Synthesis and in vitro and in vivo evaluation of antimalarial polyamines

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We recently reported that 1,14-diphenylacetamide derivatives of spermine exhibit potent nM in vitro growth inhibition properties of *Plasmodium falciparum*. In an effort to expand the structure-activity relationship of this compound class towards malaria, we have prepared and biologically tested a library that includes benzamide and 3-phenylpropanamide 'capping acid' groups, and polyamines that include spermine (PA3-4-3) and chain extended analogues PA3-8-3 and PA3-12-3. 2-Hydroxy and 2,5-dimethoxy analogues were typically found to exhibit the most potent activity towards the dual drug resistant strain K1 of *P. falciparum* with IC_{50}'s in the range of 1.3 – 9.5 nM, and selectivity indices (SI) of 42300 to 4880. In vivo evaluation of three analogues against *P. berghei* was undertaken, with one demonstrating a modest 27.9% reduction in parasitaemia.

1. Introduction

Historically, natural products have acted as either a rich source of human therapeutics or as templates from which therapeutics are developed [1]. In the specific case of malaria, the plant natural product quinine (*Cinchona* sp.) spawned the development of chloroquine, primaquine and mefloquine [2] while investigation of the active principals of the Chinese plant (*Artemisia annua*) led ultimately to the introduction of artemisinin and related semi-synthetic antimalarials [2,3]. While chloroquine and artemisinin-type endoperoxides continue to be used in the front-line treatment of malaria, the increased incidence of resistance [4] prompts the need to develop new mode-of-action antimalarials. The marine environment is proving to be a useful reservoir from which to discover new chemical scaffolds possessing antimalarial properties [5-8].

As part of our ongoing search for new antimalarial lead compounds [9], we recently reported the discovery and preliminary assessment of structure-antimalarial activity for the polyamine marine natural product orthidine F (**1**) and new synthesized structural analogues [10,11]. The study evaluated the influence on biological activity of a variety of phenylacetic acid 'capping acids' as well as the effect of variation, to smaller fragments, of the polyamine core. Two analogues, 2-hydroxyphenylacetamide **2** and 2,5-dimethoxyphenylacetamide **3**, were found to exhibit significantly more pronounced activity towards *P. falciparum*, with IC_{50} 8.6 nM and 19 nM respectively, than orthidine F (**1**) (IC_{50} 890 nM). In addition, like **1** (L6 IC_{50} > 120 μM), both **2** and **3** were found to be relatively non-toxic towards the L6 rat myoblast cell line, with IC_{50}'s of > 130 μM and 88 μM respectively [10].

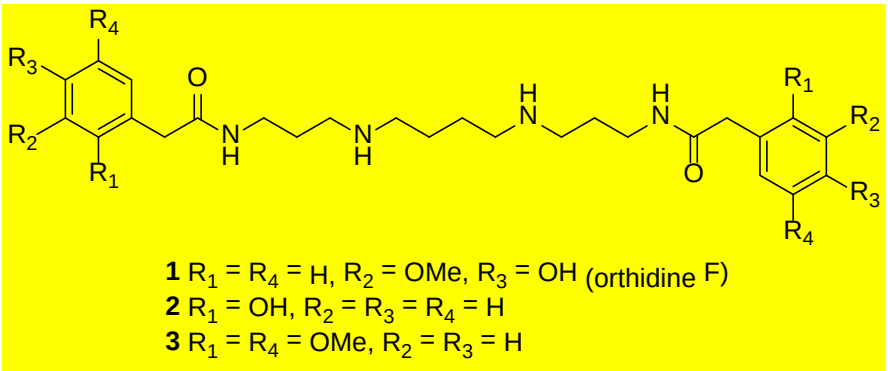


Fig. 1. Lead antimalarial polyamine structures.

In continuation of our interest in this class of antimalarial agent, we now report the synthesis of a new library of polyamine diamides that explore further polyamine fragments and alternative 'capping acids'. All analogues were evaluated for antimalarial activity against the K1 dual drug resistant strain of *P. falciparum* and for cytotoxicity towards the non-malignant L6 rat myoblast cell

line. Three analogues were also tested for their in vivo antimalarial activity against *Plasmodium berghei* in mice.

2. Chemistry

Using similar methodology previously reported by us for the preparation of spermine-phenylacetamide analogues [10,11], reaction of spermine with three benzoic acid derivatives (benzoic acid, 2-hydrobenzoic acid and 2,5-dimethoxybenzoic acid) and four 3-phenylpropanoic acid derivatives (3-phenylpropanoic acid, 3-(2-hydroxyphenyl)propanoic acid, 3-(2,5-dimethoxyphenyl)propanoic acid and 3-(3,4-dimethoxyphenyl)propanoic acid) using PyBOP as the coupling agent afforded, after chromatographic purification, analogues **4**–**10** in yields of 47%, 28%, 59%, 92%, 84%, 14% and 72%, respectively (Figure 2).

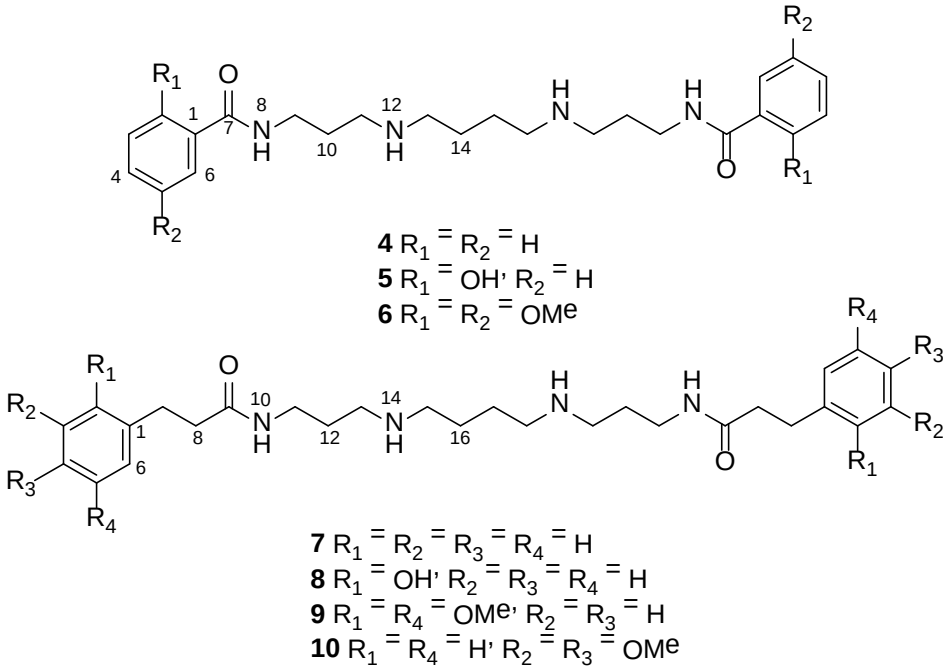
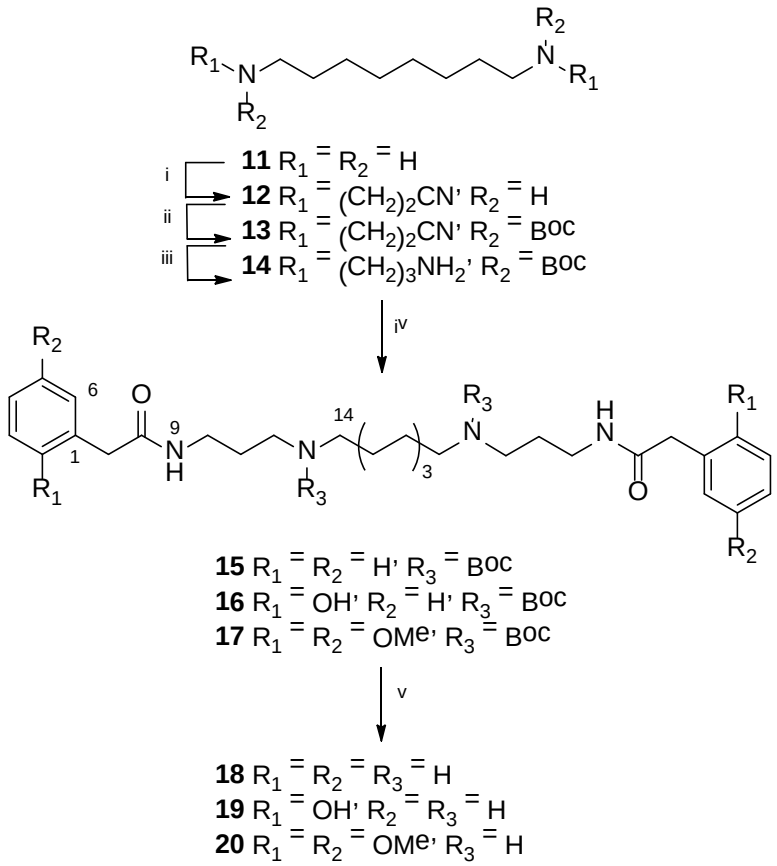


Fig. 2. Benzamide and 3-phenylpropanamide analogues of spermine.

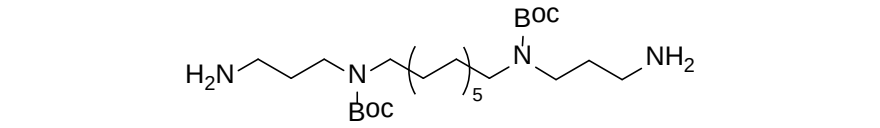
We next sought to explore the influence of polyamines of extended length on antimalarial activity with the preparation of spermine analogues bearing longer central hydrocarbon chains. The *tert*-butylcarbamate protected octane-1,8-bis(3-aminopropyl) polyamine **14** required for the preparation of analogues **15**–**20** was synthesised by a modification of previously reported methods [12,13]. Reaction of 1,8-diaminooctane (**11**) with two equivalents of acrylonitrile (to give **12**), followed by Boc protection (**13**) and reduction (Ni-Al alloy) gave the desired bis-*tert*-butoxylcarbamate protected tetraamine **14** (Scheme 1). Subsequent reaction of **14** with phenylacetic acid, 2-hydrophenylacetic acid and 2,5-dimethoxyphenylacetic acid, again using PyBOP as a coupling

agent, afforded Boc protected diamides **15**–**17** in yields of 75%, 55% and 64% respectively. Removal of the Boc groups with TFA in CH_2Cl_2 gave tetraamine-diamides **18**–**20** as TFA salts.

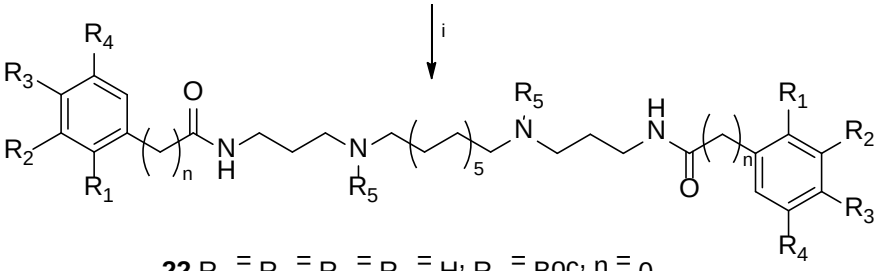


Scheme 1. Preparation of polyamines **15**–**20**. Reagents and conditions: (i) acrylonitrile, EtOH, N_2, reflux, 3h. (ii) di-*tert*-butyl dicarbonate, Et_3N, CH_2Cl_2, N_2, 22.5 h. (iii) LiOH, 10% Pd/C, 50% Ni-Al alloy, H_2, 50^{o}C, 21 h. (iv) carboxylic acid (2 equiv), PyBOP, DMF, Et_3N, N_2, 23 h. (v) CH_2Cl_2, TFA, N_2, 2 h.

The preparation of a further set of chain extended polyamine analogues made use of the previously reported Boc protected dodecane-1,12-(3-aminopropyl) tetraamine **21** [12,13] (Scheme 2). Using our general PyBOP-mediated amide coupling reaction methodology, reaction of **21** with three benzoic acids (benzoic acid, 2-hydroxybenzoic acid, and 2,5-dimethoxybenzoic acid), five phenylacetic acids (phenylacetic acid, 2-hydroxyphenylacetic acid, 2-methoxyphenylacetic acid, 4-methoxyphenylacetic acid and 2,5-dimethoxyphenylacetic acid) and four 3-phenylpropanoic acids (3-phenylpropanoic acid, 3-(2-hydroxyphenyl)propanoic acid, 3-(2,5-dimethoxyphenyl)propanoic acid and 3-(3,4-dimethoxyphenyl)propanoic acid) gave Boc protected analogues **22**–**33**. Subsequent removal of the Boc groups with TFA in CH_2Cl_2 gave tetraamine-diamides **34**–**45** as TFA salts.



