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PAPER

Syntheses of pseudoceramines A–D and a new synthesis of spermatinamine, bromotyrosine natural products from marine sponges†

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Herein we report the total syntheses of pseudoceramine A-D (**2–5**) and spermatinamine (**1**) isolated from the marine sponge *Pseudoceratina* sp. Direct acyl substitution of α-hydroxyiminoesters with amine nucleophiles was developed as a key transformation. The synthetic compounds confirm the reported structures and importantly gives access to non-symmetrical spermine based natural products carrying two different bromotyrosine building blocks. Our new synthesis of spermatinamine is two steps shorter and more efficient than the previously reported sequence.

Introduction

Marine sponges of the order Verongida are known to produce a multitude of bromotyrosine derived secondary metabolites, many with antimicrobial and cytotoxic activities.[1] In a recent bioassay-guided screening of a natural product extract library, the novel bromotyrosine alkaloid spermatinamine (**1**; Fig. 1) was isolated from an extract of *Pseudoceratina* sp. as an inhibitor of isoprenylcysteine carboxyl methyltransferase (Icmt).[2] The structure of **1** features a bromotyrosyl–spermine–bromotyrosyl sequence, constituting a rare example of a spermine alkaloid from a marine source. More recently, a bioassay-guided screening for inhibitors of the type III secretion (T3S) system of the Gram-negative bacterium *Yersinia pseudotuberculosis* led to the isolation and characterisation of four novel bromotyrosine alkaloids, pseudoceramines A–D (**2–5**; Fig. 1), from the same natural source.[3] The screening assay has the capacity to identify putative inhibitors of the T3S system as well as general antibiotics targeting bacterial growth.[4] In addition to pseudoceramines A–D the initial screen also identified **1** as a putative T3S inhibitor. Further biological assays, however, indicated that the observed T3S inhibition most likely results from a general growth inhibition rather than specific interactions with components of the T3S system. Pseudoceramines A (**2**) and B (**3**) were found to share the bromotyrosyl–spermine–bromotyrosyl sequence of **1**, while pseudoceramine C (**4**) was the first example of a bromotyrosine coupled to a spermidine derivative.

Two total syntheses of spermatinamine (**1**) have been reported in the literature. The first featured separate syntheses of the bromotyrosine building block and the spermine derivative followed by carbodiimide-mediated coupling of the two.[5] The convergent synthesis gave **1** in 15.4% yield over five steps (longest sequence) and offered a short and potentially efficient entry to synthetic analogues. A more recent approach gave **1** in 30.6% overall yield in six steps.[6] This latter synthesis utilised a protected bromotyrosine, readily available from 3,4-dibromo-4-hydroxybenzaldehyde, in the key step. No synthetic analogues of **1** have, to the best of our knowledge, been described using the aforementioned routes and no syntheses of the pseudoceramines **2–5** have been reported to date.

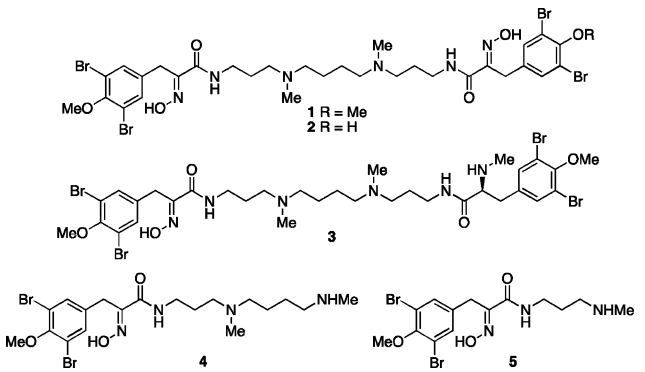

Fig. 1 Structures for spermatinamine (**1**) and pseudoceramines A–D (**2–5**).

The total syntheses of the pseudoceramines are needed to provide proof of their structures, in particular **3**, which had its absolute (*S*) configuration assigned tentatively during the course of its isolation. In addition, whilst the structure of spermatinamine is symmetrical, the pseudoceramines are all non-symmetrical, which rules out direct application of any of the previous total syntheses of spermatinamine to the pseudoceramines. This prompted the development of new, general synthetic routes that could be used for any natural bromotyrosine–polyamine derivative and which could easily be branched out in diverted total syntheses of synthetic symmetrical or non-symmetrical analogues. Finally,

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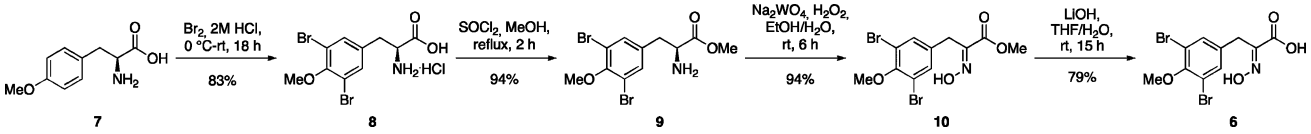
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for compounds **2–5**, **13**, **14**, **17–19**, **21**, **26–33**, and ^{1}H NMR spectrum for compound **34**. See DOI: 10.1039/c1ob06722b



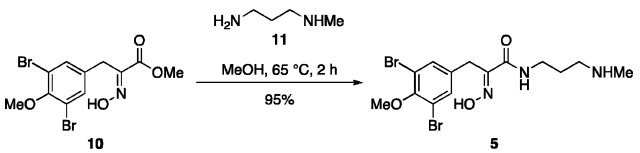
Scheme 1 Synthesis of the bromotyrosine oxime building block **6**.

the pseudoceramines together with spermatinamine provide a structural class with diverse biological profiles and which could provide interesting templates for the synthesis of new drug-like synthetic analogues. Here is reported the first syntheses of pseudoceramines A–D (**2–5**), as well as a new improved synthesis of spermatinamine (**1**).

Results and discussion

All five natural products have in common an *O*-methyltyrosine derived fragment that was readily available in the form of acid **6** by emulating the procedure of de Lera *et al.* (Scheme 1).[5] *O*-Methyl-L-tyrosine (**7**) was first brominated and then protected as the ester before tungstate mediated oxidation delivered the oxime **10**. Finally, ester hydrolysis using LiOH produced the acid **6**. This sequence turned out to be gratifyingly robust, and in our hands the yields leading up to **6** were even higher overall than those reported previously.[5]

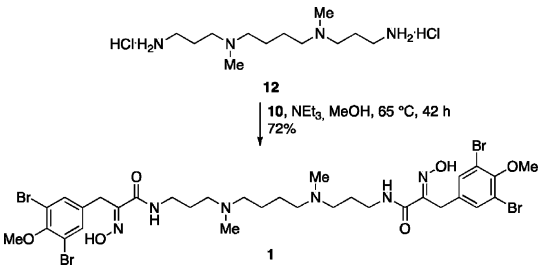
The natural next step was to attempt the synthesis of the simplest structure, pseudoceramine D (**5**), by coupling **6** with diamine **11** in the presence of dicyclohexylcarbodiimide (DCC) and *N*-hydroxyphtalimide (NHP), again using the work of de Lera and co-workers as the standard.[5] All our attempts, however, led to complex mixtures of products. Substituting for a different coupling reagent, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), or pre-forming the relevant acid chloride or pentafluorophenol (Pfp) ester for direct acyl substitution by the amine did not improve the results. Finally it was found that direct acyl substitution of the ester **10** with **11** could be achieved, analogous to the transformation demonstrated by the group of Spilling (Scheme 2).[7] Heating the two components in methanol in a sealed tube or at reflux for 2–48 h (scaling up the reaction led to shorter reaction times) not only gave **5** in high (95%) yield in the final step, but also shortened the synthesis sequence by one step (69.7% overall, 4 steps).



Scheme 2 Synthesis of pseudoceramine D (**5**).

We applied the same method in the synthesis of spermatinamine (**1**) by heating **10** with the known ammonium salt **12**[5] in methanol with added triethylamine to liberate the amines (Scheme 3). After heating for 42 h, **1** was obtained in 72% yield (58.3% longest sequence, 4 linear steps).

