = 7.7 Hz, 2H-8), 7.24–7.22 (1H, m, H-10), 6.93 (1H, dd, J = 10.4, 9.7 Hz, H-5), 5.79 (1H, d, J = 9.7 Hz, H-6), 5.08 (1H, br s, NH-1), 3.83 (2H, d, J = 6.0 Hz, H_2-2), 1.42 (9H, s, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.6 (C-3), 156.3 ($CO_2C(CH_3)_3$), 135.5 (C-7), 129.2 (2C-9), 128.0 (2C-8), 127.1 (C-10), 121.3 (C-5), 111.1 (C-6), 80.9 ($CO_2C(CH_3)_3$), 44.9 (C-2), 28.4 ($CO_2C(CH_3)_3$); (+)-HRESIMS $[M + Na]^+$ 299.1370 (calcd for $C_{15}H_{20}N_2NaO_3$, 299.1366).

(*Z*)-2-Amino-*N*-styrylacetamide (8). A solution of piperidine (0.9 mL, 20% in DMF) was added to **10** (0.086 g, 0.22 mmol) and stirred under nitrogen for 20 min. EtOAc (20 mL) was added and washed with H_2O (5 mL), the organic layer separated and the solvent removed *in vacuo*. Purification using silica gel column chromatography (eluting with *n*-hexane to EtOAc to CH_2Cl_2/MeOH 9 : 1) gave the desired product **8** as a yellow oil (0.032 g, 84%).

Alternatively, a solution of TFA/TIS/H_2O (0.5 mL, 95 : 2.5 : 2.5) was added to **12** (0.042 g, 0.15 mmol) and the solution was stirred at 0 °C under nitrogen for 20 min. The reaction was concentrated *in vacuo*. Purification using silica gel column chromatography (eluting with *n*-hexane/EtOAc 9 : 1 to EtOAc to CH_2Cl_2/MeOH 9 : 1) gave the desired product **8** as a yellow oil (0.021 g, 78%).

R_f 0.68 (*n*-hexane/EtOAc 1 : 1); IR (ATR) ν_{max} 3305, 3023, 1677, 1645, 1503, 1477, 1442 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.85 (1H, br s, NH-4), 7.38 (2H, t, J = 7.3 Hz, 2H-9), 7.33 (2H, d, J = 7.3 Hz, 2H-8), 7.24 (1H, t, J = 7.3 Hz, H-10), 6.95 (1H, dd, J = 11.9, 9.6 Hz, H-5), 5.76 (1H, d, J = 9.6 Hz, H-6), 3.43 (2H, s, H_2-2); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.7 (C-3), 136.0 (C-7), 129.0 (2C-9), 128.0 (2C-8), 126.9 (C-10), 121.2 (C-5), 110.6 (C-6), 44.6 (C-2); (+)-HRESIMS $[M + H]^+$ 177.1023 (calcd for $C_{10}H_{13}N_2O$, 177.1022).

Fmoc-His(Trt)-DOPA(TBDMS)$_2$-OH (7). A solution of Fmoc-DOPA(TBDMS)$_2$-OH[12] (2.00 g, 3.09 mmol) in CH_2Cl_2 (25 mL) was added to 2-chlorotrityl chloride resin (loading at 0.5 mmol g^{-1}, 6.18 g), followed by DIPEA (0.54 mL, 3.09 mmol). After the resin mixture was agitated for 10 min, DIPEA (0.81 mL, 4.63 mmol) was added and the mixture was further shaken for 1 h. The solution was drained off and the resin was washed with DMF (20 mL). A solution of CH_2Cl_2/MeOH/DIPEA (40 mL, 80 : 15 : 5) was added to the mixture and shaken for 20 min. The solution was drained and the procedure was repeated. The resin was then washed with DMF (20 mL). Piperidine in DMF (15 mL, 1 : 4) was added to the resin mixture and shaken for 10 min. The liquid was drained off and the piperidine washing was repeated for another 20 min. The amino acid-loaded resin was thoroughly washed with DMF (20 mL), isopropanol (20 mL) and *n*-hexane (20 mL). The resin was dried under vacuum for 30 min and stored in a desiccator overnight. CH_2Cl_2 (40 mL) was added to the amino acid-loaded resin which was left to swell for 1 h. The solution was drained and a solution of HBTU (4.39 g, 11.6 mmol), HOBt (1.57 g, 11.6 mmol), Fmoc-His(Trt)-OH (4.79 g, 7.72 mmol) and DIPEA (2.69 mL, 15.4 mmol) in DMF (11.6 mL) was added. The amino acid resin mixture was agitated for 2 h. The solution was then drained and washed with DMF (20 mL), isopropanol (20 mL) and *n*-hexane (20 mL). The resin was extensively dried and stored in a desiccator overnight. 2,2,2-Trifluoroethanol in CH_2Cl_2 (11.7 mL, 1 : 4) was added to the amino acid-loaded resin and agitated for 1 h. The solution was drained and the organic solvent was removed *in vacuo* to afford **7** as a yellowish-brown foam (2.70 g, 85% yield). M.p. 129–130 °C; $[\alpha]_D^{21.9}$ −5.7 (*c* 0.71, CH_2Cl_2); R_f 0.61 (CH_2Cl_2/MeOH 9 : 1); IR (ATR) ν_{max} 3320, 2930, 2857, 1723, 1655, 1509, 1446, 1251, 1128 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.94 (1H, br s, NH-10), 7.75 (2H, d, J = 7.3 Hz, 2H-FmocAr), 7.55–7.51 (3H, m, H-6 and 2H-FmocAr), 7.38 (2H, t, J = 7.3 Hz, 2H-FmocAr), 7.29–7.28 (2H, m, 2H-FmocAr), 7.26–7.23 (9H, m, 9H-TrtAr), 7.03–7.01 (6H, m, 6H-TrtAr), 6.75 (2H, br s, H-14 and H-17), 6.67–6.65 (2H, m, H-18 and H-8), 6.02 (1H, d, J = 7.8 Hz, NH-1), 4.72–4.66 (2H, m, H-2 and H-11), 4.29 (1H, dd, J = 12.9, 11.0 Hz, CO_2CH_2CH-a), 4.09–4.05 (2H, m, CO_2CH_2CH-b and CO_2CH_2CH), 3.33 (1H, d, J = 12.7 Hz, H_2-3a), 3.07 (1H, dd, J = 13.6, 4.7 Hz, H_2-12a), 3.01–2.97 (1H, m, H_2-12b), 2.64 (1H, dd, J = 12.7, 12.7 Hz, H_2-3b), 0.92–0.89 (18H, m, $2SiC(CH_3)_3$), 0.11–0.08 (12H, m, $2Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.6 (C-19), 170.7 (C-9), 155.7 (CO_2CH_2CH), 146.4 (C-16), 145.5 (C-15), 144.1 (C-FmocAr), 143.9 (C-FmocAr), 141.5 (3C-TrtAr), 141.3 (2C-FmocAr), 137.7 (C-6), 134.8 (C-4), 130.6 (C-13), 129.8 (6C-TrtAr), 128.5 (3C-TrtAr), 128.4 (6C-TrtAr), 127.8 (2C-FmocAr), 127.2 (2C-FmocAr), 125.6 (C-FmocAr), 125.3 (C-FmocAr), 122.7 (C-14 or C-18), 122.5 (C-14 or C-18), 120.9 (C-17), 120.3 (C-8), 120.0 (2C-FmocAr), 76.3 (CAr_3), 67.1 (CO_2CH_2CH), 55.3 (C-2 or C-11), 55.0 (C-2 or C-11), 47.3 (CO_2CH_2CH), 38.4 (C-12), 32.4 (C-3), 26.1 ($SiC(CH_3)_3$), 26.0 ($SiC(CH_3)_3$), 18.5 ($SiC(CH_3)_3$), 18.4 ($SiC(CH_3)_3$), −3.9 ($Si(CH_3)_2$), −4.0 ($Si(CH_3)_2$); (+)-HRESIMS $[M + H]^+$ 1027.4890 (calcd for $C_{61}H_{71}N_4O_7Si_2$, 1027.4856).