21



22 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 0$
23 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 0$
24 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$, $R_5 = Boc$, $n = 0$
25 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 1$
26 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 1$
27 $R_1 = OMe$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 1$
28 $R_1 = R_2 = R_4 = H$, $R_3 = OMe$, $R_5 = Boc$, $n = 1$
29 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$, $R_5 = Boc$, $n = 1$
30 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 2$
31 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, $n = 2$
32 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$, $R_5 = Boc$, $n = 2$
33 $R_1 = R_4 = H$, $R_2 = R_3 = OMe$, $R_5 = Boc$, $n = 2$

ii

34 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $n = 0$
35 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, $n = 0$
36 $R_1 = R_4 = OMe$, $R_2 = R_3 = R_5 = H$, $n = 0$
37 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $n = 1$
38 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, $n = 1$
39 $R_1 = OMe$, $R_2 = R_3 = R_4 = R_5 = H$, $n = 1$
40 $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OMe$, $n = 1$
41 $R_1 = R_4 = OMe$, $R_2 = R_3 = R_5 = H$, $n = 1$
42 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $n = 2$
43 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, $n = 2$
44 $R_1 = R_4 = OMe$, $R_2 = R_3 = R_5 = H$, $n = 2$
45 $R_1 = R_4 = R_5 = H$, $R_2 = R_3 = OMe$, $n = 2$

Scheme 2. Preparation of polyamine analogues **22**–**45**. Reagents and conditions: (i) carboxylic acid (2 equiv), PyBOP, DMF, Et_3N, N_2, 23 h. (ii) CH_2Cl_2, TFA, N_2, 2 h.

3. Biological results and discussion

3.1. In vitro antimalarial activity

The resultant library of polyamine analogues were evaluated for the ability to inhibit the growth of *Plasmodium falciparum* (strain K1, IEF stage), and in order to establish selectivity indices, the cytotoxicity of compounds towards the rat skeletal myoblast cell line L6 were also determined. Summaries of these assay results are presented in Table 1. Of the spermine analogues evaluated, benzamides **4**–**6** (entries 1-3) were either less active or equipotent to our original natural product lead compound [10] while 3-phenylpropanamides **7**–**10** (entries 4-7) were typically more active. Of particular note was the potency (*Pf* IC_{50} 6.1 nM) and selectivity (SI 16230) of the 2,5-dimethoxyphenylacetamide **9** (entry 6). Evaluation of analogues bearing a longer PA3-8-3 [14] polyamine chain identified that Boc-protected derivatives **15**–**17**, whilst being modestly active towards *P. falciparum,* exhibited markedly enhanced levels of cytotoxicity (entries 8-10), while the corresponding de-Boc analogues **18**–**20** exhibited good to potent levels of antimalarial activity, with negligible cytotoxicity (entries 11-13). A similar observation was made for the PA3-12-3 [14] library of analogues, in that Boc protected benzamides (**22**–**24**), phenylacetamides (**25**–**29**) and 3-phenylpropanamides (**30**–**33**) exhibited only modest antimalarial activity combined with enhanced cytotoxicity (entries 14-25). Removal of the Boc protecting group led to no change in *Pf* activity or selectivity for benzamide analogues **34**–**36** (entries 26-28), while de-protected phenylacetamide and 3-phenylpropanamide analogues **37**–**45** typically were as active or more potent towards *Pf* and exhibited similar or less potent cytotoxicity (entries 29-37). The pronounced anti-*Pf* activity of 2-hydroxy (**8**, **19**, **38**, **43**) and 2,5-dimethoxy (**9**, **20**) phenylacetamide and phenylpropanamide analogues is noteworthy.

3.2. In vivo antimalarial evaluation

Three analogues, 2-hydroxyphenylacetamido-spermine **2**, 2-hydroxyphenylpropanamido-spermine **8** and phenylacetamido-PA383 **18** were selected for preliminary in vivo evaluation in *Plasmodium berghei* infected mice. Preliminary ip acute toxicity of the three test compounds was determined to be 150 mg/kg (**2**) and 100 mg/kg (**18**) whereas **8** showed no toxicity up to the highest test dose of 150 mg/kg. Using a standard test protocol [15], repeated ip doses of 50 (mg/kg)/day (for **8**) or 20 (mg/kg)/day (for **18**) for four days failed to demonstrate any antimalarial efficacy and no increase in mean survival time was observed. Repeated 30 (mg/kg)/day ip dosing of **2** did lead to a 27.9% reduction in parasitaemia, but again, no increase in mean survival time was observed.

4. Conclusions

A series of diamido-polyamine derivatives have been identified that exhibit potent in vitro activity against *P. falciparum*. In addition to a range of different 'capping acids', different lengths of polyamine chains and the presence (or absence) of mid-chain nitrogen substitution were investigated for their effects on the growth inhibition of *P. falciparum*. While mid-chain Boc derivatives were typically found to have enhanced cytotoxicity, 2-hydroxy-substituted phenylacetamide or 3-phenylpropanamide polyamine analogues were found to be particularly potent antimalarials. In vitro activity did not translate to in vivo efficacy however, suggesting elements of poor pharmacokinetics are associated with this current set of compounds. Further work is needed to identify analogues that can exhibit in vivo activity – research towards this goal is currently ongoing.

5. Experimental

5.1. General Experimental.

Mass spectra were recorded on a Bruker micrOTOF Q II mass spectrometer. Infrared spectra were run as dry films on an ATR crystal and acquired with a Perkin Elmer Spectrum One Fourier Transform infrared spectrometer with a Universal ATR Sampling Accessory. Melting points were obtained on an Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR (300.13 or 400.13 MHz) and ^{13}C NMR (75.47 or 100.62 MHz) spectra were run on a Bruker Avance 300 MHz or a Bruker DRX 400 MHz spectrometer. Chemical shifts are expressed in parts per million (ppm) relative to TMS in $CDCl_3$ ^{1}H NMR and to deuterated solvent in ^{1}H NMR (DMSO-d_6: 2.50 ppm, CD_3OD: 3.31 ppm) and to deuterated solvent signals in ^{13}C NMR ($CDCl_3$: 77.16 ppm, DMSO-d_6: 39.52 ppm, CD_3OD: 49.00 ppm). Complete assignment of ^{1}H and ^{13}C NMR resonances was based on interpretation of standard 2D NMR data. Pressurized (flash) column chromatography was performed on Kieselgel 60 0.063–0.200 mesh (Merck) silica gel or C_8/C_{18}/CN/LH-20 solid supports. Analytical thin layer chromatography (TLC) was carried out on 0.2 mm thick plates of Kieselgel F_{254} (Merck). Analytical reversed phase HPLC analyses were run on either a Waters 600 HPLC photodiode array system or a Dionex UltiMate 3000 with diode array detection using a C_8 column (Grace Platinum 3 μm, 33 x 7 mm Rocket) eluting with a linear gradient of H_2O (+0.05% TFA) to MeCN over 13.5 min at 2 mL/min with monitoring at 278 nm. Reactions were heated by immersion in oil or by use of DrySyn^{TM} MULTI reaction block kit while the temperature was taken from a thermometer touching the bottom of the pyrex bath. Polyamine **21** was prepared using literature methods [12,13].

*5.2. Synthesis of diamides **4**–**10***

5.2.1. General procedure A: Amide bond formation.

To a solution of carboxylic acid (2.05 equiv.), diamine (1 equiv.), and PyBOP (2.05 equiv.) in DMF (1 mL) was added Et_3N (3 equiv.). The reaction mixture was allowed to stir under N_2 at room temperature for 23 h. The solution was dried in vacuo and the crude reaction product purified by C_8 reversed-phase column chromatography (20-30% MeOH/H_2O (+0.05%TFA)) to afford the target diamide as the bis-trifluoroacetate salt or by silica gel column chromatography to afford the target diamide as the free base.

*5.2.2. N^1,N^4-Bis(3-benzamidopropyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**4**).*

Using general procedure A, benzoic acid (100 mg, 0.82 mmol), spermine (83 mg, 0.41 mmol), PyBOP (426 mg, 0.82 mmol) and Et_3N (340 μL, 2.46 mmol) afforded **4** as a white solid (124 mg, 47% yield). Mp 150°C; R_f (20% MeOH/CH_2Cl_2) 0.22; IR ν_{max} (ATR) 3289, 3069, 2851, 1668, 1638, 1130, 719 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.65 (1H, t, J = 5.8 Hz, NH-8), 8.60 (2H, br s, NH_2-12), 7.87–7.84 (2H, m, H-2 and H-6), 7.56–7.52 (1H, m, H-4), 7.49–7.45 (2H, m, H-3 and H-5), 3.34 (2H, td, J = 6.5, 5.8 Hz, H_2-9), 2.94 (4H, br s, H_2-11 and H_2-13), 1.86 (2H, tt, J = 6.5, 6.5 Hz, H_2-10), 1.63 (2H, br s, H_2-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 166.6 (C-7), 134.2 (C-1), 131.3 (C-4), 128.3 (C-3 and C-5), 127.2 (C-2 and C-6), 46.1 (C-13), 44.8 (C-11), 36.3 (C-9), 26.1 (C-10), 22.7 (C-14); (+)-HRESIMS *m/z* 411.2744 $[M+H]^+$ (calcd for $C_{24}H_{35}N_4O_2$, 411.2755); Purity 97% t_R = 5.91 min.

*5.2.3. N^1,N^4-Bis(3-(2-hydroxybenzamido)propyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**5**).*

Using general procedure A, 2-hydroxybenzoic acid (100 mg, 0.72 mmol), spermine (73 mg, 0.36 mmol), PyBOP (396 mg, 0.76 mmol) and Et_3N (105 μL, 0.76 mmol) afforded **5** as a clear colorless gum (68 mg, 28% yield). R_f (20% MeOH/CH_2Cl_2) 0.17; IR ν_{max} (ATR) 3347, 2989, 2844, 1674, 1638, 1596, 1201, 1133, 722 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 12.51 (1H, s, OH-2), 8.96 (1H, t, J = 5.9 Hz, NH-8), 8.67 (2H, br s, NH_2-12), 7.84 (1H, dd, J = 7.9, 1.6 Hz, H-6), 7.40 (1H, ddd, J = 7.9, 7.9, 1.6 Hz, H-4), 6.92–6.86 (2H, m, H-3 and H-5), 3.37 (2H, td, J = 6.6, 5.9 Hz, H_2-9), 2.94 (4H, br s, H_2-11 and H_2-13), 1.88 (2H, tt, J = 6.6, 6.6 Hz, H_2-10), 1.63 (2H, br s, H_2-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 169.2 (C-7), 159.9 (C-2), 133.7 (C-4), 127.8 (C-6), 118.6 (C-5), 117.4 (C-3), 115.3 (C-1), 46.1 (C-13), 44.7 (C-11), 36.2 (C-9), 25.8 (C-10), 22.7 (C-14); (+)-HRESIMS *m/z* 443.2642 $[M+H]^+$ (calcd for $C_{24}H_{35}N_4O_4$, 443.2653); Purity 99% t_R = 5.18 min.

*5.2.4. N^1,N^4-Bis(3-(2,5-dimethoxybenzamido)propyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**6**).*

Using general procedure A, 2,5-dimethoxybenzoic acid (100 mg, 0.55 mmol), spermine (56 mg, 0.27 mmol), PyBOP (322 mg, 0.62 mmol) and Et_3N (228 μL, 1.65 mmol) afforded **6** as a clear colorless gum (123 mg, 59% yield). R_f (10% MeOH/CH_2Cl_2) 0.43; IR ν_{max} (ATR) 3374, 2951, 2839, 1672, 1494, 1175, 720 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.61 (2H, br s, NH_2-12), 8.40 (1H, t, J = 6.1 Hz, NH-8), 7.28 (1H, d, J = 3.0 Hz, H-6), 7.09–7.03 (2H, m, H-3 and H-4), 3.83 (3H, s, OMe-16), 3.72 (3H, s, OMe-15), 3.35 (2H, td, J = 6.5, 6.1 Hz, H_2-9), 2.94 (4H, br s, H_2-11 and H_2-13), 1.85 (2H, tt, J = 6.5, 6.5 Hz, H_2-10), 1.64 (2H, br s, H_2-14); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.3 (C-7), 153.0 (C-5), 151.1 (C-2), 123.5 (C-1), 117.5 (C-4), 115.2 (C-6), 113.5 (C-3), 56.4 (C-16), 55.5 (C-15), 46.1 (C-13), 44.7 (C-11), 36.3 (C-9), 26.1 (C-10), 22.8 (C-14); (+)-HRESIMS *m/z* 531.3179 $[M+H]^+$ (calcd for $C_{28}H_{43}N_4O_6$, 531.3177); Purity 99% t_R = 5.27 min.

*5.2.5. N^1,N^4-Bis(3-(3-phenylpropanamido)propyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**7**).*

Using general procedure A, 3-phenylpropanoic acid (120 mg, 0.80 mmol), spermine (81 mg, 0.40 mmol), PyBOP (437 mg, 0.84 mmol) and Et_3N (332 μL, 2.40 mmol) afforded **7** as a pale yellow gum (255 mg, 92% yield). R_f (10% MeOH/CH_2Cl_2) 0.44; IR ν_{max} (ATR) 3260, 3030, 2834, 1666, 1639, 1201, 1141, 839, 722 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.64–8.58 (2H, m, NH_2-14), 8.06 (1H, t, *J* = 5.9 Hz, NH-10), 7.29–7.25 (2H, m, H-3, and H-5), 7.19–7.15 (3H, m, H-2, H-4, and H-6), 3.11 (2H, td, *J* = 6.7, 5.9 Hz, H_2-11), 2.87 (2H, br s, H_2-15), 2.84–2.80 (4H, m, H_2-7, and H_2-13), 2.40 (2H, t, *J* = 8.1 Hz, H_2-8), 1.69 (2H, tt, *J* = 6.7 Hz, H_2-12), 1.61 (2H, br s, H_2-16); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.0 (C-9), 141.2 (C-1), 128.3 (C-2, C-6), 128.2 (C-3, and C-5), 126.0 (C-4), 46.1/46.0 (C-15), 44.6/44.5 (C-13), 36.9/36.8 (C-8), 35.6/35.4 (C-11), 31.0 (C-7), 26.1 (C-12), 22.7 (C-16); (+)-HRESIMS *m/z* 467.3371 $[M+H]^+$ (calcd for $C_{28}H_{43}N_4O_2$, 467.3381); Purity 99% t_R = 4.81 min.

