Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Synthesis and Evaluation of Cationic Norbornanes as Peptidomimetic Antibacterial Agents

Shane M. Hickey,[†] Trent D. Ashton,[†] Simren K. Khosa,[†] Ryan N. Robson,[†] Jonathan M. White,[‡] Jian Li,[§] Roger L. Nation,[§] Heidi Y. Yu,[§] Alysha G. Elliott,[#] Mark S. Butler,[#] Johnny X. Huang,[#] Matthew A. Cooper,[#] and Frederick M. Pfeffer*[,†]

[†]*Research Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Deakin University, Waurn Ponds, Victoria, 3216, Australia,*

[‡]*Bio21 Institute, School of Chemistry, University of Melbourne, Parkville, Victoria, 3010, Australia,*

[§]*Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Science, Royal Parade, Parkville, Victoria, 3052, Australia, and,*

[#] *Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland, 4072, Australia.*

fred.pfeffer@deakin.edu.au

Abstract

A series of structurally amphiphilic biscationic norbornanes have been synthesised as rigidified, low molecular weight peptidomimetics of cationic antimicrobial peptides. A variety of charged hydrophilic functionalities were attached to the norbornane scaffold including aminium, guanidinium, imidazolium amd pyridinium moieties. Additionally, a range of hydrophobic groups of differing sizes were incorporated by an acetal linkage. The compounds were evaluated for antibacterial activity against both Gram-negative and Gram-positive bacteria. Activity was observed across the series; the most potent of which exhibited MIC's $\leq$ 1 μg/mL against *Streptococcus pneumoniae*, *Enterococcus faecalis* and several strains of *Staphylococcus aureus*, including multi-resistant methicillin resistant (mMRSA), glycopeptide-intermediate (GISA) and vancomycin-intermediate (VISA) *S. aureus.*

Introduction

There is consensus amongst health professionals that a scenario is imminent whereby strains of pathogenic bacteria will be resistant to all current antibiotic treatments.[1] In fact, resistance has been observed for every antibiotic in current clinical use.[2] The development of new antibacterial agents is therefore a priority for continued human health.[3] Naturally occurring cationic antimicrobial peptides are well studied in medicinal chemistry as both antibiotics in their own right[4] and as lead compounds for the development of peptidomimetic antibacterial agents.[3,5] The mode of action of polymyxin peptide antibiotics (such as Colistin and Polymyxin B) initially involves electrostatic binding to the lipid A

portion of lipopolysaccharide (LPS) followed by disruption of the Gram-negative cellular membrane ultimately leading to cell lysis.[6] In order to elicit this response it is accepted that peptides (and peptidomimetics) of this nature must either inherently possess, or adopt, an amphiphilic structure.[7]

Previous work has demonstrated that the norbornene framework, when functionalised with thiourea groups, was capable of binding to phosphoanionic species.[8] As an extension of this study, bisguanidinyl norbornane **1** (Figure 1) was designed as an amphiphile capable of binding lipid A (a phosphoanionic component of the Gram-negative bacterial cell wall) and exhibited antibacterial activity against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[9] Both of these bacterial species present a global medical challenge due to increasing resistance to almost all currently available antibiotics.[2b] More recently, a series of compounds bearing two hydrophobic benzyl groups attached to the norbornane framework *via* an ether link (compound **2**, Figure 1), were shown to exhibit modest antibacterial activity against a range of Gram-negative and Gram-positive bacterial strains.[10]

Herein, the synthesis of a larger, more diverse set of functionalised norbornane acetals is presented. The antibacterial activity of these compounds was evaluated against a range of Gram-negative and Gram-positive bacteria including members of the ESKAPE pathogens[1a] using disk diffusion assay (50 μg/disk) and micro-broth dilution assay to ascertain the minimum inhibitory concentrations (MICs). From this data a putative structure activity relationship (SAR) has been identified.

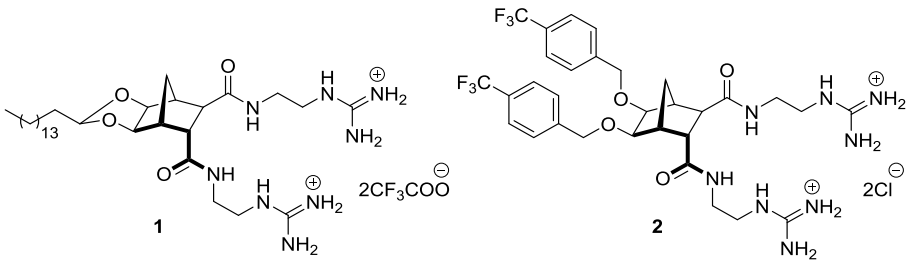


Figure 1: Previously reported antibacterial agents based on the norbornane scaffold.

Results and Discussion

Chemistry

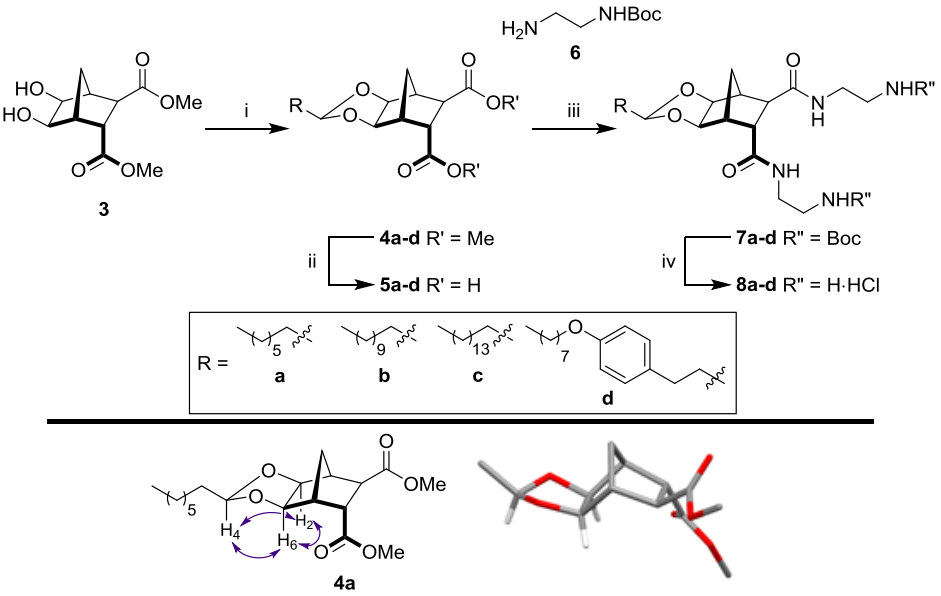
Design

A lipophilic moiety is an essential component of antibacterial agents that act *via* membrane disruption. Hence, the design of the family of compounds involved variation of the hydrophobic section to include alkyl and mixed alkyl/aryl functionality linked through an acetal to the parent norbornane. To mimic the lysine and arginine residues present in naturally occurring cationic antibacterial peptides, amines

and guanidines were used as the cationic groups. Furthermore, alkylated imidazolium and pyridinium, as well as charge neutral anion recognition groups, thioureas and squaramides, were also incorporated.

Synthesis

Initially, a synthetic route that allowed for late stage installation of the *lipophilic* group was investigated; however, the isolation of key intermediates in reproducible yields was problematic. A synthetic route to access the desired amphiphiles **8a–d** (Scheme 1), which allowed for late stage introduction of the *hydrophilic* (or anion binding) group using 2-(*tert*-butoxycarbonylamino)ethylamine **6**,[11] proved to be more robust. Following the method reported by Pandey and co-workers,[12] stirring the appropriate aldehyde with diol **3** in PhMe with catalytic TsOH (0.05 equiv) at 110 °C gave acetals **4a-d** (48–92%).



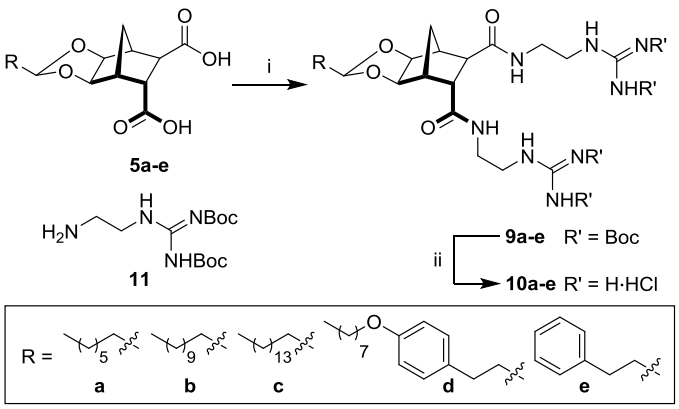
Scheme 1: *Reagents and conditions*: i) RCHO, TsOH·H_2O, $MgSO_4$, PhMe, 3 h, 110 °C, **4a–d** (48–92%); ii) NaOH, THF/H_2O, 16 h, 21 °C, **5a–d** (78–86%); iii) **6**, EDCI, HOBt, $CHCl_3$, MW: 30 min, 50 °C, **7a–d** (35–58%); iv) AcCl, MeOH, 24 h, 21 °C, **8a–d** (94–99%). ^{1}H NOESY correlations of H-2, H-4 and H-6 (bottom left) and crystal structure of **4a** with the aliphatic chain and hydrogens (except for H-2, H-4 and H-6) have been omitted for clarity (bottom right).

Unfortunately, in some instances condensation of benzaldehydes resulted in a thermodynamic mixture of inseparable *exo*/*endo* acetal isomers. After further functionalisation of the norbornane scaffold, separation of the isomers could sometimes be achieved using column chromatography (see ESI for further discussion and NMR spectra of individual isomers). It is reasonable to suggest that the stability of the intermediate oxonium species was enhanced by the adjacent aromatic system thus facilitating isomerisation. Given that the final step in the synthesis involved acid mediated Boc deprotection, product degradation[13] was likely to occur. As such this subset of analogues was abandoned.