(9*H*-Fluoren-9-yl)methyl((*S*)-1-(((*S*)-3-(3,4-bis((*tert*-butyldimethylsilyl)oxy)phenyl)-1-oxo-1-((2-oxo-2-(((*Z*)-styryl)amino)ethyl)amino)propan-2-yl)amino)-1-oxo-3-(1-trityl-1*H*-imidazol-4-yl)propan-2-yl)carbamate (13). To a solution of **7** (74.2 mg, 72.4 μmol), HBTU (54.9 mg, 0.140 mmol) and HOBt (19.6 mg, 0.14 mmol) dissolved in DMF (0.50 mL) was added DIPEA (37.8 μL, 0.22 mmol). The mixture was stirred at r.t. under nitrogen for 1 h before a solution of **8** (12.7 mg, 72.4 μmol) in DMF (0.50 mL) was added. The reaction mixture was stirred at r.t. for 26 h before EtOAc (20 mL) was added and washed with H_2O (10 mL), 10% aqueous HCl (10 mL), sat. aqueous $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a yellow oil. Purification using silica gel chromatography (eluting with *n*-hexane/EtOAc 8 : 2 to *n*-hexane/EtOAc 6 : 4) gave the desired product **13** as a yellow foam (30.0 mg, 35%). $[\alpha]_D^{22.9}$ −1.2 (*c* 0.83, CH_2Cl_2). M.p. 94–96 °C; R_f 0.57 (*n*-hexane/EtOAc 1 : 1); IR (ATR) ν_{max} 3301, 3025, 2929, 1652, 1509, 1493, 1252 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 9.26 (1H, br s,

NH-20), 8.51 (1H, d, J = 10.7 Hz, NH-23), 7.75 (1H, d, J = 6.5 Hz, H-FmocAr), 7.73 (1H, d, J = 5.5 Hz, H-FmocAr), 7.54 (1H, d, J = 7.5 Hz, H-FmocAr), 7.51 (1H, d, J = 7.5 Hz, H-FmocAr), 7.38–7.34 (4H, m, 2H-28 and 2H-FmocAr), 7.31–7.30 (7H, m, 6H-TrtAr and H-6), 7.26–7.25 (4H, m, 2H-27 and 2H-FmocAr), 7.23–7.19 (3H, m, 3H-TrtAr), 7.07–7.05 (7H, m, H-29 and 6H-TrtAr), 6.75 (1H, dd, J = 10.7, 10.0 Hz, H-24), 6.72 (1H, d, J = 8.1 Hz, H-17), 6.66 (1H, s, H-14), 6.59 (1H, s, H-8), 6.52 (1H, dd, J = 8.1, 1.4 Hz, H-18), 6.15 (1H, d, J = 6.5 Hz, NH-10), 6.03 (1H, d, J = 5.4 Hz, NH-1), 5.68 (1H, d, J = 10.0 Hz, H-25), 4.69 (1H, ddd, J = 6.5, 6.5, 6.5 Hz, H-11) 4.31–4.23 (2H, m, CO_2CH_2CH), 4.18–4.17 (1H, m, H-2), 4.13 (1H, t, J = 7.2 Hz, CO_2CH_2CH), 3.99 (1H, dd, J = 16.3, 5.8 Hz, H_2-21a), 3.84 (1H, dd, J = 16.3, 5.8 Hz, H_2-21b), 3.07 (1H, dd, J = 14.7, 6.5 Hz, H_2-12a), 3.00–2.95 (2H, m, H_2-3a and H_2-12b), 2.69 (1H, dd, J = 14.4, 3.6 Hz, H_2-3b), 0.96–0.94 (18H, m, $2SiC(CH_3)_3$), 0.16–0.15 (12H, m, $2Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.3 (C-19), 170.7 (C-9), 167.5 (C-22), 156.1 (CO_2CH_2CH), 147.4 (C-15), 146.2 (C-16), 143.9 (C-FmocAr), 143.8 (C-FmocAr), 142.2 (3C-TrtAr), 141.4 (2C-FmocAr), 138.7 (C-6), 135.6 (C-26), 135.4 (C-4), 129.8 (6C-TrtAr), 129.3 (C-13), 129.1 (2C-28), 128.4 (3C-TrtAr), 128.3 (2C-27 and 6C-TrtAr), 127.9 (2C-FmocAr), 127.2 (2C-FmocAr), 127.1 (C-29), 125.3 (2C-FmocAr), 122.0 (C-18), 121.9 (C-14), 121.4 (C-17 and C-24), 120.7 (C-8), 120.1 (2C-FmocAr), 111.2 (C-25), 75.7 (CAr_3), 67.2 (CO_2CH_2CH), 54.9 (C-2), 54.3 (C-11), 47.3 (CO_2CH_2CH), 44.0 (C-21), 36.3 (C-12), 31.0 (C-3), 26.1 ($2SiC(CH_3)_3$), 18.6 ($2SiC(CH_3)_3$), −3.9 ($2Si(CH_3)_2$); (+)-HRESIMS $[M + H]^+$ 1185.5726 (calcd for $C_{71}H_{81}N_6O_7Si_2$, 1185.5700).