*5.2.6. N^1,N^4-Bis(3-(3-(2-hydroxyphenyl)propanamido)propyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**8**).*

Using general procedure A, 3-(2-hydroxyphenyl)propanoic acid (100 mg, 0.60 mmol), spermine (61 mg, 0.30 mmol), PyBOP (344 mg, 0.66 mmol) and Et_3N (500 μL, 3.61 mmol) afforded **8** as a clear colorless gum (183 mg, 84% yield). R_f (50% MeOH/CH_2Cl_2) 0.36; IR ν_{max} (ATR) 3283, 3075, 2835, 1671, 1635, 1456, 1199, 1131, 721 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.40 (1H, br s, OH), 8.62 (2H, br s, NH_2-14), 8.04 (1H, t, *J* = 6.0 Hz, NH-10), 7.03 (1H, dd, *J* = 7.6, 1.6 Hz, H-6), 6.99 (1H, ddd, *J* = 7.6, 7.6, 1.6 Hz, H-4), 6.78 (1H, dd, *J* = 7.6, 1.0 Hz, H-3), 6.69 (1H, ddd, *J* = 7.6, 7.6, 1.0, H-5), 3.11 (2H, td, *J* = 6.6, 6.0 Hz, H_2-11), 2.89 (2H, br s, H_2-15), 2.83 (2H, br s, H_2-13), 2.74 (2H, t, *J* = 7.7 Hz, H_2-7), 2.36 (2H, t, *J* = 7.7 Hz, H_2-8), 1.70 (2H, tt, *J* = 6.6, 6.6 Hz, H_2-12), 1.62 (2H, br s, H_2-16); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.6 (C-9), 155.1 (C-2), 129.6 (C-6), 127.3 (C-1), 127.0 (C-4), 118.9 (C-5), 115.0 (C-3), 46.1 (C-15), 44.6 (C-13), 35.6 (C-11), 35.3 (C-8), 26.1 (C-12), 25.8 (C-7), 22.7 (C-16); (+)-HRESIMS *m/z* 499.3264 $[M+H]^+$ (calcd for $C_{28}H_{43}N_4O_4$, 499.3279); Purity 99% t_R = 4.01 min. The ^1H and ^{13}C data were in agreement with the literature values [16].

*5.2.7. N^1,N^4-Bis(3-(3-(2,5-dimethoxyphenyl)propanamido)propyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**9**).*

Using general procedure A, 3-(2,5-dimethoxyphenyl)propanoic acid (100 mg, 0.48 mmol), spermine (35 mg, 0.24 mmol), PyBOP (300 mg, 0.58 mmol) and Et_3N (198 μL, 1.43 mmol) afforded **9** as a clear colorless gum (28 mg, 14% yield). R_f (20% MeOH/CH_2Cl_2) 0.44; IR ν_{max} (ATR) 3279, 2949, 2836, 1673, 1500, 1224, 1200, 1026, 719 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.63 (2H, br s, NH_2-14), 8.04 (1H, t, *J* = 5.9 Hz, NH-10), 6.86–6.84 (1H, m, H-3), 6.74–6.71 (2H, m, H-4 and H-6), 3.72 (3H, s, OMe-17), 3.67 (3H, s, OMe-18), 3.11 (2H, td, *J* = 6.6, 5.9 Hz, H_2-11), 2.89 (2H, br s, H_2-15), 2.84 (2H, br s, H_2-13), 2.74 (2H, t, *J* = 7.4 Hz, H_2-7), 2.34 (2H, t, *J* = 7.4 Hz, H_2-8), 1.71 (2H, tt, *J* = 6.6, 6.6 Hz, H_2-12), 1.62 (2H, br s, H_2-16); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.1 (C-9), 153.0 (C-5), 151.2 (C-2), 130.1 (C-1), 115.8 (C-6), 111.5 (C-3), 111.1 (C-4), 55.7 (C-17), 55.3 (C-18), 46.1 (C-15), 44.6 (C-13), 35.6 (C-11), 35.2 (C-8), 26.1 (C-12), 25.8 (C-7), 22.7 (C-16); (+)-HRESIMS *m/z* 587.3787 $[M+H]^+$ (calcd for $C_{32}H_{51}N_4O_6$, 587.3803); Purity 99% t_R = 5.55 min.

*5.2.8. N^1,N^4-Bis(3-(3-(3,4-dimethoxyphenyl)propanamido)propyl)butane-1,4-diaminium 2,2,2-trifluoroacetate (**10**).*

Using general procedure A, 3-(3,4-dimethoxyphenyl)propanoic acid (113 mg, 0.54 mmol), spermine (52 mg, 0.26 mmol), PyBOP (268 mg, 0.51 mmol) and Et_3N (198 μL, 1.43 mmol) afforded **10** as a pale yellow gum (150 mg, 72% yield). R_f (20% MeOH/CH_2Cl_2) 0.21; IR ν_{max} (ATR) 3358, 2944, 2840, 1672, 1515, 1136, 1024, 838, 720 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.56 (2H, br s, NH_2-14), 8.04 (1H, t, *J* = 5.9 Hz, NH-10), 6.83 (1H, d, *J* = 8.2 Hz, H-5), 6.79 (1H, d, *J* = 1.9 Hz, H-2), 6.69 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 3.72 (3H, s, OMe-17), 3.70 (3H, s, OMe-18), 3.11 (2H, td, *J* = 6.6, 5.9 Hz, H_2-11), 2.87 (2H, br s, H_2-15), 2.80 (2H, br s, H_2-13), 2.75 (2H, t, *J* = 7.7 Hz, H_2-7), 2.37 (2H, t, *J* = 7.7 Hz, H_2-8), 1.69 (2H, tt, *J* = 6.6 Hz, H_2-12), 1.61 (2H, br s, H_2-16); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.1 (C-9), 148.6 (C-3), 147.1 (C-4), 133.7 (C-1), 119.9 (C-6), 112.1 (C-2), 111.9 (C-5), 55.5 (C-18), 55.4 (C-17), 46.1 (C-15), 44.6 (C-13), 37.2 (C-8), 35.5 (C-11), 30.7 (C-7), 26.1 (C-12), 22.7 (C-16); (+)-HRESIMS *m/z* 587.3771 $[M+H]^+$ (calcd for $C_{32}H_{51}N_4O_6$, 587.3803); Purity 99% t_R = 4.41 min.

*5.3. Synthesis of diamides **15**–**17***

*5.3.1. 3,3'-(Octane-1,8-diylbis(azanediyl))dipropanenitrile (**12**).*

To a solution of 1,8-diaminooctane (**11**) (100 mg, 0.69 mmol) in EtOH (2 mL) was added acrylonitrile (92 μL, 1.39 mmol). The reaction mixture was purged with N_2 and heated to reflux for 3 h. The crude product was concentrated in vacuo and purified by Et_3N-deactivated silica gel column chromatography (10% MeOH/CH_2Cl_2) and reversed-phase cyanopropyl column chromatography (25% MeOH/H_2O (TFA)) to give **12** as a white solid (117 mg, 38% yield). Mp 143°C; R_f (10% MeOH/CH_2Cl_2) 0.85; IR ν_{max} (ATR) 3091, 2940, 2863, 2258, 1663, 1462, 1416, 1195, 1162, 1122, 719 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.02 (1H, br s, NH-4), 3.26 (2H, t, *J* = 7.0 Hz, H_2-2), 2.95–2.90 (4H, m, H_2-3 and H_2-5), 1.59–1.55 (2H, m, H_2-6), 1.27 (4H, br m, H_2-7 and H_2-8); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 117.8 (C-1), 46.7 (C-5), 42.0 (C-2), 28.2 (C-8), 25.8 (C-7), 25.3 (C-6), 14.4 (C-3); (+)-HRESIMS *m/z* 251.2236 $[M+H]^+$ (calcd for $C_{14}H_{27}N_4$, 251.2230).

*5.3.2. Di-tert-butyl octane-1,8-diylbis((2-cyanoethyl)carbamate) (**13**).*

To a solution of **12** (190 mg, 0.76 mmol) and di-*tert*-butyl dicarbonate (414 mg, 1.90 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (420 μL, 3.04 mmol). The reaction mixture was allowed to stir under N_2 at room temperature for 22.5 h. After this time, H_2O (50 mL) was added to the reaction mixture, the organic phase separated and dried ($MgSO_4$) and solvent removed in vacuo to give the desired product **13** as a light yellow oil (321 mg, 94% yield) which was used in the following step without further purification. R_f (10% MeOH/CH_2Cl_2) 0.79; IR ν_{max} (ATR) 2976, 2930, 2858, 2249, 1686, 1413, 1366, 1154, 773 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.46 (2H, t, *J* = 6.7 Hz, H_2-3), 3.25 (2H, t, *J* = 7.2 Hz, H_2-5), 2.62 (2H, br s, H_2-2), 1.54–1.44 (2H, m, H_2-6), 1.49 (9H, s, $3H_3$-11), 1.30–1.27 (4H, m, H_2-7 and H_2-8); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.4:154.6 (C-9), 118.5:117.9 (C-1), 80.3 (C-10), 48.7:47.9 (C-5), 44.0:43.6 (C-3), 29.4 (C-8), 28.9 (C-6), 28.5 (C-11), 26.8 (C-7), 17.7:17.1 (C-2); (+)-HRESIMS *m/z* 473.3089 $[M+Na]^+$ (calcd for $C_{24}H_{42}N_4NaO_4$, 473.3098).

*5.3.3. Di-tert-butyl octane-1,8-diylbis((3-aminopropyl)carbamate) (**14**).*

To a solution of **13** (379 mg, 0.84 mmol) in dioxane/H_2O (4:1, 30 mL) was added LiOH (141 mg, 3.36 mmol), 10% Pd/C (450 mg, 0.42 mmol), and 50% nickel-aluminium alloy (790 mg, 6.73 mmol). The reaction mixture was heated to 50°C and allowed to stir overnight (21 h) under H_2 atmosphere. After cooling to room temperature, a solution of 1% K_2CO_3 (50 mL) was added to the reaction mixture and the product was extracted with CH_2Cl_2 (3 × 50 mL). The organic fractions were combined, dried ($MgSO_4$) and solvent removed in vacuo to give the desired product **14** as a pale yellow oil (272 mg, 71% yield). R_f (10% MeOH/CH_2Cl_2) 0.23; IR ν_{max} (ATR) 3367, 2973,

2928, 2857, 1683, 1416, 1364, 1158, 771 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 3.14 (2H, t, *J* = 6.6 Hz, H_2-3), 3.08 (2H, t, *J* = 7.2 Hz, H_2-5), 2.48–2.46 (2H, m, H_2-1), 1.53–1.48 (2H, m, H_2-2), 1.44–1.43 (2H, m, H_2-6), 1.38 (9H, s, $3H_3$-11), 1.25 (2H, br m, H_2-8), 1.22–1.20 (2H, m, H_2-7); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 154.7 (C-9), 78.0 (C-10), 46.3 (C-5), 44.2:43.8 (C-3), 39.1 (C-1), 32.5:31.8 (C-2), 28.7 (C-8), 28.1 (C-11), 27.9 (C-6), 26.2 (C-7); (+)-HRESIMS *m/z* 459.3891 $[M+H]^+$ (calcd for $C_{24}H_{51}N_4O_4$, 459.3905).

*5.3.4. Di-tert-butyl octane-1,8-diylbis((3-(2-phenylacetamido)propyl)carbamate) (**15**).*

Using general procedure A, reaction of phenylacetic acid (59 mg, 0.44 mmol), **14** (100 mg, 0.22 mmol), PyBOP (284 mg, 0.55 mmol) and Et_3N (181 μL, 1.31 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **15** as clear colorless gum (114 mg, 75% yield). R_f (10% MeOH/CH_2Cl_2) 0.77; IR ν_{max} (ATR) 3292, 2974, 2929, 2857, 1687, 1647, 415, 1154, 721, 696 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.26 (5H, m, H-2, H-3, H-4, H-5 and H-6), 6.70 (1H, br s, NH-9), 3.55 (2H, s, H_2-7), 3.18–3.17 (4H, m, H_2-10 and H_2-12), 3.06 (2H, t, *J* = 7.0 Hz, H_2-14), 1.61–1.67 (2H, m, H_2-11), 1.49–1.44 (2H, m, H_2-15), 1.41 (9H, s, $3H_3$-20), 1.26–1.22 (4H, m, H_2-16 and H_2-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-8), 156.6 (C-18), 135.4 (C-1), 129.5 (C-2 and C-6), 128.9 (C-3 and C-5), 127.2 (C-4), 79.6 (C-19), 47.1 (C-14), 44.2 (C-7), 43.4 (C-12), 36.0 (C-10), 29.5 (C-17), 28.5 (C-15 and C-20), 27.8 (C-11), 26.9 (C-16); (+)-HRESIMS *m/z* 695.4725 $[M+H]^+$ (calcd for $C_{40}H_{63}N_4O_6$, 695.4742); Purity 95% t_R = 7.44 min.