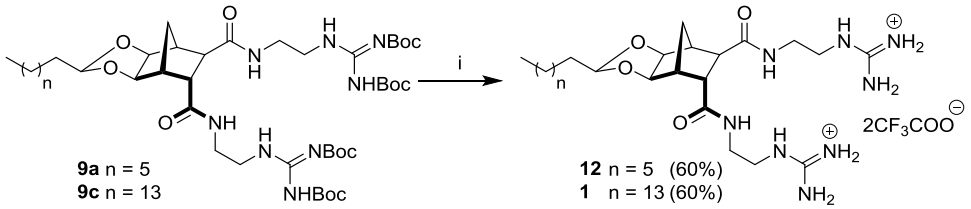
Aliphatic acetals were not prone to the same isomerisation/complications as their aryl counterparts. The *exo*-orientation of the alkyl chain was confirmed for norbornane dimethyl ester **4a** (Scheme 1) using nuclear Overhauser effect (NOE) spectroscopy. Clear correlations were observed between the acetal H-4 methine and the *endo*-methine protons (H-2 and H-6, see ESI for full NOE spectra) which can only exist if the aliphatic chain has *exo*-orientation. Single crystal X–ray crystallographic analysis (Scheme 1, see ESI for details of crystallisation) also clearly depicts the *exo*-isomer.

Methyl ester hydrolysis gave diacids **5a–d** in good yields (78–86%, Scheme 1), which were then coupled to amine **6**[11] using EDCI/HOBt and microwave heating (50 °C for 30 min) to give compounds **7a–d** (35–58%). Removal of the Boc-groups was effected using *in situ* generated HCl (AcCl/MeOH) to afford the diamines **8a–d** as hydrochloride salts in excellent yields (94–99%). All compounds were isolated as the desired *exo*-acetal with no evidence of isomerisation.

Guanidines **10a–e** were accessed by attaching 2-[2,3-bis(*tert*-butoxycarbonyl)guanidino]ethylamine **11**[14] using the previously described microwave-mediated coupling protocol, followed by removal of the Boc-protecting groups using AcCl in MeOH (Scheme 2). In order to investigate the importance of the counterion in relation to antibacterial activity two analogues (**10a** and **10c**) were also synthesised as their trifluoroacetate salts (**12** and **1** respectively) (Scheme 3).



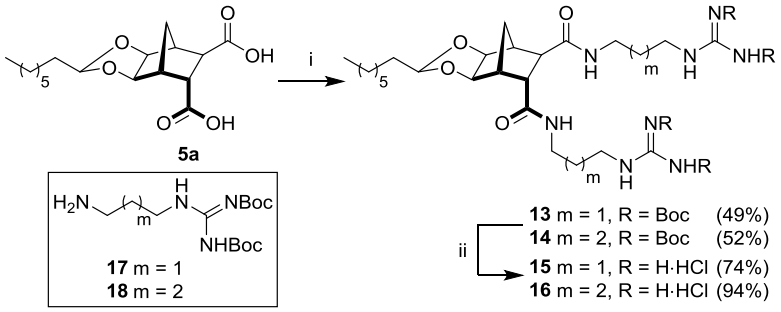
Scheme 2: *Reagents and conditions*: i) **10**, EDCI, HOBt, DMF, MW: 30 min, 50 °C, **9a–e** (41–74%); ii) AcCl, MeOH, 16 h, 21 °C, **10a–e** (74–95%).



Scheme 3: *Reagents and conditions*: i) TFA, CH_2Cl_2, 1–2 d, 21 °C.

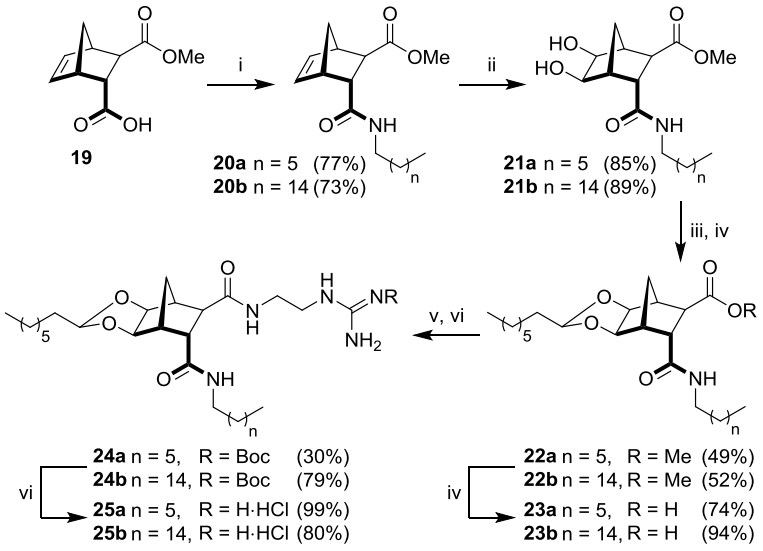
In an earlier communication, computational modelling indicated that an ethyl linker between the norbornane scaffold and the guanidine was sufficient for effective binding of Lipid A.[8a,15] However the dependence of activity on spacer length was never experimentally tested, therefore, in this study

homologated analogues were prepared. Aminopropylguanidine **17** and aminobutylguanidine **18**[14] were attached to the norbornane framework to give diamides **13** (49%) and **14** (52%) (Scheme 4). Subsequent deprotection using AcCl/MeOH gave the desired diguanidines **15** (74%) and **16** (94%) as HCl salts (Scheme 4).



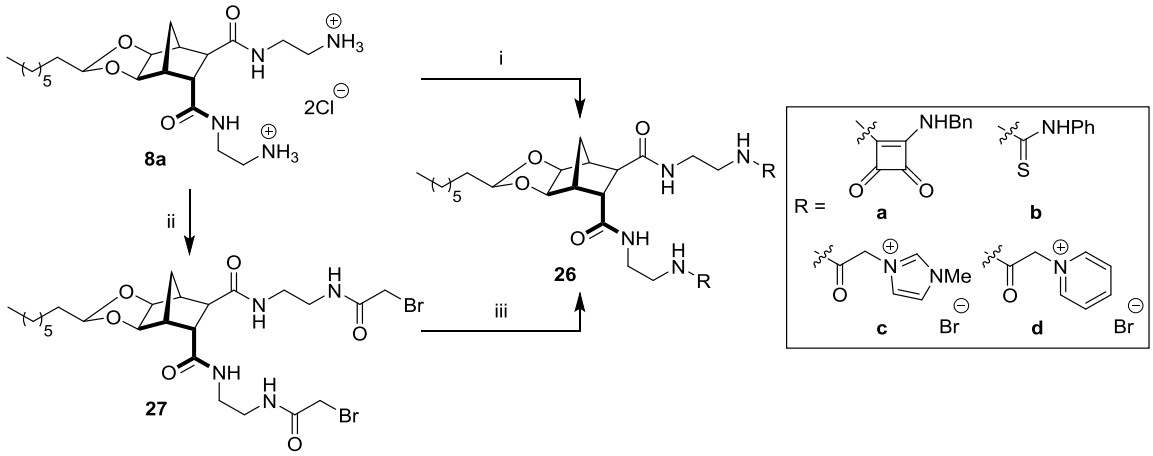
Scheme 4: *Reagents and conditions*: i) **17** (or **18**), EDCI, HOBt, DMF, MW: 30 min, 50 °C; ii) AcCl, MeOH, 16 h, 21 °C.

To investigate the influence of net ionic charge, two singularly charged analogues were synthesised. Discrimination between the norbornane *exo*- and *endo*-carboxylic acids was achieved *via* iodolactonisation (95%) of norbornene diacid (compound **S1**, see ESI).[16] Following Fischer esterification (90%) and Zn-mediated reductive elimination the norbornene **19** was obtained in 93% (Scheme 5).[16a] The free *endo*-carboxylic acid was then converted to the *n*-heptylamide **20a** (77%) using microwave-assisted amide coupling conditions. Subsequent dihydroxylation of the alkene gave **21a** in 85% yield. Acetal **22a** was then synthesised in 94% yield, before hydrolysis of the *exo*-methyl ester afforded carboxylic acid **23a** in 64% yield. Incorporation of Boc-protected aminoethylguanidine **11** and subsequent deprotection gave the desired HCl salt **25a** in near quantitative yield (99%). Guanidine **25b** was synthesised in an analogous fashion, using *n*-hexadecylamine rather than *n*-heptylamine.



Scheme 5: *Reagents and conditions*: i) *n*-heptylamine or *n*-hexadecylamine, EDCI, HOBt, $CHCl_3$, MW: 30 min, 50 °C; ii) OsO_4, NMO, acetone/H_2O, 3 d, 21 °C; iii) octanal, $TsOH \cdot H_2O$, $MgSO_4$, PhMe, 3 h, 110 °C; iv) NaOH, THF/H_2O, 16 h, 21 °C; v) **11**, EDCI, HOBt, DMF, MW: 30 min, 50 °C; vi) AcCl, MeOH, 16 h, 21 °C

Analogues with other anion recognition groups (both charged and charge-neutral) were also synthesised. Both the imidazolium and pyridiunium analogues **26c** and **26d** were accessed in reasonable yield (74 and 64% respectively) through the α-bromoamide (**27**, see ESI) following the procedure reported by Gathergood.[17] Incorporation of squaramides and thioureas onto the norbornane framework was also performed as both have previously been used to bind phosphoanions.[15,18] Stirring diamine **8a** and squaramate **S2** (synthesised from squaric acid[19], see ESI for details) at ambient temperature gave the desired disquaramide **26a** (40%, Scheme 6). In a similar fashion, bis-thiourea **26b** was accessed in excellent yield (95%) by treating diamine **8a** with phenylisothiocyanate at ambient temperature.