(*S*)-2-Amino-*N*-((*S*)-3-(3,4-bis((*tert*-butyldimethylsilyl)oxy) phenyl)-1-oxo-1-((2-oxo-2-(((*Z*)-styryl)amino)ethyl)amino)propan-2-yl)-3-(1-trityl-1*H*-imidazol-4-yl)propanamide (14). Piperidine (0.32 mL, 20% in DMF) was added to **13** (0.078 g, 0.066 mmol) and stirred at r.t. under nitrogen atmosphere. After 1 h, the brown solution was added to EtOAc (20 mL) and washed with water (10 mL). The aqueous layer was further washed with EtOAc (2 × 20 mL), the organic layers were combined and dried *in vacuo*. Purification by silica gel column chromatography (CH_2Cl_2/MeOH 9 : 1), afforded the desired product **14** as a yellow oil (59.4 mg, 94%). $[\alpha]_D^{21.9}$ −24.2 (c 0.91, CH_2Cl_2); R_f 0.49 (CH_2Cl_2/MeOH 9 : 1); IR (ATR) ν_{max} 3312, 2929, 2857, 1650, 1508, 1444, 1252, 1128 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.60 (1H, dd, J = 5.7, 5.6 Hz, NH-20), 8.51 (1H, d, J = 10.8 Hz, NH-23), 7.38–7.27 (15H, m, 9H-TrtAr, H-6, NH-10, 2H-27 and 2H-28), 7.20 (1H, t, J = 7.4 Hz, H-29), 7.09–7.06 (6H, m, 6H-TrtAr), 6.78 (1H, dd, J = 10.8, 9.8 Hz, H-24), 6.71 (1H, d, J = 8.1 Hz, H-17), 6.65 (1H, d, J = 2.0 Hz, H-14), 6.59 (1H, s, H-8), 6.54 (1H, dd, J = 8.1, 2.0 Hz, H-18), 5.71 (1H, d, J = 9.8 Hz, H-25), 4.45 (1H, ddd, J = 7.1, 7.1, 7.1 Hz, H-11), 3.93 (1H, dd, J = 16.3, 5.6 Hz, H_2-21a), 3.87 (1H, dd, J = 16.3, 5.7 Hz, H_2-21b), 3.40 (1H, dd, J = 5.5, 5.5 Hz, H-2), 3.09 (1H, dd, J = 14.1, 7.1 Hz, H_2-12a), 2.98 (1H, dd, J = 14.1, 7.1 Hz, H_2-12b), 2.82 (1H, dd, J = 14.9, 5.5 Hz, H_2-3a), 2.71 (1H, dd, J = 14.9, 5.5 Hz, H_2-3b), 0.97–0.96 (18H, m, $2SiC(CH_3)_3$), 0.17–0.16 (12H, m, $2Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.3 (C-9)[a], 172.5 (C-19), 167.6 (C-22), 147.0 (C-15), 145.9 (C-16), 142.3 (3C-TrtAr), 138.8 (C-6), 136.4 (C-4), 135.5 (C-26), 130.2 (C-13), 129.8 (6C-TrtAr), 129.1 (2C-28), 128.3 (9C-TrtAr and 2C-27), 127.0 (C-29), 122.2 (C-18), 122.0 (C-14), 121.4 (C-24), 121.2 (C-17), 120.2 (C-8), 111.4 (C-25), 75.6 (CAr_3), 55.2 (C-11), 54.7 (C-2), 44.0 (C-21), 36.1 (C-12), 32.3 (C-3), 26.1 ($2SiC(CH_3)_3$), 18.6 ($SiC(CH_3)_3$), 18.5 ($SiC(CH_3)_3$), −3.9 ($2Si(CH_3)_2$); (+)-HRESIMS $[M + H]^+$ 963.5055 (calcd for $C_{56}H_{71}N_6O_5Si_2$, 963.5019). [a] assignment by HMBC correlation.

(*S*)-2-Amino-*N*-((*S*)-3-(3,4-dihydroxyphenyl)-1-oxo-1-((2-oxo-2-(((*Z*)-styryl)amino)ethyl)amino)propan-2-yl)-3-(1-trityl-1*H*-imidazol-4-yl)propanamide (15). Compound **14** (54.8 mg, 56.9 μmol) was dissolved in THF (0.50 mL) under nitrogen atmosphere and the resulting yellow solution was stirred in an ice bath. Triethylamine trihydrofluoride (27.8 μL, 0.170 mmol) was then added dropwise to the yellow solution and continued to stir for 45 min. The reaction mixture was dried under a stream of N_2 gas, after which, water (15 mL) was added and the aqueous layer was extracted with CH_2Cl_2 (4 × 20 mL), the organic layers were combined and solvent was removed *in vacuo* to give a yellow foam. Purification by silica gel column chromatography (eluting with EtOAc to MeOH/CH_2Cl_2, 1 : 9), afforded **15** as a yellow oil (30.5 mg, 73%). $[\alpha]_D^{22.7}$ −21.9 (c 1.42, CH_2Cl_2); R_f 0.26 (CH_2Cl_2/MeOH 9 : 1); IR (ATR) ν_{max} 3277, 3057, 2926, 1651, 1508, 1486, 1260 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.42 (1H, d, J = 1.0 Hz, H-6), 7.36–7.33 (13H, m, 2H-27, 2H-28 and 9H-TrtAr), 7.20–7.17 (1H, m, H-29) 7.14–7.12 (6H, m, 6H-TrtAr), 6.74 (1H, d, J = 9.7 Hz, H-24), 6.70 (1H, s, H-8), 6.64 (1H, d, J = 1.9 Hz, H-14), 6.58 (1H, d, J = 8.1 Hz, H-17), 6.44 (1H, dd, J = 8.1, 1.9 Hz, H-18), 5.77 (1H, d, J = 9.7 Hz, H-25), 4.49 (1H, dd, J = 9.0, 5.6 Hz, H-11), 3.98 (1H, d, J = 16.8 Hz, H_2-21a), 3.84 (1H, d, J = 16.8 Hz, H_2-21b), 3.72 (1H, t, J = 5.6 Hz, H-2), 2.98 (1H, dd, J = 13.8, 5.6 Hz, H_2-12a), 2.78 (1H, dd, J = 14.8, 5.6 Hz, H_2-3a), 2.73–2.66 (2H, m, H_2-3b and H_2-12b); ^{13}C NMR (CD_3OD, 125 MHz) δ 174.3 (C-9 or C-19), 174.2 (C-9 or C-19), 169.7 (C-22), 146.3 (C-15), 145.3 (C-16), 143.6 (3C-TrtAr), 140.0 (C-6), 136.9 (C-4), 136.7 (C-26), 130.9 (6C-TrtAr), 129.8 (3C-TrtAr), 129.5 (C-13), 129.4 (2C-27 or 2C-28), 129.34 (2C-27 or 2C-28), 129.28 (6C-TrtAr), 128.0 (C-29), 122.1 (C-24), 121.64 (C-8 or C-18), 121.57 (C-8 or C-18), 117.4 (C-14), 116.3 (C-17), 113.4 (C-25), 76.9 (CAr_3), 56.3 (C-11), 55.2 (C-2), 43.7 (C-21), 38.1 (C-12), 32.7 (C-3); (+)-HRESIMS $[M + H]^+$ 735.3292 (calcd for $C_{44}H_{43}N_6O_5$, 735.3289).