Next, tyrosine derivatives **13** and **14** were prepared. These compounds are needed as building blocks for targets **2** and **3** (Scheme 4, top and bottom, respectively). Building block **13** was available in five steps from commercially available bromotyrosine



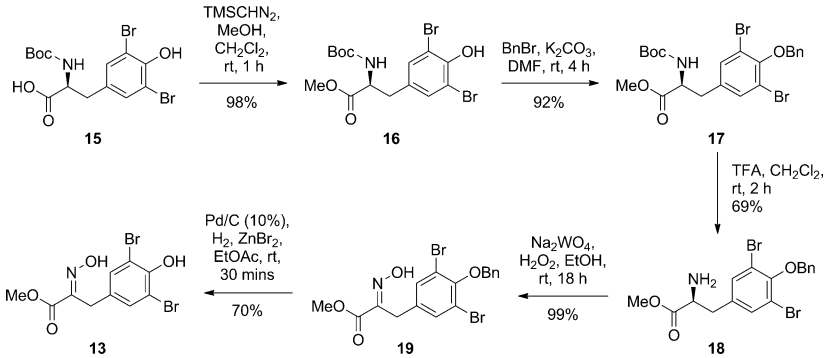
Scheme 3 Synthesis of spermatinamine **1**.

derivative **15**, starting with a sequence of esterification, benzylation, Boc-group cleavage and tungstate mediated oxidation of a primary amine to give oxime **19**. The final hydrogenolysis step to give **13** in 70% yield was achieved using a palladium catalyst that had been poisoned with zinc(II)bromide, thereby avoiding hydrodebromination of the aryl bromide moieties.[8,9] Building block **14** was available from tyrosine **20** by bromination followed by esterification.

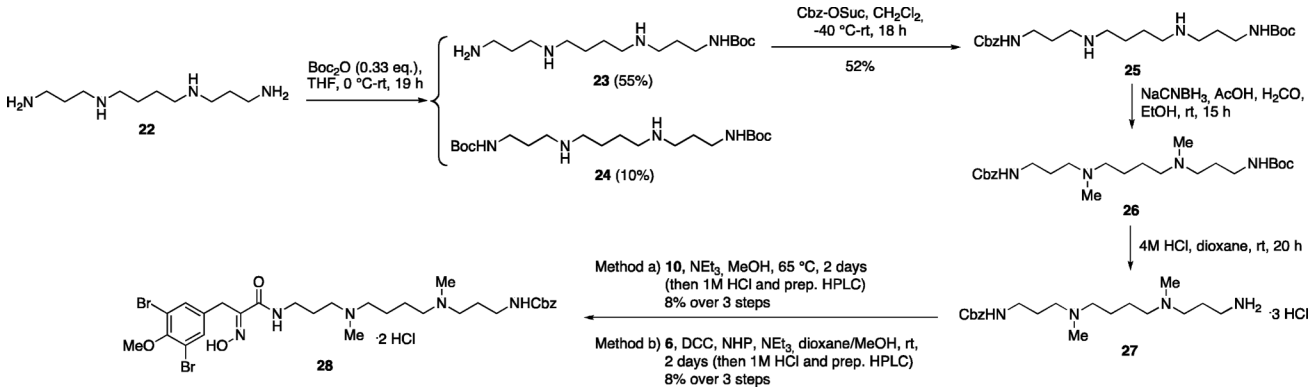
In order to obtain the non-symmetrical pseudoceramines **2** and **3**, it was envisioned that selectively protecting one of the primary amine moieties in **12** would allow for the acyl substitution of first **10**, then **13** or **14**, respectively, after deprotection of the common intermediate. Thus, spermine **22** was selectively mono-Boc-protected using the method of Blagbrough and co-workers (Scheme 5).[10] In our hands, the best result achieved was 55% of the desired intermediate **23** along with 10% of the di-Boc-protected product **24** (the latter was used to make polyamine **12** according to previously reported[5] procedures). Selective Cbz-protection of the other primary amine moiety in **23** was achieved in 52% yield using the procedure of Adamczyk *et al.*[11] From this point onwards, purification of the intermediates proved difficult, and repeated attempted purification by chromatography on silica gel generally resulted in reduced yields. Thus, intermediate **28** could be isolated in low yields only after preparative HPLC. It was observed that any attempts to isolate polyamine intermediates in their free base forms in general led to considerable decomposition; this is in accordance with observations made by Moya and Blagbrough.[12]

Although the convergent synthesis of intermediate **28** provided a branching point for the synthesis of the two remaining bistyrosine natural products, an improved synthesis was clearly desirable. Changing directions to a slightly more linear approach, it was found that acyl substitution of ester **10** with monoprotected spermine **23** gave an isolable product **29** in 85% yield (Scheme 6). This intermediate could be subjected to reductive amination followed by protecting group cleavage to give the ammonium salt **30** in 67% over two steps. Attempts to isolate the intermediate after the first step led to a lowered overall yield.

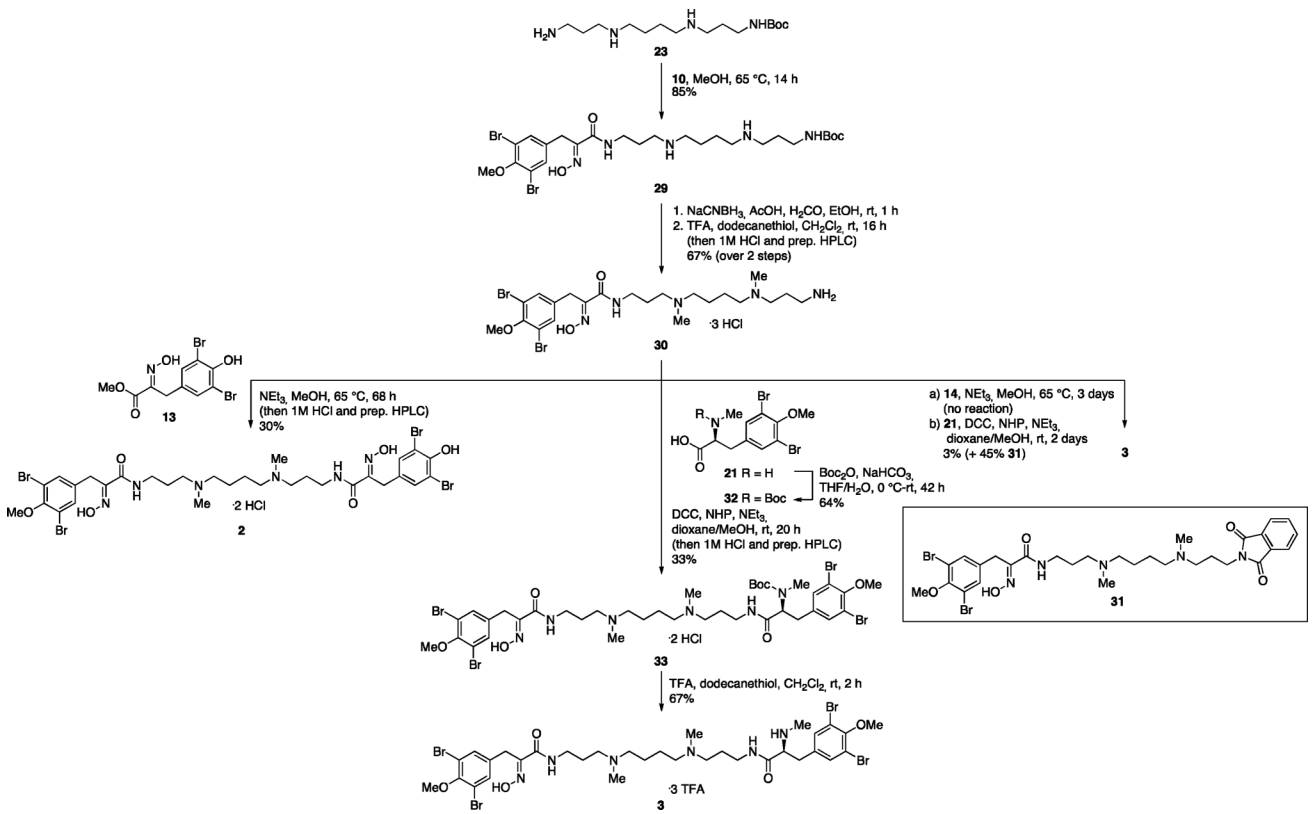
The final acyl substitution reaction between **30** and **13** to give pseudoceramine A **2** proceeded in 30% yield (12.5% longest



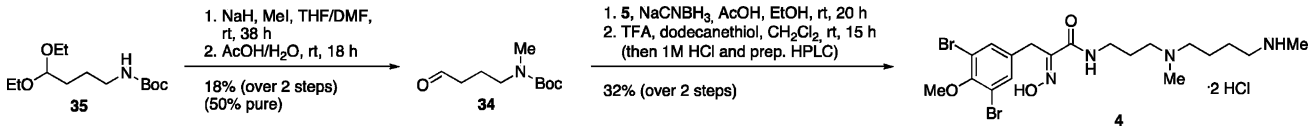
Scheme 4 Synthesis of the bromotyrosine building blocks **13** and **14**.