Scheme 6: *Reagent and conditions:* i) **S2** or phenylisothiocyante, Et_3N, MeOH or CH_2Cl_2, 21 °C, **26a** (40%) or **26b** (95%); ii) bromoacetyl bromide, Et_3N, CH_2Cl_2, 5 h, −78 °C, **27** (65%); iii) 1-methylimidazole or pyridine, THF, **26c** (74%) or **26d** (64%).

Biological evaluation

The antibacterial activity of these compounds was evaluated against a range of Gram-negative and Gram-positive bacteria, including members of the ESKAPE pathogens;[1a] first using the disk diffusion assay (Kirby-Bauer) to identify active compounds then micro-broth dilution assays to ascertain minimum inhibitory concentrations (MICs).[20]

Zones of inhibition (ZOI) were observed for diamine dihydrochloride **8a** (50 μg/mL) against *Pseudomonas aeruginosa* (16 mm) and *Klebsiella pneumoniae* (11 mm) in the disk diffusion assay. Similar activity was observed for diamines **8b** (10 and 12mm respectively) and **8d** (7mm against *K. pneumoniae*), which had larger hydrophobic portions, including activity against Gram-positive

methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE).

Compounds bearing two guanidinium groups displayed similar activity to their diamine counterparts; diguanidines **10a**, **10b** and **10d** were comparable to diamines **8a**, **8b** and **8d** respectively (Table 1). However, for the imidazolium (**26c**) and pyridinium (**26d**) analogues, only minor inhibition was observed against MRSA (ZOI = 7 and 8 mm, respectively). Furthermore, the effect of the counterion was not significant; trifluoroacetate salt (**12**) possessed similar antibacterial activity to its HCl salt counterpart (**10a**, Table 1).

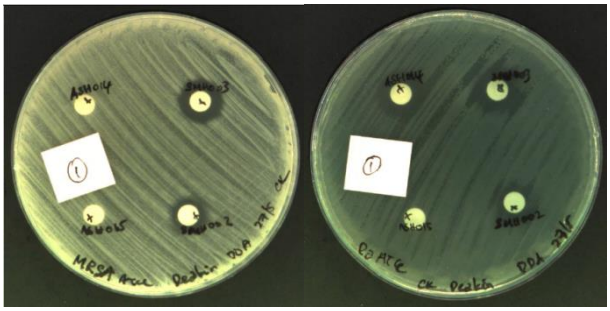


Figure 2: Antibacterial activity of **10a** against MRSA (LHS) and *P. aeruginosa* (RHS)

The effect of the linker between the norbornane framework and the guanidinium moiety was minimal as the three related diguanidine analogues **10a** (ethyl), **15** (propyl) and **16** (butyl), each had a ZOI ≥ 12 mm against *P. aeruginosa*, *K. pneumoniae* and MRSA. When only a single cationic group was present only moderate, or no, activity was observed eg **25a** (ZOI = 8 and 7 mm for MRSA and VRE, respectively, Table 1) and **25b** (inactive). This trend continued for the charge neutral disquaramide **26a** and bis-thiourea **26b** analogues in which only moderate inhibition observed against MRSA (ZOI = 7 and 8 mm, respectively) was noted.

Table 1: Antibacterial activity measured with disk diffusion[a]

Compound	*A. baumannii* **ATCC 19606**	*P. aeruginosa* **ATCC 27853**	*K. pneumoniae* **ATCC 13883**	*S. aureus* **MRSA ATCC 43300**	*E. faecium* **VRE ATCC 700221**
			Amines		
8a	-	16	11	8	-
8b	-	10	12	12	14
8c	-	-	-	-	-
8d	9	-	7	9	-
			Guanidines		

10a	NT[c]	15	12	13	12
12	NT	11	11	10	10
10b	-	8	8	9	10
10c	-	-	-	-	-
10d	9	-	8	8	-
10e	10	-	-	-	-
15	-	14	12	16	-
16	-	14	14	14	8
25a	-	-	-	8	7
25b	-	-	-	-	-
			Other anion recognition groups		
26a	-	-	-	7	-
26b	-	-	-	8	-
26c	-	-	-	7	-
26d	-	-	-	8	-
COL[b]	*20*	*19*	*20*	-	-

[a)] Measured after incubation of disk (6 mm diameter, 50 μg/disk) at 37 °C for 20 h.

[b)] Tested at 10 μg/disk.

[c)] NT = not tested

Both the norbornane acetals that showed promising results in the disk diffusion assay and the more lipophilic acetals (**8c**, **10c** and **25b**, Table 1) were subject to micro-broth dilution assay to quantify MICs (Table 2). Again the activity of the aminium and guanidinium analogues was comparable. For example, analogues with a hexadecyl chain (diamine **8c** and diguanidine **10c**) showed almost identical MIC values against all bacterial pathogens (e.g. 32 μg/mL against *E.coli* and 0.5 and 1 μg/mL against MRSA respectively, Table 2).

Weak activity in the form of identical MIC values (32 μg/mL against MRSA, Table 2) were observed for both compound **16** (which used a butyl linker to separate the guanidinuim charge from the norbornane scaffold) and **10a** (which employed an ethyl linker). No activity was observed for the corresponding propyl analogue (**15**). These results are supported by the disk diffusion results discussed earlier (Table 1). The importance of the net overall cationic charge was again emphasised with singularly charged norbornane guanidines **25a** and **25b** showing only modest potency; **25a** displayed MIC values of 32 μg/mL against all strains tested apart from MRSA (4 μg/mL), whilst **25b** was only active against MRSA (32 μg/mL, Table 2).

Diamines **8c** (hexadecyl chain) and **8d** (3-[4-(octyloxy)phenyl]propyl chain) both had MIC values of 1 μg/mL against MRSA. Indeed, compound **8d** was active against a variety of isolates tested with MIC values of 2, 16 and 32 μg/mL against *E. coli*, *K. pneumoniae* and *P. aeruginosa* respectively (Table 2).

When a dodecanyl tail was present with two guanidine moieties (**10b**) activity was observed against *E. coli* and MRSA (MIC values of 2 and 4 µg/mL respectively, Table 2). Similarly diguanidine **10d**, which contains both alkyl and aryl portions in its hydrophobic tail, showed activity against all bacterial strains tested; highest activity was exhibited against MRSA (1 µg/mL, Table 2). It is also worth noting that a large calculated LogP (cLogP) range is evident for the active compounds (−3.07–6.56, Table 2), which is in accordance with antibacterial compounds typically showcasing a broader cLogP range when compared to other pharmaceuticals.[21]

Table 2: MIC values (µg/mL) and cLogP values[a]

Compound	*A. baumannii* **ATCC 19606**	*P. aeruginosa* **ATCC 27853**	*K. pneumoniae* **ATCC 700603**	*E. coli* **ATCC 25922**	*S. aureus* **MRSA ATCC 43300**	cLogP
			Amines			
8a	>32	>32	>32	>32	>32	−0.56
8b	>32	**16-32**	>32	**4-8**	**2**	1.46
8c	**32**	>32	>32	**32**	**0.5**	3.48
8d	>32	**16-32**	**16**	**1-2**	**1**	1.82
			Guanidines			
10a	>32	>32	>32	>32	**32**	−1.93
12	>32	>32	>32	>32	**32**	−1.93
10b	>32	**16**	>32	**4**	**2**	0.09
10c	**16-32**	>32	>32	**32**	**1**	2.11
1	**8-16**	>32	>32	**32**	**0.5**	2.11
10d	**32**	**16**	**32**	**4-8**	**1**	−1.39
10e	>32	>32	>32	>32	>32	0.45
15	>32	>32	>32	>32	>32	−0.85
16	>32	>32	>32	>32	**32**	2.04
25a	**32**	**32**	**32**	**32**	**4**	6.56
25b	>32	>32	>32	>32	**32**	−3.07
COL	*0.06*	*0.25*	*0.03*	0.06	>32	

[a)] Calculated using www.molinspiration.com software.

Compounds with MIC values ≤ 2 µg/mL against Gram-positive MRSA (Table 2), were subjected to a second round of micro-broth dilution assay against additional Gram-positive bacterial isolates including: multi-resistant methicillin resistant *S. aureus* (mMRSA), glycopeptide-intermediate *S. aureus* (GISA), Vancomycin-intermediate *S. aureus* (VISA), *Streptococcus pneumoniae* and *Enterococcus faecalis* (Table 3). Compounds with a dodecyl chain attached (diamine **8b** and diguanidine **10b**) exhibited good activity against the Gram-positive bacterial strains shown in Table 3 with MIC values ranging from 2–8 µg/mL. The larger hexadecyl-substituted analogues (diamine **8c** and

diguanidines **10c** and **1**) showed excellent activity with MIC values ≤ 2 μg/mL for all bacterial strains tested in this assay. Of particular note were diamines **8c** and **8d** which showed MIC comparable to, or better than, vancomycin against all strains tested (Table 3).