*5.3.5. Di-tert-butyl octane-1,8-diylbis((3-(2-(2-hydroxyphenyl)acetamido)propyl) carbamate) (**16**).*

Using general procedure A, reaction of 2-hydroxyphenylacetic acid (40 mg, 0.26 mmol), **14** (60 mg, 0.13 mmol), PyBOP (170 mg, 0.33 mmol) and Et_3N (109 μL, 0.78 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **16** as a yellow gum (52 mg, 55% yield). R_f (10% MeOH/CH_2Cl_2) 0.49; IR ν_{max} (ATR) 3287, 2973, 2930, 1689, 1651, 1420, 1159, 753 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.16 (1H, br s, OH), 7.53 (1H, br s, NH-9), 7.16 (1H, ddd, *J* = 7.5, 7.5, 1.7 Hz, H-4), 7.06 (1H, br d, *J* = 7.5 Hz, H-6), 6.95 (1H, dd, *J* = 7.5, 1.1 Hz, H-3), 6.81 (1H, ddd, *J* = 7.5, 1.1 Hz, H-5), 3.56 (2H, s, H_2-7), 3.24–3.17 (4H, m, H_2-10 and H_2-12), 3.09 (2H, t, *J* = 7.3 Hz, H_2-14), 1.63 (2H, br s, H_2-11), 1.47–1.41 (11H, br m, H_2-15 and $3H_3$-20), 1.27–1.24 (4H, br m, H_2-16 and H_2-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4 (C-8), 156.9 (C-18), 156.4 (C-2), 130.7 (C-6), 129.0 (C-4), 122.2 (C-1), 120.2 (C-5), 118.0 (C-3), 80.1 (C-19), 47.2 (C-14), 43.4 (C-12), 41.3 (C-7), 36.1 (C-10), 29.4 (C-17), 28.5 (C-15 and C-20), 27.5 (C-11), 26.9 (C-16); (+)-HRESIMS *m/z* 727.4622 $[M+H]^+$ (calcd for $C_{40}H_{63}N_4O_8$, 727.4640).

*5.3.6. Di-tert-butyl octane-1,8-diylbis((3-(2-(2,5-dimethoxyphenyl)acetamido)propyl)carbamate) (**17**).*

Using general procedure A, reaction of 2,5-dimethoxyphenylacetic acid (86 mg, 0.44 mmol), **14** (100 mg, 0.22 mmol), PyBOP (284 mg, 0.55 mmol) and Et_3N (181 μL, 1.31 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **17** as a clear colorless gum (114 mg, 64% yield). R_f (10% MeOH/CH_2Cl_2) 0.59; IR ν_{max} (ATR) 3310, 2973, 2930, 2856, 1685, 1501, 1225, 1157, 1048, 715 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.83–6.76 (3H, m, H-3, H-4 and H-6), 6.67 (1H, br s, NH-9), 3.81 (3H, s, OMe-22), 3.76 (3H, s, OMe-21), 3.52 (2H, s, H_2-7), 3.15 (4H, br s, H_2-10 and H_2-12), 3.05 (2H, br s, H_2-14), 1.59 (2H, br s, H_2-11), 1.49–1.44 (2H, m, H_2-15), 1.41 (9H, s, $3H_3$-20), 1.26–1.21 (4H, m, H_2-16 and H_2-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-8), 156.2 (C-18), 153.7 (C-5), 151.6 (C-2), 124.9 (C-1), 117.0 (C-6), 113.2 (C-4), 111.7 (C-3), 79.3 (C-19), 56.1 (C-22), 55.7 (C-21), 47.1 (C-14), 44.7:43.4 (C-12), 39.0 (C-7), 37.3:36.0 (C-10), 29.4 (C-17), 28.6 (C-15), 28.4 (C-20), 28.0 (C-11),

26.9 (C-16); (+)-HRESIMS *m/z* 815.5179 $[M+H]^+$ (calcd for $C_{44}H_{71}N_4O_{10}$, 815.5165); Purity 95% t_R = 7.56 min.

*5.4. Synthesis of diamides **18**–**20***

5.4.1. General procedure B: Removal of Boc protecting group.

A solution of *tert*-butyl-carbamate derivative in CH_2Cl_2 (2 mL) and TFA (0.2 mL) was stirred at room temperature under N_2 for 2 h, then dried in vacuo to afford the deprotected analogue. In some cases the product required no further purification, while in other cases, purification was achieved by C_{18} reversed-phase column chromatography eluting with 0-50% MeOH/H_2O (+0.05% TFA).

*5.4.2. N^1,N^8-Bis(3-(2-phenylacetamido)propyl)octane-1,8-diaminium 2,2,2-trifluoroacetate (**18**).*

Using general procedure B, reaction of **15** (35.0 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) with TFA (200 μL) and subsequent purification by C_8 reversed-phase column chromatography (50% MeOH/H_2O (TFA)) afforded **18** as a clear pale yellow gum (40.0 mg, quantitative yield). R_f (20% MeOH/CH_2Cl_2) 0.83; IR ν_{max} (ATR) 3285, 2938, 2860, 1673, 1556, 1201, 1178, 1132, 721 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.52 (2H, br s, NH_2-13), 8.27 (1H, t, *J* = 5.9 Hz, NH-9), 7.31–7.19 (5H, m, H-2, H-3, H-4, H-5 and H-6), 3.41 (2H, s, H_2-7), 3.12 (2H, td, *J* = 6.7, 5.9 Hz, H_2-10), 2.87–2.79 (4H, m, H_2-12 and H_2-14), 1.72 (2H, tt, *J* = 6.7, 6.7 Hz, H_2-11), 1.53 (2H, br tt, *J* = 6.9, 6.9 Hz, H_2-15), 1.26 (4H, br s, H_2-16 and H_2-17); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.7 (C-8), 136.3 (C-1), 128.9 (C-2 and C-6), 128.2 (C-3 and C-5), 126.4 (C-4), 46.7 (C-14), 44.5 (C-12), 42.3 (C-7), 35.8 (C-10), 28.3 (C-17), 26.0 (C-11), 25.8 (C-16), 25.4 (C-15); (+)-HRESIMS *m/z* 495.3696 $[M+H]^+$ (calcd for $C_{30}H_{47}N_4O_2$, 495.3694).

*5.4.3. N^1,N^8-Bis(3-(2-(2-hydroxyphenyl)acetamido)propyl)octane-1,8-diaminium 2,2,2-trifluoroacetate (**19**).*

Using general procedure B, reaction of **16** (22 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (200 μL) and subsequent purification by C_8 reversed-phase column chromatography (50% MeOH/H_2O (TFA)) afforded **19** as a clear pale yellow gum (21 mg, 92% yield). R_f (20% MeOH/CH_2Cl_2) 0.62; IR ν_{max} (ATR) 3326, 2943, 2863, 1779, 1672, 1459, 1137, 705 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 9.72 (1H, br s, OH), 8.50 (2H, br s, NH_2-13), 8.14 (1H, t, *J* = 6.0 Hz, NH-9), 7.08–7.03 (2H, m, H-4 and H-6), 6.80 (1H, d, *J* = 7.6 Hz, H-3), 6.72 (1H, ddd, *J* = 7.4, 7.4, 0.6 Hz, H-5), 3.39 (2H, s, H_2-7), 3.14 (2H, td, *J* = 6.7, 6.0 Hz, H_2-10), 2.91–2.80 (4H, m, H_2-12 and H_2-14), 1.73 (2H, tt, *J* = 6.7, 6.7 Hz, H_2-11), 1.53 (2H, br tt, *J* = 6.7, 6.7 Hz, H_2-15), 1.25 (4H, br s, H_2-16 and H_2-17); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.6 (C-8), 155.5 (C-2), 130.7 (C-6), 127.7 (C-4), 122.6 (C-1), 118.9 (C-5), 115.2 (C-3), 46.8 (C-14), 44.5 (C-12), 37.2 (C-7), 35.8 (C-10), 28.3 (C-17), 26.0 (C-11), 25.8 (C-16), 25.4 (C-15); (+)-HRESIMS *m/z* 527.3585 $[M+H]^+$ (calcd for $C_{30}H_{47}N_4O_4$, 527.3592); Purity 95% t_R = 4.93 min.

*5.4.4. N^1,N^8-Bis(3-(2-(2,5-dimethoxyphenyl)acetamido)propyl)octane-1,8-diaminium 2,2,2-trifluoroacetate (**20**).*

Using general procedure B, reaction of **17** (53 mg, 0.065 mmol) in CH_2Cl_2 (2 mL) with TFA (200 μL) and subsequent purification by C_8 reversed-phase column chromatography (50% MeOH/H_2O (TFA)) afforded **20** as a clear colorless gum (48 mg, 88% yield). R_f (20% MeOH/CH_2Cl_2) 0.55; IR ν_{max} (ATR) 2940, 2839, 1775, 1645, 1502, 1135, 1045, 798, 704 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.51 (2H, br s, NH_2-13), 7.99 (1H, t, *J* = 6.0 Hz, NH-9), 6.88–6.86

(1H, m, H-3), 6.78–6.76 (2H, m, H-4 and H-6), 3.70 (3H, s, OMe-19), 3.68 (3H, s, OMe-18), 3.37 (2H, s, H_2-7), 3.13 (2H, td, *J* = 6.7, 6.0 Hz, H_2-10), 2.89–2.80 (4H, m, H_2-12 and H_2-14), 1.73 (2H, tt, *J* = 6.7, 6.7 Hz, H_2-11), 1.55–1.50 (2H, m, *J* = 7.3, 7.3 Hz, H_2-15), 1.25 (4H, m, H_2-16 and H_2-17); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.6 (C-8), 152.9 (C-5), 151.4 (C-2), 125.4 (C-1), 117.1 (C-6), 111.8 (C-4), 111.6 (C-3), 55.9 (C-19), 55.3 (C-18), 46.7 (C-14), 44.5 (C-12), 36.8 (C-7), 35.7 (C-10), 28.3 (C-17), 26.1 (C-11), 25.8 (C-16), 25.4 (C-15); (+)-HRESIMS *m/z* 615.4089 $[M+H]^+$ (calcd for $C_{34}H_{55}N_4O_6$, 615.4116); Purity 99% t_R = 5.74 min.

*5.5. Synthesis of diamides **22**–**33***

*5.5.1. Di- tert-Butyl dodecane-1,12-diylbis(3-benzamidopropylcarbamate) (**22**).*

Using general procedure A, reaction of benzoic acid (31 mg, 0.25 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (131 mg, 0.25 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (1% MeOH/CH_2Cl_2) to afford **22** (39 mg, 54% yield) as a colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.33; IR ν_{max} (ATR) 3326, 2926, 2854, 1666, 1644, 1538, 1365, 1302, 1156, 731 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.92–7.86 (3H, m, H-2, H-4 and NH-8), 7.49–7.40 (3H, m, H-3, H-5 and H-6), 3.43 (2H, dt, *J* = 5.9, 5.8 Hz, H_2-9), 3.37–3.33 (2H, m, H_2-11), 3.14 (2H, t, *J* = 7.3 Hz, H_2-13), 1.77–1.70 (2H, m, H_2-10), 1.55–1.47 (2H, m, H_2-14), 1.47 (9H, s, 3H_3-21), 1.27 (8H, br s, H_2-15, H_2-16, H_2-17 and H_2-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.2 (C-7), 157.0 (C-19), 134.8 (C-1), 131.3 (C-6), 128.6, (C-3 and C-5), 127.2 (C-2 and C-4), 79.9 (C-20), 47.1 (C-13), 43.3 (C-11), 35.9 (C-9), 29.7, 29.7, 29.5 (C-16, C-17 and C-18), 28.7 (C-14), 28.6 (C-21), 27.8 (C-10), 27.0 (C-15); (+)-HRESIMS *m/z* 723.5039 $[M+H]^+$ (calcd for $C_{42}H_{67}N_4O_6$, 723.5055); Purity 99% t_R = 7.96 min.

*5.5.2. Di-tert-Butyl dodecane-1,12-diylbis(3-(2-hydoxybenzamido)propylcarbamate) (**23**).*

Using general procedure A, reaction of 2-hydroxybenzoic acid (35 mg, 0.25 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (131 mg, 0.25 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **23** (25 mg, 34% yield) as a colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.60; IR ν_{max} (ATR) 3331, 2925, 2854, 1665, 1643, 1541, 1479, 1303, 1232, 1157, 754 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.74 (1H, br s, OH-22), 8.36 (1H, br s, NH-8), 7.64 (1H, br d, *J* = 7.8 Hz, H-6), 7.37 (1H, ddd, *J* = 8.3, 7.8, 1.4 Hz, H-4), 6.96 (1H, dd, *J* = 8.3, 1.4 Hz, H-3), 6.87 (1H, ddd, *J* = 8.3, 7.8, 1.4 Hz, H-5), 3.45–3.39 (2H, m, H_2-9), 3.36 (2H, t, *J* = 5.7 Hz, H_2-11), 3.15 (2H, t, *J* = 7.4 Hz, H_2-13), 1.77–1.70 (2H, m, H_2-10), 1.57–1.45 (2H, m, H_2-14), 1.49 (9H, s, 3H_3-21), 1.28 (8H, br s, H_2-15, H_2-16, H_2-17 and H_2-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1 (C-7), 161.8 (C-2), 157.3 (C-19), 133.9 (C-4), 126.2 (C-6), 118.8 (C-5), 118.4 (C-3), 114.8 (C-1), 80.2 (C-20), 47.3 (C-13), 43.2 (C-11), 35.2 (C-9), 29.7, 29.7, 29.5 (C-16, C-17 and C-18), 28.7 (C-14), 28.6 (C-21), 27.7 (C-10), 27.0 (C-15); (+)-HRESIMS *m/z* 777.4746 $[M+Na]^+$ (calcd for $C_{42}H_{66}N_4NaO_8$, 777.4773); Purity 99% t_R = 8.10 min.