Scheme 5 Synthesis of the protected intermediate **28**



Scheme 6 Synthesis of pseudoceramines A (**2**) and B (**3**).



Scheme 7 Synthesis of pseudoceramine D (**4**).

sequence, 7 steps), which was considered fully acceptable given the extended reaction time and the rich substitution pattern of the substrates (Scheme 6). Intermediate **14** did not react under the acyl substitution conditions *en route* to pseudoceramine B **3**. We believe this is mainly due to the α-hydroxyiminoester being more activated towards nucleophiles than the α-aminoester and that this result suggests a limitation of the potential scope of the method. Instead, acid **21** was reacted with **30** under de Lera's conditions[5] to give pseudoceramine B **3** in 3% yield, with the major by-product isolated being imide **31**. Variations of coupling agents (HATU, bromotrispyrrolidinophosphonium hexafluorophosphate, DIC/HOAt) and the preformed Pfp ester were tested without success. Currently, we speculate that the by-product arises from neighboring group participation of the α-methylamine functionality as the intermediate *O*-acylisourea is formed, leaving unreacted NHP in the reaction mixture which then reacts with **30** to form **31**. By Boc-protection of **21** and subjecting the resultant acid **32** to the coupling conditions, imide formation was averted and protected pseudoceramine **33** was formed in 33% yield. Protecting group cleavage then furnished pseudoceramine B **3** in 67% yield (9.2% longest sequence, 7 steps). The absolute (*S*) stereochemistry was confirmed for **3** by matching (+) sign of the specific rotation measurement with the isolated natural product.

The prospect of using the newly synthesised pseudoceramine D (**5**) in the synthesis of pseudoceramine C (**4**), together with the discovery of the robust reductive amination-protecting group cleavage protocol that furnished intermediate **30**, motivated synthesis of aldehyde **34** from known precursor **35** (Scheme 7). Although in our hands **34** turned out to be unstable and could not be isolated, **34** could be used in crude form (approximately 50% pure by analysis of ^{1}H NMR integrals). When this crude material was used in excess in the reductive amination with **5** and the subsequent cleavage of the Boc-group, pseudoceramine C (**4**) was formed in 32% yield.

Conclusions

In summary, the first total syntheses of pseudoceramines A–D (**2–5**) have been achieved, utilising a direct acyl substitution of α-hydroxyiminoesters with amine nucleophiles as the key transformation. The yields in the key steps ranged from excellent (pseudoceramine D) to acceptable (pseudoceramines A and B). In addition, the most efficient synthesis of spermatinamine (**1**) to date has been completed. The spectral data for the synthetic pseudoceramines A–D are in agreement with the published structures.[3] Work is ongoing to expand the scope of the methodology and generate synthetic analogues of the pseudoceramines for biological evaluation.

Experimental

General considerations

Tyrosine derivatives were purchased from Bachem GmbH; all other commercial chemicals were acquired from Sigma Aldrich Chemical Co. All experiments were conducted under a nitrogen atmosphere, in dried glassware, using anhydrous solvents unless stated otherwise. Reagent grade tetrahydrofuran was distilled from molten potassium while dichloromethane was distilled from calcium hydride. Light petroleum (bp 40–60 °C) is referred to as petrol.

Column chromatography was carried out using Merck silica gel 60 and thin layer chromatography was performed on Merck aluminium-backed plates, pre-coated with silica gel 60 (F_{254}) and visualised by UV light and staining with alkali $KMnO_4$. Preparative reversed-phase HPLC was performed on a Gilson GX-271 system fitted with a Machery-Nagel C18 HTEC, 5 μm particle size column, using a gradient of acetonitrile/water (10–60% over 20 mins) with added formic acid (0.005%) at a flow of 20 mL min^{-1} and detection at 210 nm.

Low-resolution mass-spectra were recorded on a Waters Micromass ZG 2000 instrument with an electro spray ion source (ES+ and ES–) following liquid chromatography using an XTerra® MS C_{18} 5 μm particle size, 4.6 × 50 mm column and a water/acetonitrile/0.2% formic acid eluent system. High-resolution mass-spectra were recorded on a Bruker micrOTOF II instrument with an electrospray ion source (ES+). ^{1}H NMR and ^{13}C NMR spectra were recorded at 298 K on Bruker DRX-360 (^{1}H: 360 MHz; ^{13}C: 90 MHz), DRX-400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) or DRX-500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) spectrometers, with chemical shift values being reported in ppm relative to residual $CHCl_3$ (δ_H 7.26 and δ_C 77.16 ppm) or CD_3OH (δ_H 3.31 and δ_C 49.00 ppm) as internal standard. Peak assignments could be established from complementary HMBC, HMQC and COSY experiments. Infrared spectra were recorded for neat (oils) or thin film (solids) samples on NaCl discs using a Bruker Alpha-T spectrophotometer. Optical rotation was measured on a Perkin Elmer polarimeter 343 and are reported in 10^{-1} deg cm^2 g^{-1}. Melting points were measured using a Büchi/Dr Tottoli melting point determination apparatus and are uncorrected.

Experimental procedures

(*E*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-hydroxyimino-*N*-(3-(methylamino)propyl)propanamide, pseudoceramine D (5). To a 5 mL Biotage microwave tube was added known ester **10** (191 mg, 0.500 mmol), 3-(methylamino)propylamine **11** (0.157 mL, 1.50 mmol) and methanol (1 mL). The tube was sealed and the contents heated at 65 °C for 22 h, at which point LCMS analysis showed complete consumption of starting material. After cooling, the reaction mixture was concentrated and the residue purified by column chromatography on silica gel (89 : 10 : 1 to 20 : 5 : 1 to 4 : 2 : 1 dichloromethane/methanol/aqueous ammonia) to give the freebase form of pseudoceramine D **5** (208 mg, 95%) as a pale yellow glass; ν_{max}/cm^{-1} (film) 2928, 2863, 1656, 1527, 1471, 1421; δ_H (400 MHz, CD_3OD) 7.48 (2H, s), 3.84 (2H, s), 3.80 (3H, s), 3.35–3.29 (2H, m), 2.57 (2H, t, J = 7.1 Hz), 2.37 (3H, s),

1.72 (2H, tt, J = 7.1, 7.1 Hz); δ_C (100 MHz, CD_3OD) 165.6, 153.8, 152.1, 137.4, 134.5, 118.6, 61.0, 49.4, 37.9, 35.7, 29.7, 28.8; m/z LRMS (ES+) 440.1 (69%, $[M(^{81}Br_2) + H]^+$), 438.2 (100, $[M(^{79}Br^{81}Br) + H]^+$), 436.2 (67, $[M(^{79}Br_2) + H]^+$), 409.1 (60, $[M(^{81}Br_2) - CH_4N]^+$), 407.1 (96, $[M(^{79}Br^{81}Br) - CH_4N]^+$), 405.1 (60, $[M(^{79}Br_2) - CH_4N]^+$); HRMS (ES+) found 437.9858, $C_{14}H_{20}{}^{79}Br^{81}BrN_3O_3{}^+$ requires 437.9851.