Table 3: MIC values (μg/mL) and cell line cytotoxicity

	Compound							
	8b	**8c**	**8d**	**10b**	**10c**	**1**	**10d**	**VAN[a]**
S. aureus **mMRSA**	8	2	1	4	2	2	2	1
S. aureus **GISA, NRS 17**	2	0.5	1	2	1	0.5	1	4
S. aureus **VISA, NRS 1**	4	1	1	4	2	1	2	8
S. aureus **MRSA**	8	1	1	4	2	2	2	2
S. pneumoniae **MDR ATCC 700677**	2	0.5	1	2	1	0.5	1	2
E. faecalis ***VanA***	8	0.5	2	4	1	1	2	>32
	CC_{50}							
	8b	**8c**	**8d**	**10b**	**10c**	**1**	**10d**	**TAM[b]**
HEK293 **ATCC CRL-1573**	9	7	12	7	12	6	13	11.1
HepG2 **ATCC HB-8065**	11	6	12	10	11	9	14	18.7

[a)] VAN = vancomycin

[b)] TAM = Tamoxifen

Cytotoxicity against human embryonic kidney cells (HEK293) and hepatocellular carcinoma (HepG2) was also determined (Table 3). In all cases the compounds exhibited some cytotoxicity to both HEK293 and HepG2; however, modest selectivity for bacterial cells in preference to human cells for the compounds presented here is apparent (e.g. for compound **8d** MIC = 1–2 μg/mL, CC_{50} = 12).

Conclusions

Herein, we have described a high-yielding and scalable (to multi-gram amounts) method to access highly functionalised norbornane frameworks. A series of cationic amphiphilic antibacterial agents were synthesised which possess a variety of aminium, guandinium, imidazolium and pyridinium groups.

Detailed SAR indicates that dicationic species were significantly more active than monocationic species, which in turn were more active than charge neutral compounds. The relationship between activity and the cationic group follows the trend: aminium ≈ guanidinium > imidazolium ≈ pyridinium. The nature of the counterion appears to have, at best, a minor influence on activity.

Compounds containing larger hydrophobic groups typically led to increased antibacterial activity. The hexadecyl analogues (diamine **8c** and diguanidine **10c**) and 3-[4-(octyloxy)phenyl]propyl analogues (diamine **8d** and diguanidine **10d**) were the most potent compounds identified in this study with MIC ≤ 2 μg/mL against all Gram-positive bacterial isolates tested.

The results presented here reinforce the notion that the activity of cationic antimicrobial peptides can be mimicked by relatively small, structurally rigid amphiphiles. Indeed, when compared to other synthetic scaffolds (such as calixarenes) which are used to generate antibacterial amphiphiles,[22] they combine a relatively low molecular weight with potent antibacterial activity.

Experimental

The following compounds were prepared using literature methods and full reaction details can be found in the supplementary information; hexadecanal,[23] methyl-3-(4-hydroxyphenyl)propionate,[24] 4-(octyloxy)benzaldehyde,[25] **1**,[9] **3**,[9] **4a**,[9] **5a**,[9] **5c**,[9] **6**,[11] **8a**,[9] **9c**,[9] **11**,[14a] **17**,[14a] **18**,[14a] **19**,[16b] **S1**[26] and **S2**[19]

Methyl-5,6-dihydroxy-*endo*-3-(heptylcarbamoyl)bicyclo[2.2.1]heptane-2-*exo*-carboxylate (21a). To the stirring solution of alkene **20a** (1.12 g, 3.82 mmol), NMO·H_2O (570 mg, 4.20 mmol), and a 1:4 ratio of H_2O/acetone (9.3 mL), was added OsO_4 (490 μL, 0.08 mmol) in one addition and the reaction was stirred at ambient temperature for 3 d. The reaction mix was quenched with sat. $Na_2S_2O_5$ (15 mL), and extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with brine (30 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford a yellow oil (1.06 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, CH_3), 1.25–1.31 (8H, m, 4 × CH_2), 1.36 (1H, d, *J* = 10.9 Hz, H7*s*), 1.47–1.50 (2H, m, $NHCH_2CH_2$), 1.89 (1H, dd, *J* = 10.9, 1.2 Hz, H7*a*), 2.40 (1H, d, *J* = 3.1 Hz, H4), 2.52 (1H, br s, H1), 2.73 (1H, d, *J* = 5.7 Hz, H2), 2.92 (1H, dd, *J* = 5.6, 4.9 Hz, H3), 3.19–3.24 (2H, m, $NHCH_2$), 3.69 (3H, s, OMe), 3.87 (1H, d, *J* = 5.6 Hz, H6), 4.02 (1H, d, *J* = 5.2 Hz, H5), 5.95 (1H, t, *J* = 5.4 Hz, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.7, 27.0, 29.1, 29.7, 31.8, 32.4, 40.0, 45.1, 47.1, 47.7, 48.1, 52.5, 69.8, 73.4, 171.6, 175.0. HRMS (ESI, *m/z*) for $C_{17}H_{29}NO_5$ $[M + H]^+$ calc. 328.2119; found 328.2114.

Methyl-5,6-dihydroxy-*endo*-3-(hexadecylcarbamoyl)bicyclo[2.2.1]heptane-2-*exo*-carboxylate (21b). To the stirring solution of alkene **20b** (1.34 g, 3.18 mmol), NMO·H_2O (475 mg, 3.50 mmol), $CHCl_3$ (10 mL), and a 1:4 ratio of H_2O/acetone (8.0 mL), was added OsO_4 (4% in H_2O, 410 μL, 0.06 mmol) and the reaction was stirred at ambient temperature for 16 h, before being heated for a further

24 h at 50 °C. Further OsO_4 (4% in H_2O, 410 μL, 0.06 mmol) was added and the reaction was stirred for another 48 h before being quenched with sat. $Na_2S_2O_5$ (15 mL), and extracted with $CHCl_3$ (3 × 25 mL). The combined organic phase was washed with brine (25 mL), dried ($MgSO_4$), filtered, and dried *in vacuo* to afford a dark green wax (1.28 g, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, CH_3), 1.25–1.28 (26H, m, 13 × CH_2), 1.37 (1H, d, *J* = 10.9 Hz, H7*s*), 1.47–1.50 (2H, m, $NHCH_2CH_2$), 1.68 (2H, br s, 2 × OH), 1.90 (1H, dd, *J* = 10.9, 1.2 Hz, H7*a*), 2.29 (1H, d, *J* = 3.2 Hz, H4), 2.52 (1H, br s, H1), 2.74 (1H, d, *J* = 5.4 Hz, H2), 2.92 (1H, app. t, *J* = 5.4 Hz, H3), 3.18–3.24 (2H, m, $NHCH_2$), 3.70 (3H, s, OMe), 3.89 (1H, d, *J* = 5.8 Hz, H5), 4.04 (1H, d, *J* = 5.7 Hz, H6), 5.85 (1H, t, *J* = 5.3 Hz, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.8, 27.1, 29.4, 29.5 (2 × C), 29.69 (2 × C), 29.74, 29.80 (2 × C), 29.84 (3 × C), 32.1, 32.4, 40.0, 45.1, 47.1, 47.7, 48.1, 52.4, 69.8, 73.5, 171.5, 175.0. HRMS (ESI, *m/z*) for $C_{26}H_{47}NO_5$ $[M + H]^+$ calc. 454.3520; found 454.3542.

General procedure A: acetal formation

To a stirring suspension of the appropriate diol, $TsOH·H_2O$ (0.05 equiv), $MgSO_4$ (1.0 equiv) and PhMe, was treated with the required aldehyde (1.5 equiv) at 110 °C for 3 h. Solid $MgSO_4$ was removed by filtration and the filtrate was diluted with EtOAc (30 mL), washed with H_2O (2 × 15 mL), brine (15 mL), dried ($MgSO_4$), filtered and concentrated i*n vacuo* to give the crude material which was purified by column chromatography (as specified below) to afford the title compound.

Dimethyl 4-undecanyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxylate (4b). Compound **4b** was prepared from diol **3** (947 mg, 3.87 mmol) and dodecanal (1.3 mL, 5.86 mmol) according to general procedure A and was purified by column chromatography (5–10% EtOAc in pet. spirits) to give the title compound (1.33 g, 83%) as a white waxy solid; R_f = 0.24 (10% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, CH_3), 1.24–1.39 (19H, m, 9 × CH_2, H10*s*), 1.60–1.65 (2H, m, $CHCH_2$), 1.78 (1H, dd, *J* = 10.9, 1.4 Hz, H10*a*), 2.40–2.65 (2H, m, H1, H7), 2.72 (1H, d, *J* = 4.4 Hz, H9), 3.23 (1H, app. t, *J* = 5.1 Hz, H8), 3.70 (6H, s, 2 × OMe), 3.90 (1H, d, *J* = 5.5 Hz, H6), 4.03 (1H, d, *J* = 5.6 Hz, H2), 4.65 (1H, t, *J* = 4.9 Hz, H4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.8, 24.4, 29.5, 29.6, 29.7 (2 × C), 29.8 (2 × C), 31.8, 32.1, 32.9, 43.4, 43.8, 45.1, 45.4, 52.3, 52.5, 78.9, 81.4, 104.3, 172.9, 174.1. HRMS (ESI, *m/z*) for $C_{23}H_{38}O_6$ $[M + H]^+$ calc. 411.2741; found 411.2745.

Dimethyl 4-hexadecyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxylate (4c). Compound **4c** was prepared from diol **3** (239 mg, 0.98 mmol) and hexadecanal **S6** (353 mg, 1.47 mmol) according to general procedure A and was purified by column chromatography (5% EtOAc in pet. spirits) to give the title compound (276 mg, 60%) as a white solid; R_f = 0.31 (10% EtOAc in pet. spirits). m.p: 71.0–73.7 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 6.4 Hz, CH_3), 1.25–1.41 (27H, m, 13 × CH_2, H10*s*), 1.59–1.66 (2H, m, $CHCH_2$), 1.78 (1H, dd, *J* = 10.7, 1.4 Hz, H10*a*), 2.64–2.66 (2H, m, H1, H7), 2.72 (1H, d, *J* = 4.8 Hz, H9), 3.22 (1H, app. t, *J* = 5.0 Hz, H8), 3.70 (6H, s, 2 × OMe), 3.90

(1H, d, *J* = 5.3 Hz, H6), 4.03 (1H, d, *J* = 5.5 Hz, H2), 4.65 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.3, 22.8, 24.4, 29.5, 29.6, 29.7 (2 × C), 29.8 (6 × C), 31.8, 32.1, 32.9, 43.4, 43.8, 45.2, 45.4, 52.3, 52.5, 78.9, 81.4, 104.4, 172.9, 174.1. HRMS (ESI, *m/z*) for $C_{27}H_{46}O_6$ $[M + H]^+$ calc. 467.3367; found 467.3378.