*5.5.3. Di-tert-Butyl dodecane-1,12-diylbis(3-(2,5-dimethoxybenzamide)propylcarbamate) (**24**).*

Using general procedure A, reaction of 2,5-dimethoxybenzoic acid (46 mg, 0.25 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (131 mg, 0.25 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **24** (55 mg, 65% yield) as a colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.32; IR ν_{max} (ATR) 3365, 2926, 2854, 1683, 1651, 1493, 1154, 1044, 731 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.61 (1H, br s, NH-8), 7.76 (1H, d, *J* = 3.2 Hz, H-6), 6.98 (1H, dd, *J* = 8.9, 3.2 Hz, H-4), 6.90 (1H, d, *J* = 8.9 Hz, H-3), 3.96 (3H, s, OMe-22), 3.82 (3H, s, OMe-23), 3.48–3.40 (2H, m, H_2-9), 3.33–3.25 (2H, m, H_2-11), 3.19–3.11 (2H, m, H_2-13), 1.84–1.75 (2H, m, H_2-10), 1.55–1.47 (2H, m, H_2-14), 1.46 (9H, s, 3H_3-

21), 1.26 (8H, br s, H_2-15, H_2-16, H_2-17 and H_2-18); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.2 (C-7), 156.3 (C-19), 153.8 (C-5), 152.2 (C-2), 122.4 (C-1), 119.2 (C-4), 115.6 (C-6), 112.9 (C-3), 79.2 (C-20), 56.4 (C-22), 55.9 (C-23), 47.2 (C-13), 43.9 (C-11), 36.6 (C-9), 29.7, 29.7, 29.5 (C-16, C-17 and C-18), 28.7 (C-14), 28.6 (C-21), 28.2 (C-10), 27.0 (C-15); (+)-HRESIMS *m/z* 843.5503 $[M+H]^+$ (calcd for $C_{46}H_{75}N_4O_{10}$, 843.5478); Purity 97% t_R = 8.19 min.

*5.5.4. Di-tert-Butyl dodecane-1,12-diylbis(3-(2-phenylacetamido))propylcarbamate (**25**).*

Using general procedure A, reaction of phenylacetic acid (31 mg, 0.23 mmol), **21** [12,13] (53 mg, 0.10 mmol), PyBOP (118 mg, 0.23 mmol), and Et_3N (58 μL, 0.41 mmol) yielded a crude product that was purified by silica gel column chromatography (1.5% MeOH/CH_2Cl_2) to afford **25** (27 mg, 35% yield) as a cloudy colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.33; IR ν_{max} (ATR) 3298, 2925, 2854,1548, 1365, 1154, 726 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.27 (5H, m, H-2, H-3, H-4, H-5 and H-6), 6.72 (1H, br s, NH-9), 3.55 (2H, s, H_2-7), 3.22–3.14 (4H, m, H_2-10 and H_2-12), 3.06 (2H, t, *J* = 7.1, 7.1 Hz, H_2-14), 1.63–1.57 (2H, m, H_2-11), 1.49–1.41 (2H, m, H_2-15), 1.41 (9H, s, $3H_3$-22), 1.27–1.19 (2H, m, H_2-16), 1.20 (6H, br s, H_2-17, H_2-18 and H_2-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-8), 156.4 (C-20), 135.3 (C-1), 129.5 (C-2 and C-6), 128.9 (C-3 and C-5), 127.1 (C-4), 79.6 (C-21), 47.1 (C-14), 44.0 (C-7), 43.3 (C-12), 35.9 (C-10), 29.7, 29.6, 29.5 (C-17, C-18 and C-19), 28.5 (C-22), 28.5 (C-15), 27.8 (C-11), 27.0 (C-16); (+)-HRESIMS *m/z* 751.5356 $[M+H]^+$ (calcd for $C_{44}H_{71}N_4O_6$, 751.5368); Purity 97% t_R = 7.94 min.

*5.5.5. Di-tert-Butyl dodecane-1,12-diylbis(3-(2-(2-hydroxyphenyl)acetamido)propylcarbamate) (**26**).*

Using general procedure A, reaction of 2-hydroxyphenylacetic acid (34 mg, 0.22 mmol), **21** [12,13] (52 mg, 0.10 mmol), PyBOP (116 mg, 0.22 mmol), and Et_3N (56 μL, 0.40 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **26** (17 mg, 21% yield) as a pale yellow cloudy oil. R_f (5% MeOH/CH_2Cl_2) 0.31; IR ν_{max} (ATR) 3287, 2928, 2854, 1685, 1647, 1456, 1418, 1147, 752 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.18 (1H, br s, OH-2), 7.53 (1H, br s, NH-9), 7.17 (1H, ddd, *J* = 8.0, 7.8, 1.3 Hz, H-4), 7.06 (1H, d, *J* = 7.4 Hz, H-6), 6.96 (1H, dd, *J* = 8.0, 1.1 Hz, H-3), 6.82 (1H, ddd, *J* = 7.8, 7.4, 1.1 Hz, H-5), 3.57 (2H, s, H_2-7), 3.27–3.19 (4H, m, H_2-10 and H_2-12), 3.09 (2H, t, *J* = 7.4 Hz, H_2-14), 1.66–1.60 (2H, m, H_2-11), 1.53–1.47 (2H, m, H_2-15), 1.46 (9H, s, $3H_3$-22), 1.26 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5 (C-8), 157.0 (C-20), 156.6 (C-2), 130.7 (C-6), 129.1 (C-4), 122.3 (C-1), 120.3 (C-5), 118.2 (C-3), 80.2 (C-21), 47.3 (C-14), 43.3 (C-12), 41.5 (C-7), 36.1 (C-10), 29.7, 29.7, 29.5 (C-17, C-18 and C-19), 28.6 (C-22 and C-16), 27.5 (C-11), 27.0 (C-15); (+)-HRESIMS *m/z* 783.5258 $[M+H]^+$ (calcd for $C_{44}H_{71}N_4O_8$, 783.5266); Purity 96% t_R = 7.60 min.

*5.5.6. Di-tert-Butyl dodecane-1,12-diylbis(3-(2-(2-methoxyphenyl)acetamido)propylcarbamate) (**27**).*

Using general procedure A, reaction of 2-methoxyphenylacetic acid (36 mg, 0.21 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (111 mg, 0.21 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **27** (42 mg, 53% yield) as a colorless cloudy oil. R_f (5% MeOH/CH_2Cl_2) 0.36; IR ν_{max} (ATR) 3302, 2926, 2854, 1674, 1653, 1465, 1365, 1245, 1155, 732 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.26–7.22 (2H, m, H-4 and H-6), 6.95–6.88 (2H, m, H-3 and H-5), 6.63 (1H, br s, NH-9), 3.85 (3H, s, OMe-23), 3.55 (2H, s, H_2-7), 3.19–3.11 (4H, m, H_2-10 and H_2-12), 3.08–3.02 (2H, m, H_2-14), 1.63–1.56 (2H, m, H_2-11), 1.48–1.41 (2H, m, H_2-15), 1.41 (9H, s, $3H_3$-22), 1.25 (6H, br s, H_2-17, H_2-18 and H_2-19), 1.24–1.18 (2H, m, H_2-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.4 (C-8), 157.4 (C-2), 156.3 (C-20), 131.3 (C-6), 128.6 (C-4), 124.0 (C-1), 120.9 (C-5), 110.7 (C-3), 79.3 (C-21), 55.3 (C-

23), 47.1 (C-14), 43.5 (C-12), 38.8 (C-7), 36.1 (C-10), 29.7, 29.7, 29.5 (C-17, C-18 and C-19), 28.7 (C-15), 28.5 (C-22), 28.0 (C-11), 27.0 (C-16); (+)-HRESIMS *m/z* 811.5590 $[M+H]^+$ (calcd for $C_{46}H_{75}N_4O_8$, 811.5579); Purity 99% t_R = 8.05 min.

*5.5.7. Di-tert-Butyl dodecane-1,12-diylbis(3-(2-(4-methoxyphenyl)acetamido)propylcarbamate) (**28**).*

Using general procedure A, reaction of 4-methoxyphenylacetic acid (42 mg, 0.25 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (131 mg, 0.25 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **28** (45 mg, 52% yield) as a colorless cloudy oil. R_f (5% MeOH/CH_2Cl_2) 0.31; IR ν_{max} (ATR) 3306, 2927, 2854, 1652, 1511, 1418, 1365, 1245, 1155, 730 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.20 (2H, d, *J* = 7.8 Hz, H-2 and H-6), 6.86 (2H, d, *J* = 8.6 Hz, H-3 and H-5), 6.68 (1H, br s, NH-9), 3.79 (3H, s, OMe-23), 3.49 (2H, s, H_2-7), 3.21–3.13 (4H, m, H_2-10 and H_2-12), 3.06 (2H, t, *J* = 6.6 Hz, H_2-14), 1.63–1.56 (2H, m, H_2-11), 1.49–1.44 (2H, m, H_2-15), 1.41 (9H, s, $3H_3$-22), 1.25 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6 (C-8), 158.8 (C-4), 156.5 (C-20), 130.6 (C-2 and C-6), 127.5 (C-1), 114.3 (C-3 and C-5), 79.5 (C-21), 47.1 (C-14), 43.4 (C-12), 43.2 (C-7), 35.9 (C-10), 29.7, 29.7, 29.5 (C-17, C-18 and C-19), 28.6 (C-15), 28.5 (C-22), 27.8 (C-11), 27.0 (C-16); (+)-HRESIMS *m/z* 811.5571 $[M+H]^+$ (calcd for $C_{46}H_{75}N_4O_8$, 811.5579); Purity 98% t_R = 7.94 min.

*5.5.8. Di-tert-Butyl dodecane-1,12-diylbis(3-(2-(2,5-dimethoxyphenyl)acetamido)propylcarbamate) (**29**).*

Using general procedure A, reaction of 2,5-dimethoxyphenylacetic acid (42 mg, 0.21 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (111 mg, 0.21 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **29** (47 mg, 56% yield) as a colorless cloudy oil. R_f (5% MeOH/CH_2Cl_2) 0.38; IR ν_{max} (ATR) 3309, 2926, 2854, 1675, 1652, 1417, 1224, 1156, 1048, 715 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.83 (1H, d, *J* = 2.7 Hz, H-6), 6.79 (1H, d, *J* = 8.8 Hz, H-3), 6.77 (1H, dd, *J* = 8.8, 2.7 Hz, H-4), 6.66 (1H, br s, NH-9), 3.81 (3H, s, OMe-24), 3.76 (3H, s, OMe-23), 3.52 (2H, s, H_2-7), 3.18–3.13 (4H, m, H_2-10 and H_2-12), 3.09–3.02 (2H, m, H_2-14), 1.63–1.56 (2H, m, H_2-11), 1.47–1.43 (2H, m, H_2-15), 1.41 (9H, s, $3H_3$-22), 1.25 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2 (C-8), 156.2 (C-20), 153.8 (C-5), 151.6 (C-2), 125.0 (C-1), 117.1 (C-6), 113.2 (C-4), 111.8 (C-3), 79.3 (C-21), 56.1 (C-24), 55.8 (C-23), 47.2 (C-14), 43.5 (C-12), 38.0 (C-7), 36.1 (C-10), 29.7, 29.7, 29.5 (C-17, C-18 and C-19), 28.7 (C-15), 28.5 (C-22), 28.0 (C-11), 27.0 (C-16); (+)-HRESIMS *m/z* 871.5760 $[M+H]^+$ (calcd for $C_{48}H_{79}N_4O_{10}$, 871.5791); Purity 96% t_R = 8.05 min.

*5.5.9. Di-tert-Butyl dodecane-1,12-diylbis(3-(3-phenylpropanamido)propylcarbamate) (**30**).*

Using general procedure A, reaction of 3-phenylpropionic acid (38 mg, 0.25 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (131 mg, 0.25 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to yield **30** (48 mg, 63%) as a colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.41; IR ν_{max} (ATR) 3298, 2925, 2854, 1651, 1545, 1477, 1454, 1365, 1158, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.28–7.17 (5H, m, H-2, H-3, H-4, H-5 and H-6), 6.82 (1H, br s, NH-10), 3.21–3.13 (4H, m, H_2-11 and H_2-13), 3.08 (2H, t, *J* = 6.6 Hz, H_2-15), 2.97 (2H, t, *J* = 7.9 Hz, H_2-7), 2.49 (2H, t, *J* = 7.9 Hz, H_2-8), 1.61–1.54 (2H, m, H_2-12), 1.51–1.46 (2H, m, H_2-16), 1.44 (9H, s, $3H_3$-23), 1.27 (8H, br s, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5 (C-9), 156.6 (C-21), 141.1 (C-1), 128.7, 128.5, 128.5, 128.4, 126.1 (C-2, C-3, C-4, C-5 and C-6), 79.6 (C-22), 47.1 (C-15), 43.2 (C-13), 38.6 (C-8), 35.6

(C-11), 31.8 (C-7), 29.7, 29.6, 29.4 (C-18, C-19 and C-20), 28.5 (C-23), 28.5 (C-16), 27.7 (C-12), 26.9 (C-17); (+)-HRESIMS *m/*z 779.5643 $[M+H]^+$ (calcd for $C_{46}H_{75}N_4O_6$, 779.5681); Purity 96% t_R = 8.13 min.