(+)-(*S*)-Methyl 3-(3,5-dibromo-4-hydroxyphenyl)-2-(2-methylprop-2-yloxycarbamoyl)propanoate (16). Boc-3,5-dibromo-Tyr-OH **15** (4.00 g, 9.11 mmol) was dissolved in a 1 : 9 mixture of methanol/dichloromethane (50 mL) and (trimethylsilyl)diazomethane (2.80 mL, 5.60 mmol) was added dropwise. After stirring for 1 h at room temperature any remaining reagent was quenched by adding two drops of acetic acid. The reaction mixture was diluted with dichloromethane (100 mL), washed with saturated aqueous $NaHCO_3$ (3 × 10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated to give the ester **16** (2.49 g, 98%) as a colourless oil which was used in further synthesis with no purification necessary; $[\alpha]_D^{21}$ +24.0 (c 0.25, dichloromethane); ν_{max}/cm^{-1} (neat) 3388, 2978, 1739, 1699, 1478; δ_H (400 MHz, $CDCl_3$) 7.22 (2H, s), 5.81 (1H, s), 5.02 (1H, d, J = 7.0 Hz), 4.51 (1H, m), 3.74 (3H, s), 3.05 (1H, dd, J = 13.9, 5.9 Hz), 2.92 (1H, dd, J = 13.9, 5.9 Hz), 1.44 (9H, s); δ_C (100 MHz, $CDCl_3$) 171.9, 155.1, 148.6, 132.9, 131.0, 109.9, 80.4, 54.5, 52.6, 37.1, 28.4; m/z LRMS (ES–) 454.2 (42%, $[M(^{81}Br_2) - H]^-$), 452.2 (100, $[M(^{79}Br^{81}Br) - H]^-$), 450.2 (40, $[M(^{79}Br_2) - H]^-$); HRMS (ES+) found 475.9523, $C_{15}H_{19}{}^{79}Br^{81}BrNNaO_5{}^+$ requires 475.9507. The ^1H NMR data agree with literature values.[13]

(+)-(*S*)-Methyl 3-(4-benzyloxy-3,5-dibromophenyl)-2-(2-methylprop-2-yloxycarbamoyl)propanoate (17). Benzyl bromide (0.153 mL, 1.29 mmol) was added to a stirred suspension of ester **16** (389 mg, 0.859 mmol) and K_2CO_3 (178 mg, 1.29 mmol) in DMF (9 mL) and stirred at room temperature for 4 h, at which point TLC analysis showed complete consumption of starting material. The reaction mixture was diluted with ethyl acetate (20 mL), filtered and concentrated to give the benzyl ether **17** (430 mg, 92%) as colourless platelets which was used in further synthesis with no purification necessary; mp 54–59 °C (hexanes); $[\alpha]_D^{21}$ +21.9 (c 1.17, chloroform); ν_{max}/cm^{-1} (film) 2977, 1744, 1714, 1499, 1453; δ_H (360 MHz, $CDCl_3$) 7.60 (2H, d, J = 7.4 Hz), 7.44–7.34 (3H, m), 7.31 (2H, s), 5.07 (1H, d, J = 7.3 Hz), 5.01 (2H, s), 4.54 (1H, m), 3.76 (3H, s), 3.10 (1H, dd, J = 13.6, 5.9 Hz), 2.95 (1H, dd, J = 13.9, 5.9 Hz), 1.45 (9H, s); δ_C (90 MHz, $CDCl_3$) 171.9, 155.0, 151.9, 136.4, 135.3, 133.7, 133.6, 128.6, 118.6, 80.4, 74.8, 54.4, 52.7, 37.2, 28.4; m/z LRMS (ES+) 566.1 (10%, $[M(^{79}Br^{81}Br) + Na]^+$), 446.1 (50, $[M(^{81}Br_2) - C_5H_8O_2]^+$), 444.1 (100, $[M(^{79}Br^{81}Br) - C_5H_8O_2]^+$), 442.1 (50, $[M(^{79}Br_2) - C_5H_8O_2]^+$); HRMS (ES+) found 565.9995, $C_{22}H_{25}{}^{79}Br^{81}BrNNaO_5{}^+$ requires 565.9977.

(+)-(*S*)-Methyl 2-amino-3-(4-benzyloxy-3,5-dibromophenyl)-propanoate (18). Carbamate **17** (2.61 g, 4.81 mmol) was dissolved in a mixture of dichloromethane (10 mL) and TFA (10 mL) and stirred at room temperature for 2 h, at which point TLC analysis indicated complete conversion. The reaction mixture was diluted with dichloromethane (40 mL) and made basic with saturated aqueous $NaHCO_3$ (20 mL) and aqueous 10 M NaOH (10 mL) under vigorous stirring and cooling in an ice-bath. The resulting biphasic mixture was separated and the aqueous layer extracted with dichloromethane (2 × 10 mL). The pooled organic layers were dried (Na_2SO_4) and concentrated, and the crude residue purified by column chromatography on silica gel (190 : 9 : 1 dichloromethane/methanol/aqueous ammonia) to give the amine **18** (1.48 g, 69%) as a colourless oil; $[\alpha]_D^{21}$ +5.9 (c 0.8, dichloromethane); ν_{max}/cm^{-1} (neat) 3381, 3032, 2949, 1738, 1455; δ_H (400 MHz, $CDCl_3$) 7.60 (2H, d, J = 7.4 Hz), 7.43–7.34 (3H, m), 7.40 (2H, s), 5.01 (2H, s), 3.73 (3H, s), 3.70 (1H, dd, J = 7.9, 5.1 Hz), 3.00 (1H, dd, J = 13.7, 5.1 Hz), 2.77 (1H, dd, J = 13.7, 7.9 Hz), 1.65 (2H, br s); δ_C (100 MHz, $CDCl_3$) 175.0, 151.7, 136.5, 136.3, 133.5, 128.54, 128.46, 118.5, 74.7, 55.6, 52.3, 39.7; m/z LRMS (ES+) 446.2 (70%, $[M(^{81}Br_2) + H]^+$), 444.2 (100, $[M(^{79}Br^{81}Br) + H]^+$), 442.2 (70, $[M(^{79}Br_2) + H]^+$), 386.2 (70), 384.2 (100), 382.2 (70); HRMS (ES+) found 443.9648, $C_{17}H_{18}{}^{79}Br^{81}BrNO_3{}^+$ requires 443.9633.

(*E*)-Methyl 3-(4-benzyloxy-3,5-dibromophenyl)-2-(hydroxyimino)propanoate (19). A solution of sodium tungstate dihydrate (73.7 mg, 0.223 mmol) and 37% aqueous hydrogen peroxide (0.250 mL, 2.43 mmol) in water (2 mL) was added to a solution of amine **18** (99.0 mg, 0.223 mmol) in ethanol (2 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 30 mins, then at room temperature for 17 h. To the mixture was added saturated aqueous NH_4Cl (5 mL) and brine (2 mL) and the aqueous layer extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give the oxime **19** (101 mg, 99%) as colourless platelets; mp 167–168 °C (hexanes); ν_{max}/cm^{-1} (film) 3061, 3035, 2923, 1729, 1441; δ_H (400 MHz, $CDCl_3$) 9.47 (1H, br s), 7.59 (2H, d, J = 7.4 Hz), 7.51 (2H, s), 7.43–7.34 (3H, m), 4.99 (2H, s), 3.91 (2H, s), 3.89 (3H, s); δ_C (100 MHz, $CDCl_3$) 163.6, 151.8, 150.3, 136.4, 134.4, 133.6, 128.6, 128.5, 118.6, 74.8, 53.2, 29.4; m/z LRMS (ES+) 501.1 (50%, $[M(^{81}Br_2) + C_2H_4N]^+$), 499.1 (100, $[M(^{79}Br^{81}Br) + C_2H_4N]^+$), 497.1 (45, $[M(^{79}Br_2) + C_2H_4N]^+$), 475.2 (60), 458.1 (75, $[M(^{79}Br^{81}Br) + H]^+$); HRMS (ES+) found 479.9257, $C_{17}H_{15}{}^{79}Br^{81}BrNNaO_4{}^+$ requires 479.9245.