Dimethyl 4-[4′-(octyloxy)phenethyl]-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxylate (4d). Compound **4d** was prepared from diol **3** (275 mg, 1.13 mmol) and aldehyde **S10** (437 mg, 1.67 mmol) according to general procedure A and was purified by column chromatography (5–10–20% EtOAc in pet. spirits) to give the title compound (265 mg, 48%) as a yellow oil; R_f = 0.62 (20% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 7.1 Hz, CH_3), 1.28–1.38 (9H, m, 4 × CH_2, H10*s*), 1.41–1.47 (2H, m, CH_2), 1.73–1.78 (2H, m, CH_2), 1.81 (1H, dd, *J* = 10.8, 1.5 Hz, H10*a*), 1.91–1.95 (2H, m, CH_2), 2.65–2.69 (4H, m, H1, H7, ArCH_2), 2.74 (1H, d, *J* = 4.5 Hz, H9), 3.24 (1H, app. t, *J* = 5.0 Hz, H8), 3.70 (3H, s, Me), 3.71 (3H, s, Me), 3.90–3.93 (3H, m, H6, OCH_2), 4.05 (1H, d, *J* = 5.7 Hz, H2), 4.68 (1H, t, *J* = 4.8 Hz, H4), 6.79–6.82 (2H, m, ArH), 7.07–7.09 (2H, m, ArH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.8, 26.2, 29.4, 29.5 (3 × C), 31.8, 32.0, 34.6, 43.4, 43.8, 45.2, 45.4, 52.3, 52.5, 68.2, 79.0, 81.5, 103.5, 114.6 (2 × C), 129.3 (2 × C), 133.2, 157.6, 172.9, 174.1. HRMS (ESI, *m/z*) for $C_{28}H_{40}O_7$ $[M + K]^+$ calc. 527.2406; found 527.2410.

Dimethyl 4-ethylbenzene-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxylate (4e). Compound **4e** was prepared from diol **3** (1.01 g, 4.14 mmol) and 3-phenylpropionaldehyde (820 µL, 6.21 mmol) according to general procedure A and was purified by column chromatography (10% EtOAc in pet. spirits) to give the title compound (1.18 g, 79%) as a yellow oil; R_f = 0.22 (10% EtOAc in pet. spirits). ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.38 (1H, dt, *J* = 10.8, 1.4 Hz, H10*s*), 1.82 (1H, dd, *J* = 10.8, 1.5 Hz, H10*a*), 1.93–2.01 (2H, m, CHCH_2), 2.65–2.77 (5H, m, H1, H7, H9, ArCH_2), 3.25 (1H, app. t, *J* = 5.1 Hz, H8), 3.71 (3H, s, Me), 3.72 (3H, s, Me), 3.94 (1H, d, *J* = 5.6 Hz, H6), 4.06 (1H, d, *J* = 5.6 Hz, H2), 4.70 (1H, t, *J* = 4.8 Hz, H4), 7.16–7.31 (5H, m, ArH). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 30.4, 31.8, 34.4, 43.4, 43.8, 45.2, 45.4, 52.3, 52.5, 79.0, 81.5, 103.4, 126.1, 128.46 (2 × C), 128.53 (2 × C), 141.4, 172.9, 174.1. HRMS (ESI, *m/z*) for $C_{20}H_{24}O_6$ $[M + H]^+$ calc. 361.1646; found 361.1655.

Methyl 4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-(heptylcarbamoyl)-9-*exo*-carboxylate (22a). Compound **22a** was prepared from diol **21a** (196 mg, 0.60 mmol) and octanal (140 µL, 0.90 mmol) according to general procedure A and was purified by column chromatography (10–20% EtOAc in pet. spirits) to give the title compound (247 mg, 94%) as a clear oil; R_f = 0.45 (20% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85–0.89 (6H, m, 2 × CH_3), 1.27–1.40 (19H, m, 9 × CH_2, H10*s*), 1.47–1.50 (2H, m, $NHCH_2CH_2$), 1.60–1.65 (2H, m, CHCH_2), 1.80 (1H, d, *J* = 10.6 Hz, H10*a*), 2.53 (1H, d, *J* = 4.5 Hz, H7), 2.66 (1H, br s, H1), 2.77 (1H, d, *J* = 5.5 Hz, H9), 2.98 (1H, app. t, *J* = 5.1 Hz, H8), 3.19–3.27 (2H, m, NHCH_2), 3.69 (3H, s, OMe), 4.09 (1H, d, *J* = 5.7 Hz, H6), 4.14 (1H, d, *J* = 5.7 Hz, H2), 4.65 (1H, t, *J* = 4.9 Hz, H4), 5.63 (1H, t, *J* = 5.0 Hz, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ

14.22, 14.23, 22.7, 22.8, 24.4, 27.0, 29.0, 29.3, 29.66, 29.74, 31.8, 31.9, 32.3, 33.0, 40.0, 43.6, 44.1, 45.1, 46.7, 52.5, 78.5, 81.4, 104.1, 170.8, 174.8. HRMS (ESI, *m/z*) for $C_{25}H_{43}NO_5$ $[M + H]^+$ calc. 438.3214; found 438.3205.

Methyl 4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-(hexadecylcarbamoyl)-9-*exo*-carboxylate (22b). Compound **22b** was prepared from diol **21b** (76 mg, 0.17 mmol) and octanal (40 μL, 0.26 mmol) according to general procedure A and was purified by column chromatography (10–20% EtOAc in pet. spirits) to give the title compound (43 mg, 45%) as a clear oil; R_f = 0.47 (20% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85–0.89 (6H, m, 2 × CH_3), 1.25–1.32 (35H, m, 17 × CH_2, H10*s*), 1.35–1.44 (2H, m, CH_2), 1.47–1.50 (2H, m, CH_2), 1.60–1.64 (2H, m, CH_2), 1.80 (1H, dd, *J* = 10.7, 1.3 Hz, H10*a*), 2.52 (1H, d, *J* = 4.4 Hz, H1), 2.65 (1H, br s, H7), 2.76 (1H, dd, *J* = 5.4, 1.1 Hz, H9), 2.98 (1H, app. t, *J* = 5.1 Hz, H8), 3.18–3.27 (2H, m, NHC*H*$_2$), 3.69 (3H, s, OMe), 4.08 (1H, d, *J* = 5.6 Hz, H2), 4.14 (1H, d, *J* = 5.6 Hz, H6), 4.65 (1H, t, *J* = 4.9 Hz, H4), 5.66 (1H, t, *J* = 3.7 Hz, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.2, 14.3, 22.76, 22.83, 24.4, 27.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.67, 29.72, 29.75, 29.80, 29.82, 29.84 (3 × C), 31.9, 32.0, 32.3, 33.0, 40.0, 43.6, 44.1, 45.1, 46.7, 52.4, 78.6, 81.4, 104.1, 170.8, 174.8. HRMS (ESI, *m/z*) calculated for $C_{34}H_{61}NO_5$ $[M + H]^+$ 564.4623; found 564.4631.

General procedure B: hydrolysis of methyl esters

A biphasic solution of methyl ester in 2 M NaOH/THF (1:4) was stirred at ambient temperature for 16 h. The reaction mixture was extracted with CH_2Cl_2 (2 × 8 mL) and the isolated aqueous phase was acidified to pH = 1 using 2 M HCl and extracted with EtOAc (3 × 15 mL). The combined organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford the title compound.

4-Undecanyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxylic acid (5b). The title compound was prepared from diester **4b** (137 mg, 0.33 mmol) according to general procedure B and isolated white powder (105 mg, 83%). m.p: 138.1–139.5 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.8 Hz, CH_3), 1.29–1.42 (19H, m, 9 × CH_2, H10*s*), 1.59–1.63 (2H, m, CHC*H*$_2$), 1.75 (1H, dd, *J* = 10.6, 1.2 Hz, H10*a*), 2.56 (1H, dd, *J* = 5.5, 1.0 Hz, H1), 2.58 (1H, br s, H7), 2.64 (1H, app. t, *J* = 4.4 Hz, H9), 3.17 (1H, app. t, *J* = 5.0 Hz, H8), 3.99 (1H, d, *J* = 5.6 Hz, H6), 4.02 (1H, d, *J* = 5.6 Hz, H2), 4.67 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.5, 23.8, 25.2, 30.5 (2 × C), 30.7 (3 × C), 30.8, 32.4, 33.1, 33.9, 44.4, 44.9, 46.4, 46.6, 80.2, 82.7, 105.2, 175.5, 176.9. HRMS (ESI, *m/z*) for $C_{21}H_{34}O_6$ $[M + Na]^+$ calc. 405.2248; found 405.2254.