*5.5.10. Di-tert-Butyl dodecane-1,12-diylbis(3-(3-(2-hydroxyphenyl)propanamido)propylcarbamate) (**31**).*

Using general procedure A, reaction of 3-(2-hydroxyphenyl)propanoic acid (40 mg, 0.24 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (156 mg, 0.30 mmol), and Et_3N (55 μL, 0.40 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **31** (20 mg, 25% yield) as a colorless oil. R_f (10% MeOH/CH_2Cl_2) 0.85; IR ν_{max} (ATR) 3289, 2927, 2855, 1651, 1456, 1419, 1365, 1245, 1153, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.14 (1H, br s, OH-24), 7.10 (1H, dd, *J* = 7.4, 1.6 Hz, H-6), 7.07 (1H, ddd, *J* = 7.9, 7.9, 1.6 Hz, H-4), 6.90 (1H, dd, *J* = 7.9, 1.1 Hz, H-3), 6.82 (1H, ddd, *J* = 7.9, 7.4, 1.1 Hz, H-5), 5.74 (1H, br s, NH-10), 3.21–3.13 (4H, m, H_2-11 and H_2-13), 3.06 (2H, t, *J* = 7.4 Hz, H_2-15), 2.91 (2H, t, *J* = 5.7 Hz, H_2-7), 2.64 (2H, t, *J* = 5.7 Hz, H_2-8), 1.61–1.56 (2H, m, H_2-12), 1.50–1.44 (2H, m, H_2-16), 1.45 (9H, s, $3H_3$-23), 1.26 (8H, br s, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.2 (C-9), 156.8 (C-21), 155.2 (C-2), 130.7 (C-4), 128.3 (C-1), 128.0 (C-6), 120.3 (C-5), 117.8 (C-3), 80.0 (C-22), 47.2 (C-15), 43.5 (C-13), 37.5 (C-8), 36.2 (C-11), 29.7, 29.6, 29.5 (C-18, C-19 and C-20), 28.6 (C-16), 28.6 (C-23), 27.4 (C-12), 24.7 (C-7), 27.0 (C-17); (-)-HRESIMS *m/*z 809.5427 $[M-H]^-$ (calcd for $C_{46}H_{73}N_4O_8$, 809.5434); Purity 96% t_R = 7.64 min.

*5.5.11. Di-tert-Butyl dodecane-1,12-diylbis(3-(2,5-dimethoxyphenyl)propanamido)propylcarbamate) (**32**).*

Using general procedure A, reaction of 3-(2,5-dimethoxyphenyl)propanoic acid (63 mg, 0.30 mmol), **21** [12,13] (51 mg, 0.10 mmol), PyBOP (156 mg, 0.30 mmol), and Et_3N (111 μL, 0.80 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to yield **32** (53 mg, 59%) as a colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.76; IR ν_{max} (ATR) 3298, 2926, 2854, 1690, 1672, 1646, 1499, 1417, 1222, 1156, 1049, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.77–6.74 (3H, m, H-3, H-6 and NH-10), 6.69 (1H, dd, *J* = 8.8, 2.9 Hz, H-4), 3.79 (3H, s, OMe-24), 3.74 (1H, s, OMe-25), 3.21–3.14 (4H, m, H_2-11 and H_2-13), 3.11–3.06 (2H, m, H_2-15), 2.92 (2H, t, *J* = 7.8 Hz, H_2-7), 2.47 (2H, t, *J* = 7.8 Hz, H_2-8), 1.62–1.55 (2H, m, H_2-12), 1.52–1.45 (2H, m, H_2-16), 1.44 (9H, s, $3H_3$-23), 1.26 (8H, br s, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.7 (C-9), 156.7 (C-21), 153.6 (C-5), 151.8 (C-2), 130.6 (C-1), 116.3 (C-6), 111.8 (C-4), 111.4 (C-3), 79.6 (C-22), 56.0 (C-24), 55.8 (C-25), 47.1 (C-15), 43.3 (C-13), 37.0 (C-8), 35.7 (C-11), 29.7, 29.6, 29.5 (C-18, C-19 and C-20), 28.5 (C-23), 28.5 (C-16), 27.8 (C-12), 27.1 (C-7), 27.0 (C-17); (+)-HRESIMS *m/*z 921.5889 $[M+Na]^+$ (calcd for $C_{50}H_{82}N_4NaO_{10}$, 921.5923); Purity 97% t_R = 8.13 min.

*5.5.12. Di-tert-Butyl dodecane-1,12-diylbis(3-(3-(3,4-dimethoxyphenyl)propanamido)propylcarbamate) (**33**).*

Using general procedure A, reaction of 3-(3,4-dimethoxyphenyl)propionic acid (53 mg, 0.25 mmol), **21** [12,13] (50 mg, 0.10 mmol), PyBOP (131 mg, 0.25 mmol), and Et_3N (54 μL, 0.39 mmol) yielded a crude product that was purified by silica gel column chromatography (2% MeOH/CH_2Cl_2) to afford **33** (46 mg, 51% yield) as a colorless oil. R_f (5% MeOH/CH_2Cl_2) 0.33; IR ν_{max} (ATR) 3311, 2926, 2854, 1650, 1514, 1417, 1235, 1155, 1028, 764 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.82 (1H, br s, NH-10), 6.79–6.73 (3H, m, H-2, H-5 and H-6), 3.86 (3H, s, OMe-24), 3.84 (3H, s, OMe-25), 3.21–3.13 (4H, m, H_2-11 and H_2-13), 3.11–3.05 (2H, m, H_2-15), 2.92 (2H, t, *J* = 7.6 Hz, H_2-7), 2.47 (2H, t, *J* = 7.6 Hz, H_2-8), 1.61–1.54 (2H, m, H_2-12), 1.52–1.45 (2H, m, H_2-16),

1.44 (9H, s, 3H_3-23), 1.26 (8H, br s, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3 (C-9), 156.7 (C-21), 148.9 (C-3), 147.4 (C-4), 133.8 (C-1), 120.3 (C-6), 111.8 (C-2), 111.4 (C-5), 79.6 (C-22), 56.0 (C-24), 55.9 (C-25), 47.0 (C-15), 43.1 (C-13), 38.9 (C-8), 35.5 (C-11), 31.5 (C-7), 29.7, 29.6, 29.4, 26.9 (C-17, C-18, C-19 and C-20), 28.6 (C-16), 28.5 (C-23), 27.7 (C-12); (+)-HRESIMS *m/z* 899.6076 $[M+H]^+$ (calcd for $C_{50}H_{83}N_4O_{10}$, 899.6104); Purity 99% t_R = 7.95 min.

*5.6. Synthesis of diamides **34**–**45***

*5.6.1. N^1,N^{12}-Bis(3-benzamidopropyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**34**).*

Using general procedure B, reaction of **22** (23 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **34** as a colorless oil (19 mg, 80% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.84; IR ν_{max} (ATR) 3314, 2928, 2855, 1671, 1634, 1542, 1312, 1199, 1136, 720 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.85 (2H, dd, *J* = 7.8, 1.4 Hz, H-2 and H-6), 7.55 (1H, dddd, *J* = 8.2, 8.2, 1.4, 1.4 Hz, H-4), 7.47 (2H, ddd, *J* = 8.2, 7.8, 1.4 Hz, H-3 and H-5), 3.51 (2H, t, *J* = 6.5 Hz, H_2-9), 3.05 (2H, t, *J* = 7.1 Hz, H_2-11), 3.00 (2H, t, *J* = 7.7 Hz, H_2-13), 1.99 (2H, tt, *J* = 7.1, 6.5 Hz, H_2-10), 1.70 (2H, tt, *J* = 7.7, 7.3 Hz, H_2-14), 1.40–1.33 (8H, m, H_2-15, H_2-16, H_2-17 and H_2-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.1 (C-7), 135.0 (C-1), 133.0 (C-4), 129.6 (C-3 and C-5), 128.3 (C-2 and C-6), 49.0 (C-13), 46.4 (C-11), 37.5 (C-9), 30.6, 30.5, 30.2 (C-16, C-17 and C-18), 27.8 (C-10), 27.5 (C-15), 27.3 (C-14); (+)-HRESIMS *m/z* 523.3990 $[M+H]^+$ (calcd for $C_{32}H_{51}N_4O_2$, 523.4007); Purity 99% t_R = 6.67 min.

*5.6.2. N^1,N^{12}-Bis(3-(2-hydroxybenzamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**35**).*

Using general procedure B, reaction of **23** (20 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) followed by purification by C_{18} reversed-phase column chromatography (50% MeOH/H_2O (+ 0.05% TFA)) afforded **35** as a colorless oil (19 mg, 92% yield). R_f (10% MeOH/CH_2Cl_2) 0.36; IR ν_{max} (ATR) 3315, 2929, 2856, 1670, 1634, 1547, 1200, 1133, 722 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.77 (1H, dd, *J* = 7.8, 1.6 Hz, H-6), 7.39 (1H, ddd, *J* = 7.4, 7.4, 1.5 Hz, H-4), 6.93–6.87 (2H, m, H-3 and H-5), 3.53 (2H, t, *J* = 6.5 Hz, H_2-9), 3.06 (2H, t, *J* = 7.2 Hz, H_2-11), 3.00 (2H, t, *J* = 7.7 Hz, H_2-13), 2.00 (2H, tt, *J* = 7.2, 6.5 Hz, H_2-10), 1.70 (2H, tt, *J* = 7.7, 7.7 Hz, H_2-14), 1.43–1.35 (2H, m, H_2-15), 1.33 (6H, br s, H_2-16, H_2-17 and H_2-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.7 (C-7), 160.9 (C-2), 135.0 (C-4), 129.2 (C-6), 120.2 (C-5), 118.4 (C-3), 116.9 (C-1), 49.2 (C-13), 46.4 (C-11), 37.0 (C-9), 30.6, 30.5, 30.2 (C-16, C-17 and C-18), 27.7 (C-10), 27.5 (C-15), 27.3 (C-14); (+)-HRESIMS *m/z* 555.3873 $[M+H]^+$ (calcd for $C_{32}H_{51}N_4O_4$, 555.3910); Purity 99% t_R = 6.91 min.

*5.6.3. N^1,N^{12}-Bis(3-(2,5-dimethoxybenzamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**36**).*

Using general procedure B, reaction of **24** (38.0 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) followed by purification by C_{18} reversed-phase column chromatography (50% MeOH/H_2O (+ 0.05% TFA)) afforded **36** as a colorless oil (34.0 mg, 87% yield). R_f (10% MeOH/CH_2Cl_2) 0.82; IR ν_{max} (ATR) 3375, 2930, 2856, 1674, 1640, 1494, 1200, 1174, 721 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.48 (1H, d, *J* = 1.8 Hz, H-6), 7.10–7.05 (2H, m, H-3 and H-4), 3.92 (3H, s, OMe-19), 3.78 (3H, s, OMe-20), 3.54 (2H, t, *J* = 6.5 Hz, H_2-9), 3.05 (2H, t, *J* = 7.2 Hz, H_2-11), 3.00 (2H, t, *J* = 7.7 Hz, H_2-13), 1.99 (2H, tt, *J* = 7.2, 6.5 Hz, H_2-10), 1.70 (2H, tt, *J* = 7.7, 7.2 Hz, H_2-14), 1.45–1.36 (2H, m, H_2-15), 1.32 (6H, br s, H_2-16, H_2-17 and H_2-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 169.0 (C-7), 155.1 (C-5), 153.3 (C-2), 123.0 (C-1), 119.7 (C-4), 116.8 (C-6), 114.2 (C-3), 56.9 (C-19), 56.2 (C-20), 49.2 (C-13), 46.3 (C-11), 37.3 (C-9), 30.6, 30.5, 30.2 (C-16, C-17 and

C-18), 27.7 (C-10), 27.5 (C-15), 27.3 (C-14); (+)-HRESIMS *m/z* 643.4394 $[M+H]^+$ (calcd for $C_{36}H_{59}N_4O_6$, 643.4435); Purity 99% t_R = 6.92 min.

*5.6.4. N^1,N^{12}-Bis(3-(2-phenylacetamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**37**).*

Using general procedure B, reaction of **25** (22 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **37** (22 mg, 96% yield) as a colorless oil which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.35; IR ν_{max} (ATR) 3273, 2929, 2856, 1643, 1554, 1435, 1136, 720 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.33–7.29 (4H, m, H-2, H-3, H-5 and H-6), 7.28–7.22 (1H, m, H-4), 3.53 (2H, s, H_2-7), 3.30 (2H, t, *J* = 6.9 Hz, H_2-10), 2.91–2.85 (4H, m, H_2-12 and H_2-14), 1.85 (2H, tt, *J* = 6.9, 6.9 Hz, H_2-11), 1.62 (2H, tt, *J* = 7.4, 7.1 Hz, H_2-15), 1.30 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.4 (C-8), 136.9 (C-1), 130.1 (C-2 and C-6), 129.7 (C-3 and C-5), 128.1 (C-4), 49.3 (C-14), 46.1 (C-12), 43.8 (C-7), 36.9 (C-10), 30.6 (C-17, C-18 and C-19), 27.6 (C-11), 27.5 (C-16), 27.2 (C-15); (+)-HRESIMS *m/z* 551.4286 $[M+H]^+$ (calcd for $C_{34}H_{55}N_4O_2$, 551.4325); Purity 99% t_R = 6.48 min.