(*E*)-Methyl 3-(4-hydroxy-3,5-dibromophenyl)-2-(hydroxyimino)propanoate (13). Benzyl ether **19** (229 mg, 0.501 mmol), zinc(II)bromide (338 mg, 1.50 mmol) and 10% palladium on charcoal (53.0 mg, 49.8 μmol) were suspended in dry ethyl acetate (20 mL). The reaction vessel was purged under reduced pressure and back-filled with hydrogen gas three times, then stirred vigorously under hydrogen (1 atm) for 30 mins, at which point TLC analysis showed complete consumption of starting material. The mixture was dryloaded on silica and subjected to chromatography on silica gel (1 : 3 ethyl acetate/heptanes) to give the phenol **13** (128 mg, 70%) as colourless platelets; mp 174–175 °C (ethyl acetate); ν_{max}/cm^{-1} (film) 3409, 2949, 1731, 1474; δ_H (400 MHz, CD_3OD) 7.37 (2H, s), 3.80 (2H, s), 3.79 (3H, s); δ_C (100 MHz, CD_3OD) 165.7, 151.0, 150.8, 133.8, 132.0, 112.0, 52.9, 29.7; m/z LRMS (ES–) 365.9 (65%, $[M(^{79}Br^{81}Br) - H]^-$), 253.0 (48, $[M(^{81}Br_2) - C_4H_6NO_3]^-$), 251.0 (100, $[M(^{79}Br^{81}Br) - C_4H_6NO_3]^-$), 249.0 (51, $[M(^{79}Br_2) - C_4H_6NO_3]^-$); HRMS (ES+) found 389.8792, $C_{10}H_9{}^{79}Br^{81}BrNNaO_4{}^+$ requires 389.8776.

(+)-(*S*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-methylaminopropanoic acid hydrochloride (21). Me-Tyr(Me)-OH **20** (500 mg, 2.39 mmol) was dissolved in 2 M HCl (12 mL) and cooled to 0 °C. Bromine (1.00 mL, 19.5 mmol) was added dropwise *via* addition funnel, the resulting mixture stirred for 15 mins and then warmed

to room temperature. Stirring was continued as a stream of air was passed through the mixture for 22 h. All solvents were evaporated and the crude residue triturated from diethyl ether and petrol to give **21** (864 mg, 90%) as tan platelets; mp 174–175 °C (petrol); $[\alpha]_D^{21}$ +16.3 (*c* 0.95, methanol); ν_{max}/cm^{-1} (film) 3406, 2932, 1737, 1472, 1423; δ_H (400 MHz, CD_3OD) 7.56 (2H, s), 4.29 (1H, dd, *J* = 6.7, 5.6 Hz), 3.85 (3H, s), 3.32 (1H, dd, *J* = 14.6, 5.6 Hz), 3.20 (1H, dd, *J* = 14.6, 6.7 Hz), 2.76 (3H, s); δ_C (100 MHz, CD_3OD) 170.0, 155.2, 135.0, 134.3, 119.4, 62.6, 61.1, 34.8, 32.9; *m/z* LRMS (ES–) 368.0 (44%, $[M(^{81}Br_2) - H]^-$), 366.0 (100, $[M(^{79}Br^{81}Br) - H]^-$), 364.0 (44, $[M(^{79}Br_2) - H]^-$), 335.0 (52, $[M(^{79}Br^{81}Br) - CH_4N]^-$); HRMS (ES+) found 367.9328, $C_{11}H_{14}{}^{79}Br^{81}BrNO_3{}^+$ requires 367.9320.

(+)-(*S*)-Methyl 3-(3,5-dibromo-4-methoxyphenyl)-2-methylaminopropanoate (14). Acid **21** (40 mg, 0.10 mmol) was dissolved in methanol (5 mL) and thionyl chloride (0.14 mL, 2.0 mmol) was added dropwise. The resulting mixture was refluxed for 22 h, at which point LCMS analysis indicated consumption of starting material. Solvents were removed under reduced pressure and the residue dissolved in dichloromethane (5 mL) and washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was back-extracted with dichloromethane (2 × 5 mL), the pooled organic layers dried (Na_2SO_4) and concentrated under reduced pressure and the residue purified by chromatography on silica gel (1 : 3 ethyl acetate/heptanes, then 89 : 10 : 1 dichloromethane/methanol/aqueous ammonia) to give **14** (25 mg, 66%) as a pale brown film; $[\alpha]_D^{21}$ +9.1 (*c* 1.0, dichloromethane); ν_{max}/cm^{-1} (film) 2949, 2930, 1736, 1473, 1423; δ_H (400 MHz, $CDCl_3$) 7.32 (2H, s), 3.86 (3H, s), 3.70 (3H, s), 3.40 (1H, t, *J* = 6.7 Hz), 2.88 (1H, dd, *J* = 13.3, 6.7 Hz), 2.88 (1H, dd, *J* = 13.3, 6.7 Hz), 2.38 (3H, s); δ_C (100 MHz, $CDCl_3$) 174.3, 153.0, 136.2, 133.4, 118.1, 64.2, 60.8, 52.0, 38.0, 34.8; *m/z* LRMS (ES+) 382.1 (20%, $[M(^{79}Br^{81}Br) + H]^+$), 324.1 (48, $[M(^{81}Br_2) - C_2H_3O_2]^+$), 322.1 (100, $[M(^{79}Br^{81}Br) - C_2H_3O_2]^+$), 320.1 (50, $[M(^{79}Br_2) - C_2H_3O_2]^+$); HRMS (ES+) found 381.9489, $C_{12}H_{16}{}^{79}Br^{81}BrNO_3{}^+$ requires 381.9476.

Benzyloxy-*N*-(12-(2-methylprop-2-oxy)carbamoyl-4,9-diaza-4,9-dimethyldodecyl)carbamate (26). *N1*-Cbz-*N14*-Boc-spermine **25** (578 mg, 1.32 mmol), 37% aqueous formaldehyde (2.20 mL, 29.4 mmol), acetic acid (2.50 mL, 43.7 mmol) and $NaCNBH_3$ (665 mg, 10.6 mmol) were added to ethanol (15 mL) at room temperature and the resulting mixture stirred for 15 h, at which point LCMS analysis showed completion. The mixture was diluted with dichloromethane (50 mL) and 10 M aqueous NaOH (10 mL) and stirred 10 mins. The biphasic mixture was extracted with dichloromethane (2 × 50 mL), washed with brine (10 mL), dried (Na_2SO_4) and concentrated and the residue purified by column chromatography on silica gel (1 : 1 ethyl acetate/heptanes, then 40 : 5 : 1 dichloromethane/methanol/aqueous ammonia) to give **26** (458 mg) as a colourless syrup, approximately 80–90% pure by analysis of ^1H NMR integrals, used in the next step without further purification; ν_{max}/cm^{-1} (film) 3337, 3033, 2795, 1703, 1526; δ_H (400 MHz, $CDCl_3$) 7.35–7.30 (5H, m), 5.09 (2H, s), 3.30–3.23 (2H, m), 3.18–3.14 (2H, m), 2.43–2.33 (8H, m), 2.20 (3H, s), 2.18 (3H, s), 1.70–1.59 (4H, m), 1.49–1.46 (4H, m), 1.43 (9H, s); δ_C (90 MHz, $CDCl_3$) 156.7, 156.3, 137.0, 128.6, 128.3, 128.2, 79.0, 66.5, 57.8, 57.7, 56.5, 56.2, 54.0, 52.0, 42.0, 39.9, 28.6, 26.9, 26.5, 25.04, 24.98; *m/z* LRMS (ES–) 465.4 (20%, $[M + H]^+$), 277.4 (35, $[M - C_9H_{19}N_2O_2]^+$), 143.4 (100); HRMS (ES+) found 465.3445, $C_{25}H_{45}N_4O_4{}^+$ requires 465.3441.

***N*-(12-Amino-4,9-diaza-4,9-dimethyldodecyl)-benzyloxycarbamate trishydrochloride (27).** A 100/458 mg aliquot of crude spermine derivative **26** (100 mg) was dissolved in a mixture of aqueous 4 M HCl (2 mL) and 1,4-dioxane (2 mL) and stirred at room temperature for 20 h. Solvents were evaporated under reduced pressure and the residue triturated from diethyl ether to give **27** (105 mg) as a colourless gum, approximately 70–80% pure by analysis of ^1H NMR integrals, used in the next step without further purification; ν_{max}/cm^{-1} (film) 3405, 2964, 1705, 1630, 1529, 1474; δ_H (400 MHz, CD_3OD) 7.43–7.31 (5H, m), 5.09 (2H, s), 3.41–3.06 (12H, m), 2.92 (3H, s), 2.91 (3H, s), 2.22–2.14 (4H, m), 2.02–1.83 (4H, m); δ_C (100 MHz, CD_3OD) 159.3, 138.4, 129.7, 129.2, 129.0, 67.8, 61.7, 56.8, 56.7, 55.3, 54.3, 40.8, 40.7, 38.8, 38.1, 26.2, 23.6, 22.5; *m/z* LRMS (ES–) 365.5 (15%, $[M + H]^+$), 143.4 (100); HRMS (ES+) found 365.2921, $C_{20}H_{37}N_4O_2{}^+$ requires 365.2917.