4-((*S*)-2-Ammonio-3-(((*S*)-3-(3,4-dihydroxyphenyl)-1-oxo-1-((2-oxo-2-(((*Z*)-styryl)amino)ethyl)amino)propan-2-yl)amino)-3-oxopropyl)-1*H*-imidazol-3-ium dichloride (2). A solution of 0.01 N HCl/HFIP–TIS/H_2O (1 mL, 95 : 2.5 : 2.5) was added to **15** (35.0 mg, 47.7 μmol) and the solution was stirred at r.t. for 1 h. The solution was then dried under nitrogen and the crude product was purified by C_8 column chromatography (eluting with H_2O to H_2O/MeOH 6 : 4) to afford the desired product **2** as a white solid (18.0 mg, 77%). M.p. 240 °C (decomposed); $[\alpha]_D^{20.9}$ −5.7 (c 3.12, MeOH); R_f 0.66 (butan-1-ol/acetic acid/water 2 : 1 : 1); IR (ATR) ν_{max} 3023, 2924, 1653, 1517, 1493, 1445, 1260, 1078, 1031 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.54 (1H, d, J = 10.2 Hz, NH-23), 8.65 (1H, br s, H-20), 8.08 (1H, br s, NH-10), 7.54 (1H, s, H-6), 7.39–7.34 (4H, m, 2H-27 and

2H-28), 7.22 (1H, tt, J = 6.8, 1.8 Hz, H-29), 6.81 (1H, br s, H-8), 6.76 (1H, dd, J = 10.2, 10.0 Hz, H-24), 6.60–6.59 (2H, m, H-14 and H-17), 6.41 (1H, dd, J = 8.2, 2.0 Hz, H-18), 5.69 (1H, d, J = 10.0 Hz, H-25), 4.44 (1H, br s, H-11), 3.96 (1H, dd, J = 16.7, 5.4 Hz, H_2-21a), 3.89 (1H, dd, J = 16.7, 5.4 Hz, H_2-21b), 3.34 (1H, dd, J = 8.2, 4.1 Hz, H-2), 2.90 (1H, dd, J = 13.9, 4.3 Hz, H_2-12a), 2.78 (1H, dd, J = 14.4, 4.1 Hz, H_2-3a), 2.67 (1H, dd, J = 13.9, 9.0 Hz, H_2-12b), 2.48 (1H, d, J = 8.2 Hz, H_2-3b); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 174.1 (C-9), 171.9 (C-19), 168.1 (C-22), 144.8 (C-15), 143.7 (C-16), 135.4 (C-26), 134.9 (C-6), 128.6 (2C-28), 128.4 (C-13), 128.2 (2C-27), 126.5 (C-29), 121.7 (C-24), 120.0 (C-18), 116.6 (C-14), 115.2 (C-17), 110.2 (C-25), 54.9 (C-2), 53.7 (C-11), 42.4 (C-21), 37.1 (C-12), 32.1 (C-3); (+)-HRESIMS $[M + H]^+$ 493.2182 (calcd for $C_{25}H_{29}N_6O_5$, 493.2194).

***tert*-Butyl 6-bromo-3-iodo-1*H*-indole-1-carboxylate (19).** 6-Bromo-3-iodo-indole **18**[16,17] (0.66 g, 2.0 mmol), DMAP (0.02 g, 0.2 mmol) and di-*tert*-butyl dicarbonate (0.67 g, 3.1 mmol) were dissolved in CH_2Cl_2 (4 mL) and stirred for 75 min at r.t. under nitrogen. 10% aqueous HCl (20 mL) was added and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were combined, dried ($MgSO_4$) and filtered. The solvent was then removed *in vacuo*. The crude product was subjected to silica gel column chromatography (*n*-hexane) to yield **19** as an orange solid (0.86 g, 71% yield). M.p. 148–149 °C; R_f 0.70 (*n*-hexane/EtOAc 9 : 1); IR (ATR) ν_{max} 2986, 1732, 1602, 1427, 1365, 1244, 1115 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (1H, br s, H-7), 7.68 (1H, s, H-2), 7.42 (1H, dd, J = 8.3, 1.8 Hz, H-5), 7.25 (1H, d, J = 8.3 Hz, H-4), 1.67 (9H, s, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.4 ($CO_2C(CH_3)_3$), 135.5 (C-7a), 131.2 (C-3a), 130.7 (C-2), 126.8 (C-5), 122.8 (C-4), 119.5 (C-6), 118.4 (C-7), 85.1 ($CO_2C(CH_3)_3$), 65.0 (C-3), 28.2 ($CO_2C(CH_3)_3$); (+)-HRESIMS $[M + Na]^+$ 443.9060 (calcd for $C_{13}H_{13}{}^{79}Br^{126}INNaO_2$, 443.9067).

***tert*-Butyl 6-bromo-3-((trimethylsilyl)ethynyl)-1*H*-indole-1-carboxylate (20).** Triethylamine (1.40 mL) was added to a solution of *tert*-butyl 6-bromo-3-iodo-1*H*-indole-1-carboxylate (**19**) (0.59 g, 1.40 mmol) in DMF (1.40 mL) and was degassed in a sonic bath for 30 min, under nitrogen. Bis(triphenylphosphine)palladium(II) dichloride (19.7 mg, 28.1 μmol), copper(I) iodide (10.7 mg, 56.2 μmol) and ethynyltrimethylsilane (174.6 μL, 1.26 mmol) were added to the solution. After the solution was stirred at 60 °C for 2 h, it was quenched with water (30 mL) and extracted with EtOAc (3 × 25 mL). The organic layers were combined and dried ($MgSO_4$). The organic solvent was then removed *in vacuo*. The crude product was passed through a short plug of silica (*n*-hexane/EtOAc 20 : 1) to afford **20** as a brown oil (0.489 g, 99% yield). R_f 0.93 (*n*-hexane/EtOAc 9 : 1); IR (ATR) ν_{max} 2980, 2162, 1734, 1432, 1364, 1247, 1154, 1092 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (1H, br s, H-7), 7.72 (1H, s, H-2), 7.51 (1H, d, J = 8.4 Hz, H-4), 7.41 (1H, dd, J = 8.4, 1.9 Hz, H-5), 1.66 (9H, s, $CO_2C(CH_3)_3$), 0.28 (9H, s, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.8 ($CO_2C(CH_3)_3$), 135.3 (C-7a), 129.9 (C-2), 129.5 (C-3a), 126.6 (C-5), 121.4 (C-4), 119.1 (C-6), 118.6 (C-7), 103.6 (C-3), 98.8 (C-9), 96.2 (C-8), 85.0 ($CO_2C(CH_3)_3$), 28.2 ($CO_2C(CH_3)_3$), 0.2 ($Si(CH_3)_3$); (+)-HRESIMS $[M + Na]^+$ 414.0495 (calcd for $C_{18}H_{22}{}^{79}BrNNaO_2Si$, 414.0495).