4-[4′-(Octyloxy)phenethyl]-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxylic acid (5d). The title compound was prepared from diester **4d** (260 mg, 0.532 mmol) according to general procedure B and isolated as a white solid (197 mg, 80%). m.p: 144.9–147.4 °C. ^{1}H NMR (270 MHz,

DMSO-d_6) δ 0.85 (3H, t, J = 5.9 Hz, CH_3), 1.25–1.41 (11H, m, 5 × CH_2, H10*s*), 1.62–1.72 (3H, m, CH_2, H10*a*), 1.77–1.85 (2H, m, CH_2), 2.42 (1H, d, J = 5.2 Hz, H1), 2.52–2.60 (4H, m, CH_2, H7, H9), 3.02 (1H, app. t, J = 5.2 Hz, H8), 3.89–3.92 (3H, m, OCH_2, H6), 4.00 (1H, d, J = 5.6 Hz, H2), 4.64 (1H, t, J = 4.7 Hz, H4), 6.80 (2H, d, J = 8.5 Hz, ArH), 7.09 (2H, d, J = 8.5 Hz, ArH). ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 14.0, 22.1, 25.6, 28.7 (2 × C), 28.8 (2 × C), 31.2 (2 × C), 34.2, 42.6, 43.3, 44.4, 45.0, 67.3, 78.3, 80.8, 102.5, 114.3 (2 × C), 129.1 (2 × C), 132.9, 156.9, 173.3, 174.6. HRMS (ESI, *m/z*) for $C_{26}H_{36}O_7$ $[M + Na]^+$ calc. 483.2352; found 483.2364.

4-Ethylbenzene-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-carboxylic acid (5e). The title compound was prepared from diester **4e** (1.13 g, 3.12 mmol) according to general procedure B and isolated as a white solid (805 mg, 78%). m.p: 179.7–181.3 °C. ^{1}H NMR (270 MHz, DMSO-d_6) δ 1.24 (1H, d, J = 10.8 Hz, H10*s*), 1.66 (1H, d, J = 9.6 Hz, H10*a*), 1.83–1.90 (2H, m, CHCH_2), 2.42 (1H, d, J = 5.5 Hz, H9), 2.50–2.52 (1H, m, H1), 2.58 (1H, d, J = 4.7 Hz, H7), 2.64–2.69 (2H, m, $ArCH_2$), 3.02 (1H, app. t, J = 3.5 Hz, H8), 3.93 (1H, d, J = 5.6 Hz, H6), 4.01 (1H, d, J = 4.8 Hz, H2), 4.67 (1H, t, J = 4.8 Hz, H4), 7.17–7.30 (5H, m, ArH), 12.59 (2H, br s, 2 × OH). ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 29.7, 31.2, 33.9, 42.6, 43.3, 44.4, 45.0, 78.3, 80.8, 102.5, 125.9, 128.2 (2 × C), 128.3 (2 × C), 141.2, 173.3, 174.6. HRMS (ESI, *m/z*) for $C_{18}H_{20}O_6$ $[M + Na]^+$ calc. 355.1152; found 355.1150.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*(heptylcarbamoyl)-9-*exo*-carboxylic acid (23a). The title compound was prepared from ester **22a** (240 mg, 0.55 mmol) according to general procedure B and isolated as a white waxy solid (148 mg, 64%). m.p: 137.4–139.6 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 0.89–0.91 (6H, m, 2 × CH_3), 1.30–1.41 (19H, m, 9 × CH_2, H10*s*), 1.50–1.53 (2H, m, $NHCH_2CH_2$), 1.58–1.62 (2H, m, CHCH_2), 1.75 (1H, dd, J = 10.5, 1.4 Hz, H10*a*), 2.55 (1H, d, J = 4.1 Hz, H7), 2.59 (1H, br s, H1), 2.68 (1H, d, J = 4.8 Hz, H9), 3.05 (1H, app. t, J = 5.2 Hz, H8), 3.13 (1H, dt, J = 13.3, 7.0 Hz, NHCH_2), 3.25 (1H, dt, J = 13.3, 7.0 Hz, NHCH_2), 4.01 (1H, d, J = 5.6 Hz, H6), 4.03 (1H, d, J = 5.7 Hz, H3), 4.65 (1H, t, J = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 14.5, 23.66, 23.70, 25.2, 27.9, 30.1, 30.3, 30.4, 30.6, 32.9, 33.0, 33.1, 33.9, 40.5, 44.3, 45.4, 46.3, 47.8, 79.9, 82.6, 105.1, 173.4, 177.2. HRMS (ESI, *m/z*) for $C_{24}H_{41}NO_5$ $[M + H]^+$ calc. 424.3058; found 424.3044.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*(hexadecylcarbamoyl)-9-*exo*-carboxylic acid (23b). The title compound was prepared from ester **22b** (41 mg, 0.07 mmol) according to general procedure B and isolated as a brown wax (37 mg, 93%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.85–0.90 (6H, m, 2 × CH_3), 1.25–1.52 (39H, m, 18 × CH_2, H10*s*), 1.59–1.67 (2H, m, C$H$$CH_2$), 1.83 (1H, d, J = 10.1 Hz, H10*a*), 2.54 (1H, d, J = 3.3 Hz, H7), 2.71–2.73 (2H, m, H1, H9), 2.95 (1H, dd, J = 5.5, 4.8 Hz, H8), 3.19–3.28 (2H, m, NHCH_2), 4.07 (1H, d, J = 5.7 Hz, H6), 4.14 (1H, d, J = 5.7 Hz, H2), 4.66 (1H, t, J = 4.8 Hz, H4), 5.85 (1H, t, J = 4.1 Hz, NH). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.2, 14.3, 22.75, 22.82, 24.3, 27.0, 29.3, 29.4, 29.5, 29.6, 29.67 (2 × C), 29.73, 29.80 (2 × C), 29.83, 29.84 (3 × C), 31.9, 32.1,

32.5, 32.9, 40.1, 43.7, 43.9, 44.6, 46.9, 78.5, 81.4, 104.3, 171.3, 177.9. HRMS (ESI, *m/z*) for $C_{33}H_{59}NO_5$ $[M + H]^+$ calc. 550.4466; found 550.4455.