*5.6.5. N^1,N^{12}-Bis(3-(2-(2-hydroxyphenyl)acetamido)propyl)dodecan-1,12-diaminium 2,2,2-trifluoroacetate (**38**).*

Using general procedure B, reaction of **26** (20 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **38** as a colorless oil (20 mg, 96% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.34; IR ν_{max} (ATR) 3090, 2929, 2856, 1670, 1642, 1457, 1200, 1178, 1134, 721 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.14 (1H, dd, *J* = 8.7, 1.8 Hz, H-6), 7.09 (1H, dd, *J* = 7.7, 1.8 Hz, H-4), 6.82–6.79 (2H, m, H-3 and H-5), 3.54 (2H, s, H_2-7), 3.32 (2H, obsc. H_2-10), 2.96 (2H, t, *J* = 6.9 Hz, H_2-12), 2.88 (2H, t, *J* = 7.0 Hz, H_2-14), 1.85 (2H, tt, *J* = 6.9, 6.6 Hz, H_2-11), 1.62 (2H, tt, *J* = 7.4, 7.0 Hz, H_2-15), 1.32 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.2 (C-8), 156.7 (C-2), 132.3 (C-6), 129.6 (C-4), 123.2 (C-1), 120.8 (C-5), 116.2 (C-3), 49.2 (C-14), 45.9 (C-12), 39.1 (C-7), 36.6 (C-10), 30.6, 30.5, 30.2 (C-17, C-18 and C-19), 27.6 (C-11), 27.5 (C-16), 27.2 (C-15); (+)-HRESIMS *m/z* 583.4190 $[M+H]^+$ (calcd for $C_{34}H_{55}N_4O_4$, 583.4223); Purity 99% t_R = 5.70 min.

*5.6.6. N^1,N^{12}-Bis(3-(2-(2-methoxyphenyl)acetamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**39**).*

Using general procedure B, reaction of **27** (20 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **39** as a colorless oil (20 mg, 90% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.33; IR ν_{max} (ATR) 3284, 3071, 2927, 2852, 1663, 1634, 1492, 1247, 1198, 1143, 720 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.65 (1H, ddd, *J* = 8.2, 8.2, 1.5 Hz, H-4), 7.20 (1H, dd, *J* = 7.4, 1.4 Hz, H-6), 6.97 (1H, d, *J* = 8.2 Hz, H-3), 6.92 (1H, ddd, *J* = 8.2, 7.4, 1.4 Hz, H-5), 3.83 (3H, s, OMe-20), 3.54 (2H, s, H_2-7), 3.29 (2H, t, *J* = 6.7 Hz, H_2-10), 2.94 (2H, t, *J* = 7.0 Hz, H_2-12), 2.88 (2H, t, *J* = 7.7 Hz, H_2-14), 1.85 (2H, tt, *J* = 7.0, 6.7 Hz, H_2-11), 1.60 (2H, tt, *J* = 7.7, 6.7 Hz, H_2-15), 1.31 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.7 (C-8). 159.0 (C-2), 132.2 (C-6), 129.8 (C-4), 124.9 (C-1), 121.8 (C-5), 111.7 (C-3), 55.9 (C-20), 48.9 (C-14), 46.0 (C-12), 38.6 (C-7), 36.8 (C-10), 30.6, 30.5, 30.2 (C-17, C-18 and C-19), 27.7 (C-11), 27.5 (C-16), 27.2 (C-15); (+)-HRESIMS *m/z* 611.4506 $[M+H]^+$ (calcd for $C_{36}H_{59}N_4O_4$, 611.4531); Purity 99% t_R = 6.98 min.

*5.6.7. N^1,N^{12}-Bis(3-(2-(4-methoxyphenyl)acetamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**40**).*

Using general procedure B, reaction of **28** (20.0 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **40** as a colorless oil (20.0 mg, 96% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.54; IR ν_{max} (ATR) 3284, 2929, 2855, 1673, 1638, 1514, 1199, 1138, 1032, 720 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.21 (2H, dd, *J* = 8.7, 2.9 Hz, H-2 and H-6), 6.87 (2H, dd, *J* = 8.7, 2.9 Hz, H-3 and H-5), 3.77 (3H, s, OMe-20), 3.45 (2H, s, H_2-7), 3.29 (2H, t, *J* = 6.5 Hz, H_2-10), 2.90–2.85 (4H, m, H_2-12 and H_2-14), 1.84 (2H, tt, *J* = 6.5, 6.5 Hz, H_2-11), 1.65–1.58 (2H, m, H_2-15), 1.33 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.8 (C-8), 160.3 (C-4), 131.1 (C-2 and C-6), 128.8 (C-1), 115.1 (C-3 and C-5), 55.7 (C-20), 49.1 (C-14), 46.1 (C-12), 42.9 (C-7), 36.9 (C-10), 30.6, 30.5, 30.2 (C-17, C-18 and C-19), 27.6 (C-11), 27.5 (C-16), 27.2 (C-15); (+)-HRESIMS *m/z* 611.4495 $[M+H]^+$ (calcd for $C_{36}H_{59}N_4O_4$, 611.4531); Purity 98% t_R = 6.38 min.

*5.6.8. N^1,N^{12}-Bis(3-(2-(2,5-dimethoxyphenyl)acetamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**41**).*

Using general procedure B, reaction of **29** (38 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **41** as a colorless oil (28 mg, 71% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.55; IR ν_{max} (ATR) 3290, 2930, 2855, 1671, 1650, 1501, 1226, 1199, 1174, 1024, 719 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 6.89 (1H, d, *J* = 7.9 Hz, H-3), 6.82–6.78 (2H, m, H-4 and H-6), 3.79 (3H, s, OMe-20), 3.74 (3H, s, OMe-21) 3.51 (2H, s, H_2-7), 3.30 (2H, obsc. H_2-10), 2.94 (2H, t, *J* = 7.1 Hz, H_2-12), 2.88 (2H, t, *J* = 7.7 Hz, H_2-14), 1.85 (2H, tt, *J* = 7.1, 6.8 Hz, H_2-11), 1.62 (2H, tt, *J* = 7.7, 7.1 Hz, H_2-15), 1.31 (8H, br s, H_2-16, H_2-17, H_2-18 and H_2-19); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.5 (C-8), 155.1 (C-5), 153.1 (C-2), 125.9 (C-1), 118.6 (C-6), 113.8 (C-4), 112.7 (C-3), 56.5 (C-20), 56.1 (C-21), 49.3 (C-14), 45.9 (C-12), 38.7 (C-7), 36.7 (C-10), 30.6, 30.5, 30.2 (C-17, C-18 and C-19), 27.7 (C-11), 27.5, (C-16), 27.2 (C-15); (+)-HRESIMS *m/z* 671.4716 $[M+H]^+$ (calcd for $C_{38}H_{63}N_4O_6$, 671.4748); Purity 99% t_R = 6.30 min.

*5.6.9. N^1,N^{12}-Bis(3-(3-phenylpropanamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**42**).*

Using general procedure B, reaction of **30** (31 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **42** as a colorless oil (31 mg, 97% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.53; IR ν_{max} (ATR) 3290, 2928, 2856, 1671, 1646, 1200, 1174, 1138, 721 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.29–7.16 (5H, m, H-2, H-3, H-4, H-5 and H-6), 3.24 (2H, t, *J* = 6.6 Hz, H_2-11), 2.93 (2H, t, *J* = 7.5 Hz, H_2-7), 2.86 (2H, t, *J* = 7.6 Hz, H_2-15), 2.75 (2H, t, *J* = 7.0 Hz, H_2-13), 2.55 (2H, t, *J* = 7.5 Hz, H_2-8), 1.77 (2H, tt, *J* = 7.0, 6.6 Hz, H_2-12), 1.66 (2H, tt, *J* = 7.6, 7.3 Hz, H_2-16), 1.41–1.35 (2H, m, H_2-17), 1.35 (6H, br s, H_2-18, H_2-19 and H_2-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.3 (C-9), 142.0 (C-1), 129.5, 129.4, 127.3 (C-2 to C-6), 49.1 (C-15), 46.0 (C-13), 38.3 (C-8), 36.6 (C-11), 32.6 (C-7), 30.6, 30.5, 30.2 (C-18, C-19 and C-20), 27.6 (C-12), 27.5 (C-17), 27.3 (C-16); (+)-HRESIMS *m/z* 579.4597 $[M+H]^+$ (calcd for $C_{36}H_{59}N_4O_2$, 579.4633); Purity 99% t_R = 6.67 min.

*5.6.10. N^1,N^{12}-Bis(3-(3-(2-hydroxyphenyl)propanamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**43**).*

Using general procedure B, reaction of **31** (14 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) followed by purification by C_{18} reversed-phase column chromatography (50% MeOH/H_2O (TFA)) afforded **43** as a colorless oil (10 mg, 90% yield). R_f (10% MeOH/CH_2Cl_2) 0.46; IR ν_{max} (ATR) 3302, 2931, 2857, 1671, 1441, 1182, 1135, 724 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.07 (1H, dd, *J* = 7.4, 1.5 Hz, H-6), 7.02 (1H, ddd, *J* = 7.8, 7.8, 1.5 Hz, H-4), 6.77–6.71 (1H, m, H-3 and H-5), 3.25 (2H, t, *J* = 6.5 Hz, H_2-11), 2.93–2.86 (4H, m, H_2-7 and H_2-15), 2.79 (2H, t, *J* = 7.2 Hz,

H_2-13), 2.56 (2H, t, *J* = 7.4 Hz, H_2-8), 1.78 (2H, tt, *J* = 7.2, 6.5 Hz, H_2-12), 1.66 (2H, tt, *J* = 7.2, 6.4 Hz, H_2-16), 1.38 (8H, br s, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 177.2 (C-9), 156.5 (C-2), 131.1 (C-6), 128.6 (C-4), 128.1 (C-1), 120.5 (C-5), 116.1 (C-3), 49.2 (C-15), 46.0 (C-13), 36.8 (C-8), 36.6 (C-11), 30.6, 30.5, 30.2 (C-18, C-19 and C-20), 27.7 (C-12), 27.5 (C-7), 27.5 (C-17), 27.3 (C-16); (+)-HRESIMS *m/z* 611.4501 $[M+H]^+$ (calcd for $C_{36}H_{59}N_4O_4$, 611.4531); Purity 98% t_R = 6.84 min.

*5.6.11. N^1,N^{12}-Bis(3-(3-(2,5-dimethoxyphenyl)propanamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**44**).*

Using general procedure B, reaction of **32** (25 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) afforded **44** as a colorless oil (18 mg, 95% yield) which required no further purification. R_f (10% MeOH/CH_2Cl_2) 0.75; IR ν_{max} (ATR) 3285, 2931, 2855, 1672, 1650, 1500, 1200, 1127, 720 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.84 (1H, d, *J* = 7.9 Hz, H-3), 6.75–6.72 (2H, m, H-4 and H-6), 3.78 (3H, s, OMe-21), 3.72 (3H, s, OMe-22), 3.24 (2H, t, *J* = 6.5 Hz, H_2-15), 2.91–2.86 (4H, m, H_2-7 and H_2-11), 2.81 (2H, t, *J* = 7.1 Hz, H_2-13), 2.52 (2H, t, *J* = 7.3 Hz, H_2-8), 1.78 (2H, tt, *J* = 7.1, 6.5 Hz, H_2-12), 1.67 (2H, tt, *J* = 7.4, 7.1 Hz, H_2-16), 1.42–1.30 (8H, m, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.8 (C-9), 154.9 (C-5), 153.2 (C-2), 131.1 (C-1), 117.7 (C-6), 112.5 (C-4), 112.4 (C-3), 49.2 (C-15), 46.0 (C-13), 36.7 (C-8), 36.6 (C-11), 30.6, 30.5, 30.2 (C-18, C-19 and C-20), 27.7 (C-7), 27.7 (C-12), 27.5 (C-17), 27.3 (C-16); (+)-HRESIMS *m/z* 699.5001 $[M+H]^+$ (calcd for $C_{40}H_{67}N_4O_6$, 699.5055); Purity 99% t_R = 6.26 min.

*5.6.12. N^1,N^{12}-Bis(3-(3-(3,4-dimethoxyphenyl)propanamido)propyl)dodecane-1,12-diaminium 2,2,2-trifluoroacetate (**45**).*

Using general procedure B, reaction of **33** (34 mg, 0.04 mmol) in CH_2Cl_2 (2 mL) with TFA (0.2 mL) followed by purification by C_{18} reversed-phase column chromatography (50% MeOH/H_2O (TFA)) afforded **45** as a colorless oil (31 mg, 88% yield). R_f (10% MeOH/CH_2Cl_2) 0.68; IR ν_{max} (ATR) 3291, 2930, 2856, 1673, 1648, 1515, 1200, 1177, 721 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.86 (1H, d, *J* = 8.2 Hz, H-5), 6.83 (1H, d, *J* = 1.9 Hz, H-2), 6.76 (1H, dd, *J* = 8.2, 1.9 Hz, H-6), 3.84 (3H, s, OMe-21), 3.79 (3H, s, OMe-22), 3.24 (2H, t, *J* = 6.5 Hz, H_2-11), 2.89–2.83 (4H, m, H_2-7 and H_2-15), 2.76 (2H, t, *J* = 7.1 Hz, H_2-13), 2.53 (2H, t, *J* = 7.4 Hz, H_2-8), 1.78 (2H, tt, *J* = 7.1, 6.5 Hz, H_2-12), 1.65 (2H, tt, *J* = 7.1, 7.0 Hz, H_2-16), 1.34 (8H, br s, H_2-17, H_2-18, H_2-19 and H_2-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.7 (C-9), 150.4 (C-3), 149.0 (C-4), 134.9 (C-1), 121.7 (C-6), 113.7 (C-2), 113.3 (C-5), 56.6 (C-22), 56.5 (C-21), 49.0 (C-15), 46.0 (C-13), 38.5 (C-8), 36.7 (C-11), 32.2 (C-7), 30.6, 30.5, 30.2 (C-18, C-19 and C-20), 27.8 (C-12), 27.5 (C-17), 27.3 (C-16); (+)-HRESIMS *m/z* 699.5020 $[M+H]^+$ (calcd for $C_{40}H_{67}N_4O_6$, 699.5055); Purity 99% t_R = 6.51 min.