***tert*-Butyl 6-bromo-3-ethynyl-1*H*-indole-1-carboxylate (17).** *tert*-Butyl 6-bromo-3-((trimethylsilyl)ethynyl)-1*H*-indole-1-carboxylate **20** (0.54 g, 1.38 mmol) in THF (30 mL) was stirred in ice bath for 10 min before tetrabutylammonium fluoride (0.44 g, 1.68 mmol) was added and was further stirred in ice bath for 20 min, under nitrogen. Sat. aqueous NH_4Cl (25 mL) was added and extracted with diethyl ether (4 × 20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude brown oil was purified by silica gel column chromatography eluting with *n*-hexane to give **17** as brown solid (0.32 g, 72% yield). M.p. 115–117 °C; R_f 0.64 (*n*-hexane/EtOAc 9 : 1); IR (ATR) ν_{max} 3292, 2987, 1735, 1456, 1364, 1312, 1251 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.31 (1H, br s, H-7), 7.73 (1H, s, H-2), 7.47 (1H, d, J = 8.3 Hz, H-4), 7.37 (1H, dd, J = 8.3, 1.8 Hz, H-5), 3.23 (1H, s, H-9), 1.66 (9H, s, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.6 ($CO_2C(CH_3)_3$), 135.2 (C-7a), 130.2 (C-2), 129.3 (C-3a), 126.6 (C-5), 121.1 (C-4), 119.1 (C-6), 118.5 (C-7), 102.3 (C-3), 85.0 ($CO_2C(CH_3)_3$), 81.2 (C-9), 75.2 (C-8), 28.1 ($CO_2C(CH_3)_3$); (+)-HRESIMS $[M + Na]^+$ 342.0099 (calcd for $C_{15}H_{14}{}^{79}BrNNaO_2$, 342.0100).

***tert*-Butyl (*Z*)-3-(2-(2-aminoacetamido)vinyl)-6-bromo-1*H*-indole-1-carboxylate (21) and di-*tert*-butyl 3,3′-(but-1-en-3-yne-1,4-diyl)(*E*)-bis(6-bromo-1*H*-indole-1-carboxylate) (22).** Fmoc-Gly-NH_2 (**9**) (0.287 g, 0.97 mmol), *tert*-butyl 6-bromo-3-ethynyl-1*H*-indole-1-carboxylate **17** (0.618 g, 1.94 mmol), bis(2-methylallyl)(1,5-cyclooctadiene)ruthenium(II) (15.0 mg, 47.0 μmol), 1,4-bis(dicyclohexylphosphino)butane (26.0 mg, 58.0 μmol) and ytterbium triflate (24.0 mg, 38.7 μmol) were placed under vacuum and then flushed with nitrogen (four times). Subsequently, degassed DMF (3.00 mL) was added, followed by degassed water (105.0 μL, 5.82 mmol) and the mixture was further stirred under nitrogen at 70 °C for 24 h. The reaction mixture was added to sat. aqueous $NaHCO_3$ (30 mL) and the resulting mixture was extracted with EtOAc (5 × 20 mL). The organic layers were combined and washed with water (30 mL) then brine (30 mL), dried ($MgSO_4$), filtered, and the solvent removed *in vacuo*. Purification using silica gel column chromatography (eluting with *n*-hexane to *n*-hexane/EtOAc 9 : 1) gave enyne **22** as a brown oil (37.2 mg, 6% yield) and (eluting with *n*-hexane/EtOAc 9 : 1 to EtOAc) **21** as a yellow oil (0.164 g, 43% yield).

***tert*-Butyl (*Z*)-3-(2-(2-aminoacetamido)vinyl)-6-bromo-1*H*-indole-1-carboxylate (21).** R_f 0.14 (EtOAc); IR (ATR) ν_{max} 3333, 2979, 1732, 1694, 1497, 1370, 1250, 1155 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.70 (1H, d, J = 11.3 Hz, H-4), 8.35 (1H, s, H-10), 7.71 (1H, s, H-8), 7.44–7.38 (2H, m, H-12 and H-13), 7.06 (1H, dd, J = 11.3, 9.6 Hz, H-5), 5.79 (1H, d, J = 9.6 Hz, H-6), 3.47 (2H, s, H_2-2), 1.68 (9H, s, $CO_2C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4 (C-3), 149.3 ($CO_2C(CH_3)_3$), 135.8 (C-9a), 128.7 (C-13a), 126.2 (C-12), 122.9 (C-5), 122.3 (C-8), 120.6 (C-13), 119.0 (C-11), 118.6 (C-10), 115.7 (C-7), 99.7 (C-6), 84.6 ($CO_2C(CH_3)_3$), 44.6 (C-2), 28.3 ($CO_2C(CH_3)_3$); (+)-HRESIMS $[M + H]^+$ 394.0751 (calcd for $C_{17}H_{21}{}^{79}BrN_3O_3$, 394.0761).

Di-*tert*-butyl 3,3′-(but-1-en-3-yne-1,4-diyl)(*E*)-bis(6-bromo-1*H*-indole-1-carboxylate) (22). R_f 0.59 (*n*-hexane/EtOAc 9 : 1); IR (ATR) ν_{max} 2980, 2934, 2860, 1736, 1602, 1431, 1360, 1249, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (2H, d, J =

9.9 Hz, H-7 and H-15), 7.72 (1H, s, H-13), 7.65 (1H, s, H-2), 7.59 (1H, d, J = 8.4 Hz, H-4), 7.54 (1H, d, J = 8.3 Hz, H-18), 7.40 (1H, dd, J = 8.3, 1.7 Hz, H-17), 7.39 (1H, dd, J = 8.4, 1.7 Hz, H-5), 7.10 (1H, d, J = 16.4 Hz, H-8), 6.44 (1H, d, J = 16.4 Hz, H-9), 1.68 (18H, s, $3H_3$-21 and $3H_3$-24); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.0 (C-19), 148.7 (C-22), 136.7 (C-7a), 135.4 (C-14a), 132.2 (C-8), 129.3 (C-18a), 128.9 (C-13), 127.0 (C-3a), 126.6 (C-5 and C-17), 126.5 (C-5 and C-17), 125.0 (C-2), 121.3 (C-18), 121.0 (C-4), 119.1 (C-6 or C-16), 118.8 (C-3 and C-7 or C-15), 118.6 (C-7 or C-15), 118.3 (C-6 or C-16), 108.3 (C-9), 103.7 (C-12), 92.9 (C-10), 85.0 (C-20 or C-23), 84.9 (C-20 or C-23), 83.0 (C-11), 28.2 (3C-21 and 3C-24); (+)-HRESIMS $[M + Na]^+$ 661.0302 (calcd for $C_{30}H_{28}{}^{79}Br_2N_2O_4Na$, 661.0308).