General procedure C: amide formation

A microwave vial was charged with the appropriate carboxylic acid, EDCI (3.0 equiv), HOBt (0.1 equiv) and anhydrous $CHCl_3$ and was stirred at ambient temperature for 30 min. The appropriate alkylamine (3.0 equiv) was then added and the reaction was heated at 50 °C for 30 min using microwave irradiation. The resulting homogenous clear solution was diluted with $CHCl_3$ (20 mL), washed with H_2O (2 × 10 mL), brine (8 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford the crude material that was purified by column chromatography (as specified below) to give the title compound.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylamino)ethylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (7a). Compound **7a** was prepared from diacid **5a** (333 mg, 1.02 mmol) and amine **6** (490 mg, 3.06 mmol) according to general procedure C and after purification by column chromatography (50–70% EtOAc in pet. spirits–EtOAc) was isolated as a white solid (357 mg, 57%); R_f = 0.21 (70% EtOAc in pet. spirits). m.p: 121.7–123.4 °C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, CH_3), 1.25–1.44 (28H, m, 5 × CH_2, *t*-Bu), 1.57–1.65 (3H, m, CHC*H*$_2$, H10*s*), 1.80 (1H, d, *J* = 3.6 Hz, H10*a*), 2.43 (1H, d, *J* = 5.0 Hz, H1), 2.53–2.57 (2H, m, H7, H9), 2.92 (1H, app. t, *J* = 5.1 Hz, H8), 3.26–3.41 (8H, m, 4 × CH_2), 3.96 (1H, d, *J* = 5.8 Hz, H2), 4.13 (1H, d, *J* = 5.5 Hz, H6), 4.64 (1H, t, *J* = 4.8 Hz, H4), 5.01–5.08 (2H, m, 2 × NH), 6.89 (1H, br s, NH), 6.86 (1H, br s, NH). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.2, 22.8, 24.4, 28.5, 29.3, 29.6, 29.8, 31.9, 32.6, 33.0, 40.4, 40.6, 41.2, 43.4 (2 × C), 44.5, 44.7, 47.8, 78.8, 79.8, 80.0, 81.6, 104.2, 156.8, 157.1, 172.5, 174.3. HRMS (ESI, *m/z*) for $C_{31}H_{54}N_4O_8$ $[M + H]^+$ calc. 611.4014; found 611.4031.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylamino)ethylcarbamoyl]-4-undecanyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (7b). Compound **7b** was prepared from diacid **5b** (512 mg, 1.34 mmol) and amine **6** (670 mg, 4.18 mmol) according to general procedure C and after purification by column chromatography (50% EtOAc in pet. spirits–EtOAc) was isolated as a white solid (542 mg, 61%); R_f = 0.43 (EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 7.1 Hz, CH_3), 1.24–1.39 (18H, m, 9 × CH_2), 1.43–1.44 (18H, m, 2 × *t*-Bu), 1.49 (1H, d, *J* = 10.5 Hz, H10*s*), 1.60–1.64 (2H, m, CHC*H*$_2$), 1.81 (1H, d, *J* = 9.9 Hz, H10*a*), 2.40 (1H, d, *J* = 5.6 Hz, H1), 2.54 (1H, d, *J* = 3.9 Hz, H7), 2.59 (1H, br s, H9), 2.88 (1H, dd, *J* = 5.8, 4.6 Hz, H8), 3.22–3.44 (8H, m, 4 × CH_2), 3.96 (1H, d, *J* = 5.6 Hz, H2), 4.13 (1H, d, *J* = 5.6 Hz, H6), 4.64 (1H, t, *J* = 4.8 Hz, H4), 4.92–5.02 (2H, m, 2 × NH), 6.63 (1H, br s, NH), 6.77 (1H, br s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.8, 24.4, 28.5, 29.5, 29.67, 29.68, 29.71, 29.76, 29.79, 32.1, 32.6, 33.0, 40.4, 40.5, 40.7, 41.2, 43.3, 44.4, 44.5, 48.0, 78.7, 79.9, 80.1, 81.6, 104.2, 156.8, 157.1, 172.5, 174.3. HRMS (ESI, *m/z*) for $C_{35}H_{62}N_4O_8$ $[M + H]^+$ calc. 667.4640; found 667.4656.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylamino)ethylcarbamoyl]-4-hexapentyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (7c). Compound **7c** was prepared from diacid **5c** (100 mg, 0.23 mmol) and amine **6** (110 mg, 0.68 mmol) according to general procedure C and after purification by column chromatography (50–70% EtOAc in pet. spirits) was isolated as clear oil (76 mg, 46%); R_f = 0.07 (70% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, CH_3), 1.24–1.39 (27H, m, 13 × CH_2, H10*s*), 1.43–1.44 (18H, m, 2 × *t*-Bu), 1.59–1.64 (2H, m, CHCH_2), 1.81 (1H, d, *J* = 9.8 Hz, H10*a*), 2.42 (1H, d, *J* = 5.7 Hz, H1), 2.54 (1H, d, *J* = 3.7 Hz, H7), 2.57 (1H, br s, H9), 2.91 (1H, app. t, *J* = 4.9 Hz, H8), 3.22–3.43 (8H, m, 4 × CH_2), 3.96 (1H, d, *J* = 5.5 Hz, H2), 4.13 (1H, d, *J* = 4.8 Hz, H6), 4.64 (1H, t, *J* = 4.9 Hz, H4), 5.01–5.08 (2H, m, 2 × NH), 6.69 (1H, br s, NH), 6.84 (1H, br s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.8, 24.4, 28.5, 29.5, 29.67, 29.69, 29.72, 29.80 (2 × C), 29.81 (2 × C), 29.84 (2 × C), 32.1, 32.6, 33.0, 34.0, 40.5, 40.6, 41.1, 43.4, 44.5, 44.6, 47.8, 78.7, 79.8, 80.0, 81.6, 104.2, 156.8, 157.1, 172.5, 174.3. HRMS (ESI, *m/z*) for $C_{39}H_{70}N_4O_8$ $[M + H]^+$ calc. 723.5266; found 723.5263.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylamino)ethylcarbamoyl]-4-[4′-(octyloxy)phenethyl]-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (7d). Compound **7d** was prepared from diacid **5d** (51 mg, 0.11 mmol) and amine **6** (78 mg, 0.49 mmol) according to general procedure C and after purification by column chromatography (50% EtOAc in CH_2Cl_2–EtOAc) was isolated as a clear oil (29 mg, 35%); R_f = 0.34 (EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 6.9 Hz, CH_3), 1.25–1.36 (8H, m, 4 × CH_2), 1.42–1.43 (20H, m, CH_2, *t*-Bu), 1.51 (1H, d, *J* = 10.1 Hz, H10*s*), 1.72–1.77 (2H, m, CH_2), 1.81 (1H, d, *J* = 10.0 Hz, H10*a*), 1.88–1.92 (2H, m, CH_2), 2.51 (1H, d, *J* = 5.4 Hz, H1), 2.56 (1H, s, H7), 2.59 (1H, d, *J* = 3.7 Hz, H9), 2.65 (2H, t, *J* = 8.1 Hz, ArCH_2), 3.05 (1H, app. t, *J* = 4.7 Hz, H8), 3.25–3.34 (8H, m, 4 × CH_2), 3.91 (2H, t, *J* = 6.6 Hz, OCH_2), 4.00 (1H, d, *J* = 5.5 Hz, H6), 4.13 (1H, d, *J* = 5.3 Hz, H2), 4.66 (1H, t, *J* = 4.7 Hz, H4), 5.27 (2H, br s, 2 × NH), 6.79 (2H, d, *J* = 8.6 Hz, ArH), 6.97 (1H, br s, NH), 7.06 (2H, d, *J* = 8.6 Hz, ArH), 7.20 (1H, br s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.8, 26.2, 28.5, 29.4 (2 × C), 29.5, 29.8, 31.9, 32.4, 34.8, 40.4 (2 × C), 40.8 (2 × C), 43.7, 44.4, 45.5, 47.1, 68.1, 78.9, 79.7, 79.9, 81.7, 103.2, 114.5 (2 × C), 129.2 (2 × C), 133.4, 156.8, 157.0, 157.5, 172.5, 174.4. HRMS (ESI, *m/z*) for $C_{40}H_{64}N_4O_9$ $[M + H]^+$ calc. 745.4746; found 745.4768.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylguanidino)propylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (13). Compound **13** was prepared from diacid **5a** (170 mg, 0.52 mmol) and amine **17** (968 mg, 3.06 mmol) according to general procedure C and after purification by column chromatography (20–50% EtOAc in CH_2Cl_2) was isolated as a white residue (234 mg, 49%); R_f = 0.41 (50% EtOAc in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, t, *J* = 6.5 Hz, CH_3), 1.23–1.27 (8H, m, 4 × CH_2), 1.36–1.41 (2H, m, CH_2), 1.49–1.51 (36H, m, 4 × *t*-Bu), 1.57 (1H, d, *J* = 10.4 Hz, H10*s*), 1.60–1.72 (6H, m, 3 × CH_2), 1.77 (1H, d, *J* = 9.9 Hz, H10*a*), 2.54 (1H, br s, H7), 2.56 (1H, d, *J* = 5.3 Hz, H1), 2.76 (1H, d, *J* = 4.2 Hz, H9), 3.05–3.19 (3H, m, CH_2, H8), 3.29–3.42 (4H, m, 2 × CH_2), 3.47–3.63 (2H, m, CH_2), 4.03 (1H, d, *J* = 5.7 Hz, H2), 4.05 (1H, d, *J* = 5.7 Hz, H6), 4.63 (1H, t, *J* = 4.8 Hz,

H4), 6.85 (1H, t, *J* = 5.1 Hz, NH), 7.62 (1H, t, *J* = 5.7 Hz, NH), 8.45 (2H, br s, 2 × NH), 11.46 (1H, s, NH), 11.49 (1H, s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.7, 24.3, 28.2, 28.4, 28.5, 29.3, 29.7, 29.8, 30.0, 31.9, 32.3, 33.0, 35.5, 36.5, 37.4, 38.0, 43.6, 44.1, 45.5, 47.3, 79.0, 79.9, 81.8, 83.5, 83.6, 104.0, 153.29, 153.34, 156.7, 157.2, 163.2 (2 × C), 171.8, 174.0. HRMS (ESI, *m/z*) for $C_{45}H_{78}N_8O_{12}$ [M + H]$^+$ calc. 923.5812; found 923.5822.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylguanidino)butylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (14). Compound **14** was prepared from diacid **5a** (251 mg, 0.77 mmol) and amine **18** (1.03 g, 3.12 mmol) according to general procedure C and after purification by column chromatography (20–50% EtOAc in CH_2Cl_2) was isolated as a white residue (382 mg, 52%); R_f = 0.48 (50% EtOAc in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, t, *J* = 6.8 Hz, CH_3), 1.25–1.28 (10H, m, 5 × CH_2), 1.37–1.63 (47H, m, 4 × *t*-Bu, 5 × CH_2, H10*s*), 1.80 (1H, d, *J* = 9.7 Hz, H10*a*), 2.43 (1H, d, *J* = 5.6 Hz, H9), 2.56–2.57 (2H, m, H1, H7), 2.93 (1H, dd, *J* = 5.7, 4.7 Hz, H8), 3.19–3.47 (8H, m, 4 × CH_2), 3.98 (1H, d, *J* = 5.6 Hz, H2), 4.09 (1H, d, *J* = 5.6 Hz, H6), 4.64 (1H, t, *J* = 4.8 Hz, H4), 6.25–6.44 (2H, m, 2 × NH), 8.38–8.43 (2H, m, 2 × NH), 11.48 (1H, s, NH), 11.50 (1H, s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.8, 24.4, 26.5, 26.8, 26.9, 27.0, 28.2, 28.41, 28.42, 29.3, 29.7, 31.9, 32.6, 33.0, 39.3, 39.5, 40.7, 40.8, 43.4, 44.4, 44.6, 47.9, 78.8, 79.9, 80.1, 81.7, 83.5, 83.6, 104.2, 153.4 (2 × C), 156.2 (2 × C), 163.2 (2 × C), 171.9, 173.6. HRMS (ESI, *m/z*) for $C_{47}H_{82}N_8O_{12}$ [M + H]$^+$ calc. 951.6125; found 951.6137.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]-4-ethylbenzene-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (9e). Compound **9e** was prepared from diacid **5e** (203 mg, 0.61 mmol) and amine **11** (550 mg, 1.83 mmol) according to general procedure C and after purification by flash column chromatography (50% EtOAc in pet. spirits–EtOAc) was isolated as a white waxy solid (242 mg, 44%); R_f = 0.39 (70% EtOAc in pet. spirits).

^{1}H NMR (270 MHz, $CDCl_3$) δ 1.49–1.51 (37H, m, *t*-Bu, H10*s*), 1.82 (1H, d, *J* = 10.6 Hz, H10*a*), 1.90–1.98 (2H, m, CH_2), 2.46 (1H, d, *J* = 6.2 Hz, H1), 2.61 (1H, br s, H7), 2.70–2.76 (3H, m, $ArCH_2$, H9), 2.96 (1H, app. t, *J* = 5.9 Hz, H8), 3.35–3.62 (8H, m, 4 × CH_2), 3.98 (1H, d, *J* = 5.6 Hz, H2), 4.07 (1H, d, *J* = 5.3 Hz, H6), 4.66 (1H, t, *J* = 4.7 Hz, H4), 6.88 (1H, t, *J* = 5.3 Hz, NH), 7.16–7.30 (5H, m, ArH), 8.05 (1H, t, *J* = 3.1 Hz, NH), 8.51 (1H, t, *J* = 5.9 Hz, NH), 8.65 (1H, t, *J* = 5.7 Hz, NH), 11.47 (1H, s, NH), 11.48 (1H, s, NH). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 28.2, 28.4, 30.4, 32.5, 34.5, 40.0, 40.2 (2 × C), 42.3, 43.0, 44.3, 44.4, 47.8, 79.2, 79.7, 79.9, 81.8, 83.4, 83.7, 103.2, 126.1, 128.5 (4 × C), 141.6, 153.2 (2 × C), 157.1, 158.0, 163.0, 163.5, 172.0, 174.8. HRMS (ESI, *m/z*) for $C_{44}H_{67}N_8O_{12}$ [M + H]$^+$ calc. 901.5030; found 901.5052.