5.7. Biology Assays

5.7.1. In vitro Assays

In vitro anti-*P. falciparum* testing used the K1 (chloroquine and pyrimethamine resistant) strain, at the erythrocytic stage, with chloroquine as the positive control (IC_{50} of 0.20 μM (0.065 μg/mL)). Cytotoxicity assessment used the L6 rat skeletal myoblast cell line, with positive control

podophyllotoxin (IC_{50} of 0.01 μM (0.004 μg/mL)). The cytotoxicity of chloroquine against L6 cells is IC_{50} 72 μM (37.3 μg/mL). Protocols for these assays have been reported elsewhere [5].

5.7.2 In vivo Antimalarial Efficacy Studies

In vivo antimalarial activity was assessed as previously described [15]. Groups of three female NMRI mice (20–22 g) were intravenously infected with 2 x 10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA [17]. Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water and administered intraperitoneally in a volume of 10 ml kg^{-1} on four consecutive days (4, 24, 48 and 72 h post infection). Parasitemia was determined on day 4 post infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean per cent parasitaemia for the control (n=5 mice) and treated groups expressed as a per cent relative to the control group. The survival of the animals was usually monitored up to 30 days, but in the current study all mice were euthanized after the determination of parasitaemia due to inactivity. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites. All protocols and procedures used in the current study were reviewed and approved by the local veterinary authorities of the Canton Basel-Stadt.

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Supplementary Data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/. These data include copies of ^1H and ^{13}C NMR spectra and MOL files of the most important compounds described in this article.

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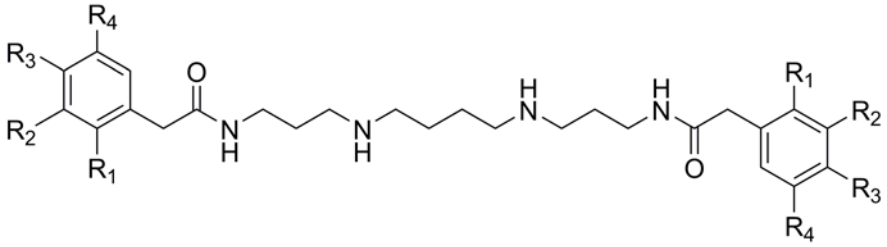
Fig. 1. Lead antimalarial polyamine structures.

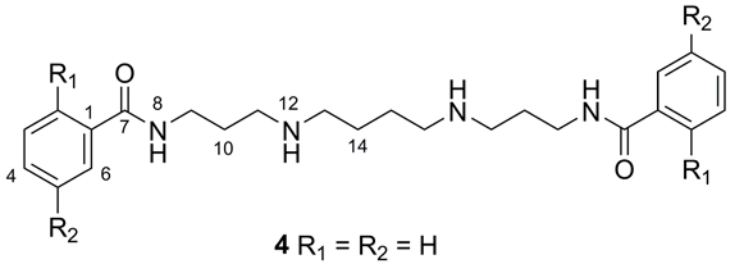
Fig. 2. Benzamide and 3-phenylpropanamide analogues of spermine.

Scheme 1. Preparation of polyamines **15**–**20**. Reagents and conditions: (i) acrylonitrile, EtOH, N_2, reflux, 3h. (ii) di-*tert*-butyl dicarbonate, Et_3N, CH_2Cl_2, N_2, 22.5 h. (iii) LiOH, 10% Pd/C, 50% Ni-Al alloy, H_2, 50^oC, 21 h. (iv) carboxylic acid (2 equiv), PyBOP, DMF, Et_3N, N_2, 23 h. (v) CH_2Cl_2, TFA, N_2, 2 h.

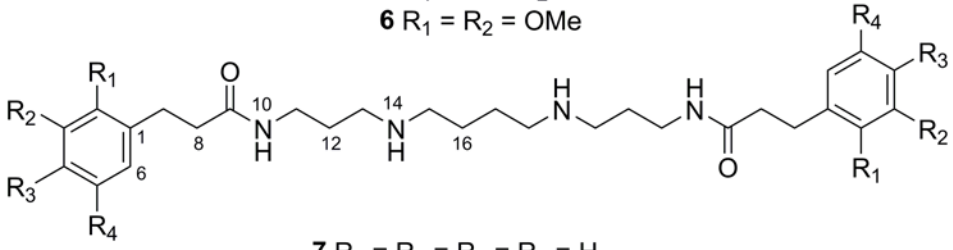
Scheme 2. Preparation of polyamine analogues **22**–**45**. Reagents and conditions: (i) carboxylic acid (2 equiv), PyBOP, DMF, Et_3N, N_2, 23 h. (ii) CH_2Cl_2, TFA, N_2, 2 h.



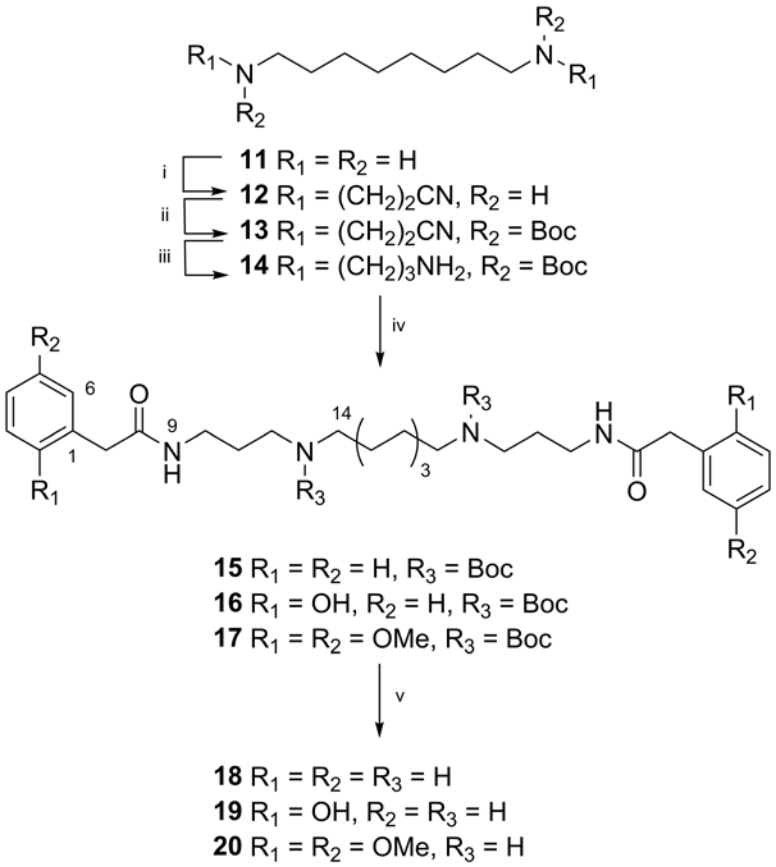
1 $R_1 = R_4 = H$, $R_2 = OMe$, $R_3 = OH$ (orthidine F)
2 $R_1 = OH$, $R_2 = R_3 = R_4 = H$
3 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$

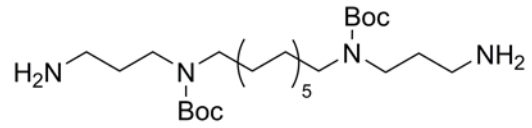


4 $R_1 = R_2 = H$
5 $R_1 = OH$, $R_2 = H$
6 $R_1 = R_2 = OMe$

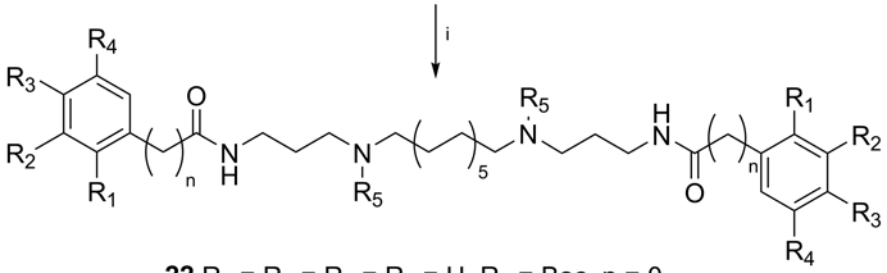


7 $R_1 = R_2 = R_3 = R_4 = H$
8 $R_1 = OH$, $R_2 = R_3 = R_4 = H$
9 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$
10 $R_1 = R_4 = H$, $R_2 = R_3 = OMe$


R2
R1
N
R2
N
R1
i
ii
iii
11 R1 = R2 = H
12 R1 = (CH2)2CN, R2 = H
13 R1 = (CH2)2CN, R2 = Boc
14 R1 = (CH2)3NH2, R2 = Boc
iv
R2
6
O
1
9
N
H
R1
14
N
R3
3
R3
N
H
N
O
R1
R2
15 R1 = R2 = H, R3 = Boc
16 R1 = OH, R2 = H, R3 = Boc
17 R1 = R2 = OMe, R3 = Boc
v
18 R1 = R2 = R3 = H
19 R1 = OH, R2 = R3 = H
20 R1 = R2 = OMe, R3 = H



21



22 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 0
23 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 0
24 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$, $R_5 = Boc$, n = 0
25 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 1
26 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 1
27 $R_1 = OMe$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 1
28 $R_1 = R_2 = R_4 = H$, $R_3 = OMe$, $R_5 = Boc$, n = 1
29 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$, $R_5 = Boc$, n = 1
30 $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 2
31 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = Boc$, n = 2
32 $R_1 = R_4 = OMe$, $R_2 = R_3 = H$, $R_5 = Boc$, n = 2
33 $R_1 = R_4 = H$, $R_2 = R_3 = OMe$, $R_5 = Boc$, n = 2

ii

34 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, n = 0
35 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, n = 0
36 $R_1 = R_4 = OMe$, $R_2 = R_3 = R_5 = H$, n = 0
37 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, n = 1
38 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, n = 1
39 $R_1 = OMe$, $R_2 = R_3 = R_4 = R_5 = H$, n = 1
40 $R_1 = R_2 = R_4 = R_5 = H$, $R_3 = OMe$, n = 1
41 $R_1 = R_4 = OMe$, $R_2 = R_3 = R_5 = H$, n = 1
42 $R_1 = R_2 = R_3 = R_4 = R_5 = H$, n = 2
43 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$, n = 2
44 $R_1 = R_4 = OMe$, $R_2 = R_3 = R_5 = H$, n = 2
45 $R_1 = R_4 = R_5 = H$, $R_2 = R_3 = OMe$, n = 2

Table 1

Polyamine analogues and their antimalarial and cytotoxic biological activities (μM)

entry	No.	*P. falc.*[a]	L6[b]	SI[c]		entry	No.	*P. falc.*[a]	L6[b]	SI[c]
	1	0.89	>120	>134		19	**27**	0.46	2.7	5.9
1	**4**	0.45	89	200		20	**28**	0.21	4.7	22
2	**5**	1.9	83	44		21	**29**	0.16	2.2	14
3	**6**	0.93	75	81		22	**30**	0.24	4.0	17
4	**7**	0.10	91	910		23	**31**	0.13	3.6	28
5	**8**	0.015	86	5700		24	**32**	0.18	2.3	13
6	**9**	0.0061	99	16230		25	**33**	0.066	2.5	38
7	**10**	0.46	73	160		26	**34**	0.16	6.9	43
8	**15**	0.47	8.6	18		27	**35**	0.38	2.5	6.6
9	**16**	0.73	16	22		28	**36**	0.39	2.2	5.6
10	**17**	0.048	1.9	40		29	**37**	0.16	22	138
11	**18**	0.015	74	4933		30	**38**	0.0086	42	4880
12	**19**	0.0013	55	42300		31	**39**	0.078	21	269
13	**20**	0.012	61	5083		32	**40**	0.200	19	95
14	**22**	0.16	8.3	52		33	**41**	0.19	23	121
15	**23**	0.50	93	186		34	**42**	0.073	6.9	95
16	**24**	0.18	1.8	10		35	**43**	0.0095	62	6530
17	**25**	0.26	8.2	32		36	**44**	0.11	6.5	59
18	**26**	0.17	10	59		37	**45**	0.071	57	803

[a] *Plasmodium falciparum*. IC_{50} (μM).

[b] L6 rat skeletal myoblast cell line. IC_{50} (μM).
[c] Selectivity index (SI) = IC_{50} L6 / IC_{50} *Pf*.
All data is the mean value from duplicate assays.