(*Z*)-2-Amino-*N*-(2-(6-bromo-1*H*-indol-3-yl)vinyl)acetamide (16). A solution of **21** (0.167 g, 0.425 mmol) in CH_2Cl_2 (1.30 mL) was stirred in a salted ice bath under nitrogen for 5 min before TFA (1.30 mL, 17.0 mmol) was added dropwise. The solution was stirred for 8 h in an ice bath. Sat. aqueous $NaHCO_3$ (20 mL) was added and the mixture was extracted with EtOAc (4 × 20 mL). The organic layers were combined and dried *in vacuo*. The crude black oil was subjected to silica gel column chromatography (eluting with EtOAc to CH_2Cl_2/MeOH 9 : 1) to give **16** as a black oil (0.10 g, 80% yield). R_f 0.90 (CH_2Cl_2/MeOH 9 : 1); IR (ATR) ν_{max} 3209, 2931, 1652, 1532, 1455, 1227, 1002 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.73 (1H, br s, NH-9), 7.69 (1H, br, s, H-8), 7.62 (1H, d, J = 1.8 Hz, H-10), 7.56 (1H, d, J = 8.5 Hz, H-13), 7.17 (1H, dd, J = 8.5, 1.8 Hz, H-12), 6.73 (1H, d, J = 9.4 Hz, H-5), 5.95 (1H, d, J = 9.4 Hz, H-6), 3.53 (2H, br s, H_2-2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.6 (C-3), 136.7 (C-9a), 125.7 (C-13a), 124.5 (C-8), 122.0 (C-12), 120.3 (C-13), 118.3 (C-5), 114.6 (C-11), 114.3 (C-10), 110.0 (C-7), 102.2 (C-6), 42.8 (C-2); (+)-HRESIMS $[M + Na]^+$ 316.0060 (calcd for $C_{12}H_{12}{}^{79}BrN_3NaO$, 316.0056).

(9*H*-Fluoren-9-yl)methyl((*S*)-1-(((*S*)-3-(3,4-bis((*tert*-butyldimethylsilyl)oxy)phenyl)-1-((2-(((*Z*)-2-(6-bromo-1*H*-indol-3-yl)vinyl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)amino)-1-oxo-3-(1-trityl-1*H*-imidazol-4-yl)propan-2-yl)carbamate (23). Fmoc-His(Trt)-DOPA(TBDMS)$_2$-OH **7** (0.267 g, 0.26 mmol), EDC·HCl (54 mg, 0.28 mmol), HOBt (51.9 mg, 0.38 mmol) and enamide **16** (76.2 mg, 0.26 mmol) were dissolved in CH_2Cl_2 (3 mL). DIPEA (0.22 mL, 1.28 mmol) was added to the solution and stirred at r.t. under a nitrogen atmosphere for 6 h. EtOAc (20 mL) was added and washed with H_2O (10 mL). The aqueous layer was washed with EtOAc (3 × 15 mL) and combined. The organic layers were dried ($MgSO_4$), filtered and the solvent removed *in vacuo* to give a yellow crude oil. Purification using silica gel chromatography (eluting with *n*-hexane/EtOAc 8 : 2 to *n*-hexane/EtOAc 4 : 6) gave **23** as a yellow oil (61.2 mg, 18% yield). R_f 0.61 (CH_2Cl_2/MeOH 9 : 1); $[\alpha]_D^{23.0}$ +12.6 (c 1.03, CH_2Cl_2); IR (ATR) ν_{max} 3317, 2930, 2857, 1657, 1506, 1446, 1251, 1229, 1158, 1129, 1041 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.06 (1H, br s, NH-28), 9.00 (1H, br s, NH-20), 8.21 (1H, d, J = 10.7 Hz, H-23), 7.74 (1H, d, J = 7.7 Hz, H-FmocAr), 7.73 (1H, d, J = 7.7 Hz, H-FmocAr), 7.52 (1H, d, J = 7.7 Hz, H-FmocAr), 7.50 (1H, d, J = 7.7 Hz, H-FmocAr), 7.49 (1H, br s, H-29), 7.41 (1H, d, J = 8.3 Hz, H-32), 7.37 (2H, t, J = 7.7 Hz, 2H-FmocAr), 7.32 (1H, s, H-6), 7.28–7.23 (11H, m, 2H-FmocAr, 9H-TrtAr), 7.21 (1H, d, J = 8.3 Hz, H-31), 7.16 (1H, br s, H-27), 6.99–6.98 (6H, m, 6H-TrtAr), 6.75–6.68 (3H, m, H-14, H-17 and H-24), 6.58 (1H, br s, H-8), 6.54 (1H, d, J = 7.6 Hz, H-18), 6.30 (1H, br s, NH-10), 5.99 (1H, br s, NH-1), 5.79 (1H, d, J = 9.1 Hz, H-25), 4.78–4.76 (1H, m, H-11), 4.33–4.30 (3H, m, H-2 and CO_2CH_2CH), 4.23–4.18 (1H, m, H_2-21a), 4.15 (1H, t, J = 6.9 Hz, CO_2CH_2CH), 3.70 (1H, dd, J = 16.7, 4.2 Hz, H_2-21b), 3.11 (1H, dd, J = 14.1, 6.1 Hz, H_2-12a), 2.98 (1H, dd, J = 14.8, 4.2 Hz, H_2-3a), 2.94 (1H, dd, J = 14.1, 6.1 Hz, H_2-12b), 2.77 (1H, dd, J = 14.8, 4.2 Hz, H_2-3b), 0.96–0.95 (18H, m, 2SiC(CH_3)$_3$), 0.16 (12H, s, 2Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.0 (C-19), 170.8 (C-9), 166.6 (C-22), 156.3 (CO_2CH_2CH), 147.4 (C-15), 146.4 (C-16), 143.8 (C-FmocAr), 143.7 (C-FmocAr), 142.0 (3C-TrtAr), 141.4 (2C-FmocAr), 138.8 (C-6), 136.6 (C-28a), 135.2 (C-4), 129.7 (6C-TrtAr), 129.0 (C-13), 128.3 (9C-TrtAr), 128.0 (2C-FmocAr), 127.2 (2C-FmocAr), 126.0 (C-32a), 125.1 (2C-FmocAr), 123.4 (C-27), 123.2 (C-31), 122.3 (C-18), 122.0 (C-14), 121.4 (C-17), 120.8 (C-8), 120.2 (2C-FmocAr and C-32), 119.3 (C-24), 116.3 (C-30), 114.4 (C-29), 111.2 (C-26), 102.8 (C-25), 75.8 (CAr_3), 67.3 (CO_2CH_2CH), 54.9 (C-2), 54.0 (C-11), 47.3 (CO_2CH_2CH), 43.9 (C-21), 36.6 (C-12), 30.9 (C-3), 26.0 (2SiC(CH_3)$_3$), 18.6 (2SiC(CH_3)$_3$), −3.9 (2Si(CH_3)$_2$); (+)-HRESIMS $[M + H]^+$ 1302.4954 (calcd for $C_{73}H_{81}{}^{79}BrN_7O_7Si_2$, 1302.4914).