(*E*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-hydroxyimino-*N*-(12-benzyloxycarbamoyl-4,9-diazadodecyl)propanamide bisformate (28). Acid **6** (37 mg, 0.10 mmol), *N*,*N*′-dicyclohexylcarbodiimide (23 mg, 0.11 mmol), *N*-hydroxyphtalimide (18 mg, 0.11 mmol) and 1,4-dioxane (0.5 mL) were combined in a 5 mL Biotage microwave tube and the resulting mixture stirred at room temperature for 24 h. To the mixture was added a solution of a 52/105 mg aliquot of crude spermine derivative **27** (52 mg) and triethylamine (31 µL, 0.22 mmol) in methanol (0.5 mL) and the resulting mixture was stirred for 14 h. Solvents were evaporated under reduced pressure and the residue purified by column chromatography on silica gel (9 : 1 : 0 to 4 : 2 : 1 dichloromethane/methanol/aqueous ammonia) and preparative HPLC to give the bisformate salt of **28** (9.0 mg, 8% from **25**) as a colourless, hygroscopic powder; ν_{max}/cm^{-1} (film) 3300, 2946, 1709, 1661, 1588; δ_H (400 MHz, CD_3OD) 8.47 (2H, s), 7.49 (2H, s), 7.36–7.28 (5H, m), 5.08 (2H, s), 3.85 (2H, s), 3.81 (3H, s), 3.33 (2H, t, *J* = 6.3 Hz), 3.21 (2H, t, *J* = 6.5 Hz), 3.00–2.90 (8H, m), 2.69 (3H, s), 2.67 (3H, s), 1.92–1.83 (4H, m), 1.74–1.69 (4H, m); δ_C (100 MHz, CD_3OD) 169.4, 166.0, 159.1, 153.9, 152.0, 138.3, 137.5, 134.5, 129.5, 129.1, 128.8, 118.6, 67.6, 61.1, 55.2, 40.6, 39.0, 37.5, 28.8, 26.4, 26.1, 23.2; *m/z* LRMS (ES+) 716.4 (35%, $[M(^{81}Br_2) + H]^+$), 714.4 (70, $[M(^{79}Br^{81}Br) + H]^+$), 712.4 (38, $[M(^{79}Br_2) + H]^+$), 494.2 (40, $[M(^{81}Br_2) - C_{12}H_{17}N_2O_2]^+$), 492.2 (83, $[M(^{79}Br^{81}Br) - C_{12}H_{17}N_2O_2]^+$), 490.2 (42, $[M(^{79}Br_2) - C_{12}H_{17}N_2O_2]^+$), 409.1 (50, $[M(^{81}Br_2) - C_{17}H_{28}N_3O_2]^+$), 407.1 (100, $[M(^{79}Br^{81}Br) - C_{17}H_{28}N_3O_2]^+$), 405.1 (50, $[M(^{79}Br_2) - C_{17}H_{28}N_3O_2]^+$), 277.4 (75); HRMS (ES+) found 714.1703, $C_{30}H_{44}{}^{79}Br^{81}BrN_5O_5{}^+$ requires 714.1689.

(*E*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-hydroxyimino-*N*-(12-(2-methylprop-2-oxy)carbamoyl-4,9-diazadodecyl)propanamide (29). Ester **10** (216 mg, 0.567 mmol) and *N*1-Boc-spermine **23** (343 mg, 1.13 mmol) were dissolved in methanol (6 mL) and the resulting mixture refluxed for 14 h, at which point LCMS analysis showed completion. After cooling, the reaction mixture was concentrated and the residue purified by column chromatography on silica gel (89 : 10 : 1 to 20 : 5 : 1 dichloromethane/methanol/aqueous ammonia) to give **29** (312 mg, 85%) as a pale yellow glass; ν_{max}/cm^{-1} (film) 3290, 2971, 2932,

2864, 1693, 1659, 1525; δ_H (400 MHz, CD_3OD) 7.48 (2H, s), 3.84 (2H, s), 3.81 (3H, s), 3.35–3.32 (2H, m), 3.09 (2H, t, J = 6.7 Hz), 2.63–2.53 (8H, m), 1.74–1.64 (4H, m), 1.60–1.50 (4H, m), 1.43 (9H, s); δ_C (100 MHz, CD_3OD) 165.6, 158.6, 153.8, 152.0, 137.5, 134.5, 118.6, 79.9, 61.0, 50.4, 48.2, 47.7, 39.1, 38.5, 30.6, 29.9, 28.8, 28.3, 28.1; m/z LRMS (ES–) 652.3 (48%, $[M(^{81}Br_2) - H]^-$), 650.4 (100, $[M(^{79}Br^{81}Br) - H]^-$), 648.4 (45, $[M(^{79}Br_2) - H]^-$), 576.2 (52, $[M(^{79}Br^{81}Br) - C_4H_9]^-$); HRMS (ES+) found 652.1562, $C_{25}H_{42}{}^{79}Br^{81}BrN_5O_5{}^+$ requires 652.1532.

(*E*)-*N*-(12-Amino-4,9-dimethyl-4,9-diazadodecyl)-3-(3,5-dibromo-4-methoxyphenyl)-2-(hydroxyimino)propanamide trishydrochloride (30). Boc-protected polyamine **29** (16 mg, 25 μmol), 37% aqueous formaldehyde (75 μL, 1.0 mmol), acetic acid (0.11 mL, 2.0 mmol) and $NaCNBH_3$ (31 mg, 0.50 mmol) were added to ethanol (1 mL) at room temperature and the resulting mixture stirred for 1 h, at which point LCMS analysis showed completion. The mixture was diluted with dichloromethane (6 mL) and aqueous 10 M NaOH (3 mL) and stirred for 10 mins. The biphasic mixture was extracted with dichloromethane (3 × 10 mL), washed with brine (5 mL), dried (Na_2SO_4) and concentrated to 5 mL total volume. To the concentrate was added *n*-dodecanethiol (0.5 mL) and TFA (0.25 mL) and the resulting solution stirred at room temperature for 16 h. All volatile solvents were removed by evaporation under reduced pressure and the residue extracted into a 1 : 1 mixture of methanol/aqueous 1 M HCl (2 mL) and purified by preparative HPLC to yield **30** (11.5 mg, 67%) as a colourless film; ν_{max}/cm^{-1} (film) 3385, 2965, 1666, 1530, 1471; δ_H (400 MHz, CD_3OD) 7.50 (2H, s), 3.86 (2H, s), 3.82 (3H, s), 3.39–3.35 (2H, m), 3.30–3.05 (10H, m), 2.91 (3H, s), 2.87 (3H, s), 2.23–2.13 (2H, m), 2.02–1.94 (2H, m), 1.90–1.80 (4H, m); δ_C (100 MHz, CD_3OD) 166.1, 153.9, 151.9, 137.5, 134.5, 118.6, 61.1, 56.7, 56.6, 55.2, 54.2, 40.6, 40.5, 37.9, 37.1, 28.9, 25.7, 23.5, 22.35, 22.33; m/z LRMS (ES+) 580.4 (50%, $[M(^{81}Br_2) + H]^+$), 578.4 (100, $[M(^{79}Br^{81}Br) + H]^+$), 576.4 (48, $[M(^{79}Br_2) + H]^+$); HRMS (ES+) found 580.1348, $C_{22}H_{38}{}^{79}Br^{81}BrN_5O_3{}^+$ requires 580.1321.

(*E*,*E*)-*N*-(12-(3-(3,5-Dibromo-4-hydroxyphenyl)-2-(hydroxyimino)propanamido)-4,9-dimethyl-4,9-diazadodecyl)-3-(3,5-dibromo-4-methoxyphenyl)-2-(hydroxyimino)propanamide bishydrochloride, pseudoceramine A (2). To a 5 mL Biotage microwave tube was added **13** (8.1 mg, 22 μmol), **30** (23 mg, 33 μmol), NEt_3 (14 μL, 100 μmol) and methanol (0.5 mL). The tube was sealed and the contents heated at 65 °C for 68 h. After cooling, the reaction mixture was diluted with aqueous 1 M HCl (0.5 mL) and purified by preparative HPLC to give the hydrochloride salt of pseudoceramine A **2** (6.5 mg, 30%) as a pale yellow powder; ν_{max}/cm^{-1} (film) 3300, 3050, 2942, 1658, 1587, 1529, 1471; δ_H (400 MHz, CD_3OD) 8.51 (1H, br s), 7.50 (2H, s), 7.35 (2H, s), 3.86 (2H, s), 3.81 (3H, s), 3.79 (2H, s), 3.35–3.32 (4H, m), 2.85–2.67 (8H, m), 2.57 (3H, s), 2.56 (3H, s), 1.91–1.78 (4H, m), 1.63–1.56 (4H, m); δ_C (100 MHz, CD_3OD) 166.2, 165.8, 153.9, 152.8, 152.6, 152.1, 137.5, 134.5, 133.7, 133.6, 130.7, 118.6, 113.0, 61.1, 57.6, 57.4, 55.3, 55.2, 40.7, 37.8, 37.4, 28.8, 28.5, 26.42, 26.39, 24.0, 23.7; m/z LRMS (ES+) 915.3 (35%, $[M(^{79}Br_2{}^{81}Br_2) + H]^+$), 409.1 (50), 407.1 (100), 405.1 (48), 393.1 (76, $[C_{12}H_{13}{}^{79}Br^{81}BrN_2O_3]^+$); HRMS (ES+) found 914.9912, $C_{31}H_{43}{}^{79}Br_2{}^{81}Br_2N_6O_6{}^+$ requires 914.9937.

(–)-(*S*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-(*N*-methyl-(2-methylprop-2-yloxycarbamoyl)propanoic acid (32). Ammonium salt **21** (270 mg, 0.669 mmol) was dissolved in water (2 mL), cooled to 0 °C and freebased by careful addition of solid $NaHCO_3$ (141 mg, 1.67 mmol). To the vortexed solution was added dropwise a solution of di-*tert*-butyl dicarbonate (175 mg, 0.802 mmol) in THF (2 mL). After warming to room temperature and stirring for 17 h the solution was again cooled to 0 °C and another portion of di-*tert*-butyl dicarbonate (115 mg, 0.527 mmol) in THF (1 mL) was added, followed by warming to room temperature and stirring for 26 h. Deemed complete by LCMS analysis, the reaction mixture was diluted with water (5 mL), acidified to pH 2 with aqueous 1 M HCl, extracted with dichloromethane (3 × 10 mL) and the combined organic layers dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by chromatography on silica gel (1 : 3 ethyl acetate/heptanes) to give **32** (200 mg, 64%) as colourless needles; mp 51–52 °C (hexanes); $[\alpha]_D^{21}$ –40.7 (*c* 2.48, dichloromethane); ν_{max}/cm^{-1} (film) 2976, 2932, 1735, 1690, 1474; δ_H (400 MHz, CD_3OD) obtained as a 5 : 4 mixture of atropisomers, 7.47 (1.1H, s), 7.45 (0.9H, s), 4.82–4.72 (1H, m), 3.83 (1.6H, s), 3.82 (1.4H, s), 3.24 (1H, dd, J = 14.6, 4.9 Hz), 3.03 (0.4H, dd, J = 14.6, 10.5 Hz), 2.98 (0.6H, dd, J = 14.6, 10.5 Hz), 2.73 (1.6H, s), 2.72 (1.4H, s), 1.39 (4H, s), 1.34 (5H, s); δ_C (100 MHz, CD_3OD) 173.7 (Major), 173.5 (minor), 157.5 (m), 157.0 (M), 154.1 (M), 154.0 (m), 138.9 (M), 138.6 (m), 134.6 (M), 134.5 (m), 118.8 (M), 118.6 (m), 81.9 (M), 81.5 (m), 62.0, 61.0 (M), 60.8 (m), 34.9 (M), 34.5 (m), 32.8 (m), 32.4 (M), 28.6 (m), 28.5 (M); m/z LRMS (ES–) 468.0 (50%, $[M(^{81}Br_2) - H]^-$), 466.0 (100, $[M(^{79}Br^{81}Br) - H]^-$), 464.0 (48, $[M(^{79}Br_2) - H]^-$), 335.0 (38, $[M(^{79}Br^{81}Br) - C_6H_{13}NO_2]^-$); HRMS (ES+) found 489.9682, $C_{16}H_{21}{}^{79}Br^{81}BrNNaO_5{}^+$ requires 489.9664.

(–)-(*S*)-(*E*)-3-(3,5-Dibromo-4-methoxyphenyl)-*N*-(12-(3-(3,5-dibromo-4-methoxyphenyl)-2-(*N*-methyl-(2-methylprop-2-yloxy)-carbamoyl)propanamido)-4,9-dimethyl-4,9-diazadodecyl)-2-(hydroxyimino)propanamide bishydrochloride (33). Acid **32** (13 mg, 28 μmol), DCC (6.3 mg, 31 μmol) and *N*-hydroxyphthalimide (5.0 mg, 31 μmol) were dissolved in 1,4-dioxane (0.5 mL) and stirred at room temperature for 2 h, at which point DCC (0.60 mg, 3.1 μmol) and *N*-hydroxyphthalimide (0.50 mg, 3.1 μmol) were added, followed by further stirring for 18 h. A solution of ammonium salt **30** (21 mg, 31 μmol) and NEt_3 (13 μL, 92 μmol) in methanol (0.5 mL) was then added and stirring continued for 7 h. The reaction mixture was concentrated and the crude residue dissolved in 1 : 1 methanol/aqueous 1 M HCl (2 mL) and purified by preparative HPLC to give the hydrochloride salt **33** (10 mg, 33%) as a colourless powder; $[\alpha]_D^{21}$ –19.6 (*c* 1.4, methanol); ν_{max}/cm^{-1} (film) 3385, 2926, 1687, 1656, 1594, 1469; δ_H (500 MHz, CD_3OD) obtained as a 5 : 4 mixture of atropisomers 8.50 (1H, br s), 7.49 (2H, s), 7.46 (2H, s), 4.80–4.70 (1H, m), 3.86 (2H, s), 3.82 (3H, s), 3.81 (3H, s), 3.35–3.21 (6H, m), 2.95–2.89 (8H, m), 2.78 (1.4H, s), 2.73 (1.6H, s), 2.67 (6H, s), 1.92–1.83 (4H, m), 1.77–1.71 (4H, m), 1.40 (5H, s), 1.31 (4H, s); δ_C (125 MHz, CD_3OD) 173.1 (Major), 172.6 (minor), 169.5, 166.0, 157.5 (M), 156.8 (m), 154.1 (m), 153.9 (M), 152.1, 138.8 (m), 138.5 (M), 137.5, 134.7, 134.5, 118.9 (m), 118.7 (M), 118.6, 82.1 (m), 81.8 (M), 62.4 (m), 61.7 (M), 61.1, 55.3 (M), 55.2 (m), 40.8, 38.1 (m), 37.6 (M), 34.5 (m), 34.2 (M), 33.0 (M), 31.5 (m), 28.9, 28.6 (M), 28.5 (m), 26.2, 23.6; m/z LRMS (ES–) 1029.8 (75%, $[M(^{79}Br^{81}Br_3) - H]^-$), 1027.9 (100, $[M(^{79}Br_2{}^{81}Br_2) - H]^-$), 1025.8 (60, $[M(^{79}Br_3{}^{81}Br) - H]^-$);

HRMS (ES+) found 1029.0957, $C_{38}H_{57}{}^{79}Br_2{}^{81}Br_2N_6O_7^+$ requires 1029.0981.