(*S*)-2-Amino-*N*-((S)-3-(3,4-bis((*tert*-butyldimethylsilyl)oxy)phenyl)-1-((2-(((*Z*)-2-(6-bromo-1*H*-indol-3-yl)vinyl)amino)-2-oxoethyl)amino)-1-oxopropan-2-yl)-3-(1-trityl-1*H*-imidazol-4-yl)propanamide (24). Piperidine (20% in DMF, 0.50 mL) was added to protected halocyamine A **23** (25.0 mg, 19.2 μmol) and was stirred under N_2 at r.t. for 20 min. EtOAc was added to the reaction solution and the mixture was washed with water (5 mL). The aqueous layer was further washed with EtOAc (3 × 15 mL) and the organic layers were then combined and dried *in vacuo*. Purification by silica gel column chromatography (eluting with EtOAc to CH_2Cl_2/MeOH 9 : 1), afforded **24** as a yellow oil (15.6 mg, 75% yield). R_f 0.59 (CH_2Cl_2/MeOH 9 : 1); $[\alpha]_D^{20.4}$ −20.5 (c 1.07, CH_2Cl_2); IR (ATR) ν_{max} 3257, 2929, 1663, 1509, 1445, 1252, 1202, 1129, 1023 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.58 (1H, br s, NH-28), 8.70 (1H, t, J = 5.9 Hz, NH-20), 8.22 (1H, d, J = 10.7 Hz, NH-23), 7.50 (1H, d, J = 1.6 Hz, H-29), 7.42 (1H, d, J = 8.5 Hz, H-32), 7.34 (1H, d, J = 0.9 Hz, H-6), 7.30–7.25 (9H, m, 9H-TrtAr), 7.22–7.19 (2H, m, H-27 and H-31), 7.01–6.99 (6H, m, 6H-TrtAr), 6.88 (1H, d, J = 7.5 Hz, NH-10), 6.73 (1H, d, J = 8.2 Hz, H-17), 6.71 (1H, dd, J = 10.7, 9.2 Hz, H-24), 6.67 (1H, d, J = 2.1 Hz, H-14), 6.60 (1H, d, J = 0.9 Hz, H-8), 6.56 (1H, dd, J = 8.2, 2.1 Hz, H-18), 5.80 (1H, d, J = 9.2 Hz, H-25), 4.68 (1H, ddd, J = 7.5, 7.5, 7.5 Hz, H-11), 4.14 (1H, dd, J = 16.9, 5.9 Hz, H_2-21a), 3.81 (1H, dd, J = 16.9, 5.9 Hz, H_2-21b), 3.54 (1H, dd, J = 5.2, 5.2 Hz, H-2), 3.08 (1H, dd, J = 14.1, 7.5 Hz, H_2-12a), 2.98 (1H, dd, J = 14.1, 7.5 Hz, H_2-12b), 2.88 (1H, dd, J = 14.8, 5.2 Hz, H_2-3a), 2.74 (1H, dd, J = 14.8, 5.2 Hz, H_2-3b), 0.97–0.96 (18H, m, 2SiC(CH_3)$_3$), 0.17–0.16 (12H, m, 2Si(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.7 (C-9), 172.3 (C-19), 166.6 (C-22), 147.1 (C-15), 146.1 (C-16), 142.0 (3C-TrtAr), 138.9 (C-6), 136.4 (C-28a), 135.7 (C-4), 129.6 (9C-TrtAr), 129.4 (C-13), 128.2 (6C-TrtAr), 125.9 (C-32a), 123.6

(C-27), 123.0 (C-31), 122.1 (C-18), 121.9 (C-14), 121.2 (C-17), 120.6 (C-8), 120.1 (C-32), 119.0 (C-24), 116.0 (C-30), 114.2 (C-29), 110.9 (C-26), 102.9 (C-25), 75.5 (*C*Ar$_3$), 54.4 (C-11), 54.2 (C-2), 43.7 (C-21), 36.5 (C-12), 33.0 (C-3), 25.9 (2SiC(*C*H$_3$)$_3$), 18.5 (SiC(CH$_3$)$_3$), 18.4 (SiC(CH$_3$)$_3$), −4.1 (2Si(*C*H$_3$)$_2$); (+)-HRESIMS [M + H]$^+$ 1080.4262 (calcd for $C_{58}H_{71}{}^{79}BrN_7O_5Si_2$, 1080.4233).

(*S*)-2-Amino-*N*-((*S*)-1-((2-(((*Z*)-2-(6-bromo-1*H*-indol-3-yl)vinyl)amino)-2-oxoethyl)amino)-3-(3,4-dihydroxyphenyl)-1-oxopropan-2-yl)-3-(1-trityl-1*H*-imidazol-4-yl)propanamide (25). To a solution of **24** (0.113 g, 0.105 mmol) in THF (2 mL) cooled in an ice bath, was added dropwise triethylamine trihydrofluoride (51 μL, 0.31 μmol). The solution was stirred at 0 °C under a nitrogen atmosphere for 1 h. The reaction was then dried under nitrogen and dissolved in H_2O (15 mL). The crude product was extracted from the aqueous layer with EtOAc (5 × 20 mL) and the organic layers were combined. Removal of solvent *in vacuo* gave the crude product as a yellow oil. Purification by silica gel column chromatography (eluting with EtOAc to MeOH/CH_2Cl_2, 1 : 9), afforded **25** as a yellow oil (56.5 mg, 63% yield). R_f 0.10 (CH_2Cl_2/MeOH 4 : 1); $[\alpha]_D^{27.3}$ −8.8 (*c* 1.96, CH_2Cl_2); IR (ATR) ν_{max} 3282, 2929, 1655, 1532, 1446, 1338, 1041 cm^{-1}; ^{1}H NMR (CD_3OD, 500 MHz) δ 7.52 (1H, d, *J* = 1.7 Hz, H-29), 7.43–7.41 (2H, m, H-27 and H-32), 7.36 (1H, br s, H-6), 7.32–7.30 (9H, m, 9H-TrtAr), 7.13 (1H, dd, *J* = 8.6 1.7 Hz, H-31), 7.08–7.06 (6H, m, 6H-TrtAr), 6.69 (1H, br s, H-8), 6.65–6.62 (3H, m, H-14, H-17 and H-24), 6.49 (1H, dd, *J* = 8.0, 1.9 Hz, H-18), 5.92 (1H, d, *J* = 9.9 Hz, H-25), 4.55 (1H, dd, *J* = 9.1, 5.2 Hz, H-11), 3.90 (1H, d, *J* = 16.7 Hz, H_2-21a), 3.86 (1H, d, *J* = 16.7 Hz, H_2-21b), 3.53 (1H, dd, *J* = 5.9, 5.9 Hz, H-2), 3.02 (1H, dd, *J* = 14.0, 5.2 Hz, H_2-12a), 2.79–2.68 (3H, m, H_2-3 and H_2-12b); ^{13}C NMR (CD_3OD, 125 MHz) δ 175.9 (C-9), 174.6 (C-19), 169.3 (C-22), 146.3 (C-15), 145.2 (C-16), 143.6 (3C-TrtAr), 139.8 (C-6), 138.3 (C-28a), 137.6 (C-4), 130.8 (6C-TrtAr), 129.7 (C-13), 129.24 (3C-TrtAr), 129.22 (6C-TrtAr), 127.2 (C-32a), 125.5 (C-27), 123.6 (C-31), 121.7 (C-18 or C-8), 121.6 (C-18 or C-8), 121.0 (C-32), 119.5 (C-24), 117.3 (C-14), 116.4 (C-30), 116.3 (C-17), 115.3 (C-29), 111.3 (C-26), 105.4 (C-25), 76.8 (*C*Ar$_3$), 56.3 (C-11), 55.4 (C-2), 44.0 (C-21), 37.9 (C-12), 33.9 (C-3); (+)-HRESIMS [M + H]$^+$ 852.2518 (calcd for $C_{46}H_{43}{}^{79}BrN_7O_5$, 852.2504).