(+)-(*S*)-(*E*)-3-(3,5-Dibromo-4-methoxyphenyl)-*N*-(12-(3-(3,5-dibromo-4-methoxyphenyl)-2-methylaminopropanamido)-4,9-dimethyl-4,9-diazadodecyl)-2-(hydroxyimino)propanamide tristrifluoroacetate, pseudoceramine B (3). Protected pseudoceramine **33** (8.6 mg, 7.8 μmol), n-dodecanethiol (11 mg, 39 μmol) and TFA (0.5 mL) were dissolved in dichloromethane (0.5 mL) and stirred at room temperature for 2 h, at which point LCMS analysis showed complete consumption of starting materials. The reaction mixture was concentrated under reduced pressure and the crude residue filtered through a Supelco fritted C18 silica plug, eluting with a gradient of methanol-water (0–70%) to give the trifluoroacetate salt of pseudoceramine B **3** (6.6 mg, 67%) as a colourless film; $[\alpha]_D^{21}$ +34.3 (*c* 0.07, methanol) (lit.,[3] +8° (*c* 0.08, methanol)); ν_{max}/cm^{-1} (film) 3357, 3224, 2961, 2706, 1666, 1545, 1471; δ_H (500 MHz, CD_3OD) 7.52 (2H, s), 7.50 (2H, s), 4.00 (1H, dd, J = 8.5, 6.0 Hz), 3.86 (2H, s), 3.85 (3H, s), 3.82 (3H, s), 3.45–3.00 (14H, m), 2.86 (3H, s), 2.85 (3H, s), 2.70 (3H, s), 1.99–1.79 (8H, m); δ_C (125 MHz, CD_3OD) 168.2, 166.3, 155.1, 153.9, 151.9, 137.5, 135.1, 134.6, 134.5, 119.3, 118.6, 63.5, 61.2, 61.1, 56.6, 55.2, 55.0, 40.4, 37.6, 37.1, 35.9, 32.4, 28.9, 25.7, 25.1, 22.29, 22.28; *m/z* LRMS (ES–) 929.8 (57%, $[M(^{79}Br^{81}Br_3) - H]^-$), 927.8 (100, $[M(^{79}Br_2{}^{81}Br_2) - H]^-$), 925.8 (60, $[M(^{79}Br_3{}^{81}Br) - H]^-$); HRMS (ES+) found 1029.0957, $C_{38}H_{57}{}^{79}Br_2{}^{81}Br_2N_6O_7^+$ requires 1029.0981.

(*E*)-2-(12-(3-(3,5-Dibromo-4-methoxyphenyl)-2-(hydroxyimino)propanamido)-4,9-dimethyl-4,9-diazadodecyl)-1,3-dioxoisoindole bishydrochloride (31). Acid **21** (9.1 mg, 23 μmol), DCC (5.1 mg, 25 μmol) and *N*-hydroxyphthalimide (4.0 mg, 25 μmol) were dissolved in 1,4-dioxane (0.5 mL) and stirred at room temperature for 4 h, at which point DCC (0.50 mg, 2.5 μmol) and *N*-hydroxyphthalimide (0.40 mg, 2.5 μmol) were added, followed by further stirring for 19 h. A solution of ammonium salt **30** (17 mg, 25 μmol) and NEt_3 (14 μL, 99 μmol) in methanol (0.5 mL) was then added and stirring continued for 23 h. The reaction mixture was concentrated and the crude residue dissolved in 1:1 methanol/aqueous 1 M HCl (2 mL) and purified by preparative HPLC to give, along with the hydrochloride salt of pseudoceramine B **3** (0.7 mg, 3%), the hydrochloride salt **31** (8.0 mg, 45%) as a colourless film; ν_{max}/cm^{-1} (film) 3386, 2959, 1711, 1660, 1589, 1471; δ_H (400 MHz, CD_3OD) 8.34 (1H, br s), 7.88–7.85 (2H, m), 7.83–7.80 (2H, m), 7.48 (2H, s), 3.85 (2H, s), 3.81 (3H, s), 3.80 (2H, t, J = 6.6 Hz), 3.35 (2H, t, J = 6.6 Hz), 3.17–3.04 (8H, m), 2.80 (3H, s), 2.79 (3H, s), 2.15–2.08 (2H, m), 1.98–1.91 (2H, m), 1.80–1.76 (4H, m); δ_C (100 MHz, CD_3OD) 169.8, 166.1, 153.9, 152.0, 137.5, 135.5, 134.5, 133.3, 124.2, 118.6, 61.1, 56.7, 55.3, 55.1, 40.6, 40.5, 37.2, 36.0, 28.8, 25.8, 25.1, 22.8, 22.7; *m/z* LRMS (ES–) 710.8 (50%, $[M(^{81}Br_2) - H]^-$), 708.7 (100, $[M(^{79}Br^{81}Br) - H]^-$), 706.5 (55, $[M(^{79}Br_2) - H]^-$); HRMS (ES+) found 710.1393, $C_{30}H_{40}{}^{79}Br^{81}BrN_5O_5^+$ requires 710.1376.

***N*-(3-Formylpropyl)-*N*-methyl-(2-methylprop-2-yloxy)carbamide (34).** Sodium hydride (washed with pentane and dried *in vacuo*, 27 mg, 1.1 mmol) was added to a solution of **35** (240 mg, 0.92 mmol) in THF (5 mL). After 1 h, iodomethane (80 μL, 1.3 mmol) was added and the resulting mixture stirred for 18 h. Deemed incomplete by ^1H NMR analysis of an aliquot, DMF (5 mL) and iodomethane (160 μL, 2.6 mmol) were added and the resulting mixture stirred for 20 h. Saturated aqueous ammonium chloride (5 mL) was then added and the resulting biphasic mixture was extracted with diethyl ether (2 × 10 mL), the combined organic layers dried (Na_2SO_4) and concentrated and the residue dissolved in acetic acid (4 mL) and water (2 mL) and stirred for 18 h. After evaporation of solvents, purification of the residue was attempted by chromatography on silica gel (acetone) to give the unstable aldehyde **34** as a colourless oil, approximately 50% pure by ^1H NMR analysis of the crude material (68 mg, 18%), which was used immediately in the next step without further purification; δ_H (400 MHz, $CDCl_3$) obtained as a 1:1 mixture of atropisomers 9.78 (1H, m), 3.27–3.14 (2H, m), 2.84 (1.5H, s), 2.83 (1.5H, s), 2.00–1.61 (4H, m), 1.45 (4.5H, s), 1.45 (4.5H, s).

(*E*)-3-(3,5-Dibromo-4-methoxyphenyl)-2-hydroxyimino-*N*-(8-(methylamino)-4-methyl-4-azaoctyl)propanamide bishydrochloride, pseudoceramine C (4). To a solution of **5** (16.1 mg, 36.8 μmol) in ethanol (1 mL) was added **34** (50% pure, 74.0 mg, 0.184 mmol), acetic acid (22.1 mg, 0.369 mmol) and $NaCNBH_3$ (11.5 mg, 0.184 mmol). The resulting mixture was stirred at room temperature for 16 h, then diluted with dichloromethane (5 mL) and aqueous 10 M NaOH (0.5 mL), stirred 5 mins and washed with brine (3 mL). The aqueous phase was extracted with dichloromethane (2 × 5 mL) and the combined organic layers dried (Na_2SO_4) and concentrated to 2 mL total volume. *n*-Dodecanethiol (0.25 mL) and TFA (1 mL) were added and the resulting mixture stirred for 15 h. All volatiles were evaporated under reduced pressure, the crude residue extracted with a 1:1 mixture of methanol/aqueous 1 M HCl (2 mL) and the extract purified by preparative HPLC to give the hydrochloride salt of pseudoceramine C **4** (6.9 mg, 32%) as a colourless film; ν_{max}/cm^{-1} (film) 3357, 2974, 2927, 2896, 1720, 1664; δ_H (500 MHz, CD_3OD) 7.50 (2H, s), 3.86 (2H, s), 3.82 (3H, s), 3.41–3.35 (2H, m), 3.22–3.03 (6H, m), 2.85 (3H, s), 2.72 (3H, s), 1.98–1.93 (2H, m), 1.83–1.72 (4H, m); δ_C (125 MHz, CD_3OD) 166.3, 153.9, 152.0, 137.4, 134.5, 118.6, 61.1, 56.6, 55.2, 40.4, 37.1, 33.6, 28.9, 25.8, 24.1, 22.3; *m/z* LRMS (ES+) 523.2 (51%, $[M(^{81}Br_2) + H]^+$), 521.3 (100, $[M(^{79}Br^{81}Br) + H]^+$), 519.2 (47, $[M(^{79}Br_2) + H]^+$); HRMS (ES+) found 523.0760, $C_{19}H_{31}{}^{79}Br^{81}BrN_4O_5^+$ requires 523.0742.

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