Halocyamine A dihydrochloride (1). A cocktail solution of 0.01 N HCl/HFIP–TIS/H_2O (2 mL, 95 : 2.5 : 2.5) was added to **25** (29.0 mg, 34.1 μmol). The reaction was stirred at r.t. for 1 h, after which, the solution was dried under a stream of nitrogen. Purification by C_8 column chromatography (eluting with H_2O to H_2O/MeOH 60 : 40) afforded **1** as a white solid (13.09 mg, 56% yield). M.p. 280 °C (decomposed); R_f 0.72 (butan-1-ol/acetic acid/H_2O 2 : 1 : 1); $[\alpha]_D^{22.3}$ +3.4 (*c* 1.07, MeOH) (lit.[4a] $[\alpha]_D^{22.3}$ +5.2 (*c* 0.5, MeOH)); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.51 (1H, br s, NH-28), 9.08 (1H, d, *J* = 10.0 Hz, NH-23), 8.88 (1H, br s, NH-20), 8.40 (1H, br s, NH-10), 7.72 (1H, s, H-27), 7.60 (1H, s, H-6), 7.58 (1H, d, *J* = 1.6 Hz, H-29), 7.56 (1H, d, *J* = 8.3 Hz, H-32), 7.16 (1H, dd, *J* = 8.3, 1.6 Hz, H-31), 6.86 (1H, s, H-8) 6.67 (1H, dd, *J* = 10.0, 9.7 Hz, H-24), 6.64 (1H, d, *J* = 1.5 Hz, H-14), 6.59 (1H, d, *J* = 8.0 Hz, H-17), 6.45 (1H, dd, *J* = 8.0, 1.5 Hz, H-18), 5.92 (1H, d, *J* = 9.7 Hz, H-25), 4.44 (1H, br s, H-11), 3.99 (1H, dd, *J* = 16.6, 5.8 Hz, H_2-21a), 3.93 (1H, dd, *J* = 16.6, 5.8 Hz, H_2-21b), 3.61 (1H, br s, H-2), 2.95 (1H, dd, *J* = 14.0, 4.1 Hz, H_2-12a), 2.91–2.89 (1H, m, H_2-3a), 2.73 (1H, dd, *J* = 14.3, 7.5 Hz, H_2-3b), 2.66 (1H, dd, *J* = 14.0, 9.6 Hz, H_2-12b); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 171.9 (C-19), 171.6 (C-9), 167.5 (C-22), 144.9 (C-15), 143.7 (C-16), 136.4 (C-28a), 135.1 (C-6), 133.5 (C-4), 128.5 (C-13), 125.7 (C-32a), 124.9 (C-27), 121.9 (C-31), 120.1 (C-32), 119.9 (C-18), 118.6 (C-24), 116.6 (C-14), 115.3 (C-17), 114.3 (C-30), 114.1 (C-29), 109.7 (C-26), 102.2 (C-25), 54.5 (C-11), 53.8 (C-2), 42.6 (C-21), 36.7 (C-12), 30.5 (C-3); (+)-HRESIMS [M + H]$^+$ 610.1393 (calcd for $C_{27}H_{29}{}^{79}BrN_7O_5$, 610.1408).

Antibiotic susceptibility testing

The susceptibility of bacterial strains to antibiotics and compounds was determined in microplates using the standard broth dilution method in accordance with the recommendations of the Comité de l'AntibioGramme de la Société Française de Microbiologie (CA-SFM).[21] Briefly, the minimal inhibitory concentrations (MICs) were determined with an inoculum of 10^5 CFU in 200 μL of MH broth containing two-fold serial dilutions of each drug. The MIC was defined as the lowest concentration of drug that completely inhibited visible growth after incubation for 18 h at 37 °C. To determine all MICs, the measurements were independently repeated at least three times. Minimum inhibitory concentration of positive control: colistin [*P. aeruginosa* (1 μM), *E. coli* (2 μM)], streptomycin [*P. aeruginosa* (21.5 μM), *E. coli* (21.5 μM), *S. aureus* (21.5 μM), *S. intermedius* (10.7 μM) and *E. faecalis* (21.5 μM)] and chloramphenicol [*S. aureus* (1.5–3 μM), *S. intermedius* (3–6 μM) and *E. faecalis* (1.5–3 μM)].

Marine bacteria susceptibility testing

The antibacterial activity assay was performed on the marine environmental bacterial strains Gram-negative *V. harveyi* ATCC 14126, *V. alginolyticus* ATCC 17749 and *L. anguillarum* ATCC 19264 by the liquid growth inhibition in 96-well microplates. A pre-culture of 5 mL marine broth (MB) was prepared by inoculating a colony of each bacterial strain and was incubated at 30 °C with stirring overnight. The concentration of the pre-culture was assessed by measuring the optical density (OD) at 620 nm and was adjusted by dilution in order to obtain a suspension of 0.03 OD. An aliquot of 200 μL of the bacterial suspension was distributed in each well and 10 μL of a serial dilution in DMSO of the pure compound were added in triplicate. The 96-well microplates were incubated at 30 °C overnight with shaking (450 rpm). The optical density of the wells was measured at 620 nm with a microplate reader and the inhibition (IC_{50}) was calculated and plotted *versus* test concentrations.

Antioxidant testing

Quantitative ORAC assay was run as previously described.[22] The result is expressed as relative Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) equivalents.

DPPH free radical scavenging assay

The potential antioxidant activity was evaluated in a 96-well microplate format assay whereby an aliquot of 10 μL of a serial dilution in MeOH of the pure compound and 190 μL of DPPH (200 μM, MeOH) were added in triplicate. After 1 h at room temperature in the dark, the absorbance was recorded at 510 nm in a microplate reader (DPPH radical has a characteristic absorption in MeOH at 510 nm, which disappears with acceptance of an electron from the antioxidant sample). Ascorbic acid was used as a positive control (IC_{50} 101 ± 8 μM). The antioxidant activity of the tested compounds was evaluated by the IC_{50}, which represents the sample concentration required to scavenge 50% of the DPPH free radical.

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