Methyl *endo*-3-(heptylcarbamoyl)bicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (20a). Compound **20a** was prepared from acid **19** (533 mg, 2.72 mmol) and *n*-heptylamine (620 μL, 4.07 mmol) according to general procedure C and after purification by column chromatography (20% EtOAc in CH_2Cl_2) was

isolated as a clear oil (615 mg, 77%); R_f = 0.32 (20% EtOAc in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.9 Hz, CH_3), 1.26–1.30 (8H, m, 4 × CH_2), 1.44–1.48 (3H, m, NHCH$_2$C*H*$_2$, H7*s*), 1.55 (1H, d, *J* = 8.7 Hz, H7*a*), 2.58 (1H, dd, *J* = 5.0, 1.8 Hz, H2), 3.10–3.25 (5H, m, NHC*H*$_2$, H1, H4, H3), 3.72 (3H, s, Me), 5.80 (1H, t, *J* = 6.0 Hz, NH), 6.17 (1H, dd, *J* = 5.7, 3.0 Hz, H6), 6.23 (1H, dd, *J* = 5.6, 3.4 Hz, H5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.7, 27.0, 29.1, 29.8, 31.9, 39.7, 45.7, 46.9, 47.8, 48.2, 49.8, 52.3, 135.7, 136.6, 172.5, 175.7. HRMS (ESI, *m/z*) for $C_{17}H_{27}NO_3$ $[M + H]^+$ calc. 294.2064; found 294.2056.

Methyl *endo*-3-(hexadecylcarbamoyl)bicyclo[2.2.1]hept-5-ene-2-*exo*-carboxylate (20b). Compound **20b** was prepared from acid **19** (511 mg, 2.60 mmol) and *n*-hexadecylamine (950 mg, 3.90 mmol) according to general procedure C and after purification by column chromatography (5% EtOAc in CH_2Cl_2) was isolated as a clear oil (793 mg, 73%); R_f = 0.41 (20% EtOAc in CH_2Cl_2). m.p: 83.5–84.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.25–1.29 (26H, m, 13 × CH_2), 1.44–1.49 (3H, m, NHCH$_2$C*H*$_2$, H7*s*), 1.55 (1H, d, *J* = 8.7 Hz, H7*a*), 2.58 (1H, dd, *J* = 5.0, 1.7 Hz, H2), 3.10–3.26 (5H, m, NHC*H*$_2$, H1, H3, H4), 3.73 (3H, s, Me), 5.79 (1H, t, *J* = 5.5 Hz, NH), 6.17 (1H, dd, *J* = 5.5, 2.8 Hz, H6), 6.24 (1H, dd, *J* = 5.6, 3.5 Hz, H5). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.8, 27.0, 29.4, 29.5, 29.69, 29.72, 29.79 (2 × C), 29.81 (2 × C), 29.83 (3 × C), 32.1, 39.7, 45.7, 46.9, 47.8, 48.2, 49.8, 52.3, 135.7, 136.6, 172.5, 175.7. HRMS (ESI, *m/z*) for $C_{26}H_{45}NO_3$ $[M + H]^+$ calc. 420.3472; found 420.3483.

9-*exo*-[2′-(2″,3″-Bis-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]-8-*endo*-(hexadecylcarbamoyl)-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (24b). Compound **24b** was prepared from acid **23b** (20 mg, 0.04 mmol) and amine **11** (21 mg, 0.07 mmol) according to general procedure C and after purification by column chromatography (10–20% EtOAc in CH_2Cl_2) was isolated as a pale yellow oil (22 mg, 79%); R_f = 0.32 (20% EtOAc in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85–0.88 (6H, m, 2 × CH_3), 1.24–1.50 (57H, m, 19 × CH_2, 2 × *t*-Bu, H10*s*), 1.59–1.63 (2H, m, CH_2), 1.78 (1H, d, *J* = 9.6 Hz, H10*a*), 2.39 (1H, d, *J* = 5.6 Hz, H9), 2.52 (1H, d, *J* = 4.1 Hz, H1), 2.56 (1H, br s, H7), 2.94 (1H, dd, *J* = 5.8, 4.6 Hz, H8), 3.13–3.27 (2H, m, CH_2), 3.40 (2H, dt, *J* = 5.7, 5.3 Hz, CH_2), 3.51–3.64 (2H, m, CH_2), 3.98 (1H, d, *J* = 5.7 Hz, H2), 4.19 (1H, d, *J* = 5.7 Hz, H6), 4.63 (1H, t, *J* = 4.9 Hz, H4), 6.19 (1H, t, *J* = 5.6 Hz, NH), 7.06 (1H, t, *J* = 4.9 Hz, NH), 8.54 (1H, t, *J* = 5.7 Hz, NH), 11.49 (1H, s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 14.3, 22.75, 22.83, 24.4, 27.1, 28.2, 28.4, 29.2, 29.3, 29.4, 29.5, 29.67, 29.68, 29.73, 29.80, 29.83 (3 × C), 31.9 (2 × C), 32.1 (2 × C), 32.3, 33.0, 39.9, 40.0, 40.7, 43.3, 44.5, 44.9, 47.4, 78.7, 79.8, 81.6, 83.6, 104.1, 153.2, 157.3, 163.4, 171.7, 174.1. HRMS (ESI, *m/z*) for $C_{46}H_{83}N_5O_8$ $[M + Na]^+$ calc. 856.6134; found 856.6134.

General procedure D: amidation of diacids

A microwave vial was charged with the appropriate carboxylic acid, EDCI (1.5 equiv), HOBt (0.05 equiv) and dry DMF and was stirred at ambient temperature for 30 min. The appropriate alkylamine

(1.5 equiv) was then added and the reaction was irradiated to 50 °C for 30 min. The resulting homogenous mixture was diluted with EtOAc (15 mL), washed with H_2O (3 × 8 mL), brine (8 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford a solid that was purified by column chromatography (as specified below) which gave the title compound.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (9a). Compound **9a** was prepared from diacid **5a** (156 mg, 0.48 mmol) and amine **11** (434 mg, 1.43 mmol) according to general procedure D and after purification by column chromatography (20–70% EtOAc in pet. spirits) was isolated as a white solid (274 mg, 64%); R_f = 0.38 (70% EtOAc in pet. spirits). m.p: 102.9–127.5 °C (slow decomposition). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.86 (3H, t, *J* = 6.7 Hz, CH_3), 1.25 (12H, br s, 6 × CH_2), 1.48–1.63 (37H, m, *t*-Bu, H10*s*), 1.72–1.83 (1H, m, H10*a*), 2.44 (1H, d, *J* = 5.9 Hz, H1), 2.57 (1H, br s, H7), 2.70 (1H, d, *J* = 3.8 Hz, H9), 2.94 (1H, app. t, *J* = 4.5 Hz, H8), 3.31–3.40 (4H, m, 2 × CH_2), 3.51–3.61 (4H, m, 2 × CH_2), 3.95 (1H, d, *J* = 5.5 Hz, H2), 4.03 (1H, d, *J* = 5.5 Hz, H6), 4.60 (1H, t, *J* = 4.8 Hz, H4), 6.87 (1H, t, *J* = 5.4 Hz, NH), 8.04 (1H, t, *J* = 4.1 Hz, NH), 8.51 (1H, t, *J* = 5.3 Hz, NH), 8.64 (1H, t, *J* = 5.7 Hz, NH), 11.45 (1H, s, NH), 11.48 (1H, s, NH). ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 14.2, 22.8, 24.3, 28.2, 28.4, 29.3, 29.7, 31.9, 32.5, 33.0, 40.0, 40.1, 40.2, 42.3, 43.0, 44.3, 44.4, 47.8, 79.0, 79.6, 79.9, 81.7, 83.4, 83.7, 104.1, 153.2 (2 × C), 157.1, 157.9, 163.0, 163.5, 172.0, 174.3. HRMS (ESI, *m/z*) for $C_{43}H_{74}N_8O_{12}$ $[M + H]^+$ calc. 895.5499; found 895.5511.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]-4-undecanyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (9b). Compound **9b** was prepared from diacid **5b** (95 mg, 0.25 mmol) and amine **11** (230 mg, 0.78 mmol) according to general procedure D and after purification by column chromatography (50–70% EtOAc in pet. spirits) was isolated as a clear oil (116 mg, 49%); R_f = 0.50 (70% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.2 Hz, CH_3), 1.24–1.37 (22H, m, 11 × CH_2), 1.49–1.50 (37H, m, *t*-Bu, H10*s*), 1.57–1.62 (2H, m, CHCH_2), 1.77 (1H, d, *J* = 10.4 Hz, H10*a*), 2.45 (1H, d, *J* = 5.8 Hz, H1), 2.58 (1H, br s, H7), 2.69 (1H, d, *J* = 4.1 Hz, H9), 2.94 (1H, app. t, *J* = 5.2 Hz, H8), 3.34–3.44 (4H, m, 2 × CH_2), 3.55–3.59 (4H, m, 2 × CH_2), 3.95 (1H, d, *J* = 5.7 Hz, H2), 4.02 (1H, d, *J* = 5.7 Hz, H6), 4.60 (1H, t, *J* = 4.9 H4), 6.88 (1H, br s, NH), 8.04 (1H, t, *J* = 4.0 Hz, NH), 8.55 (1H, br s, NH), 8.65 (1H, br s, NH), 11.45 (1H, s, NH), 11.48 (1H, s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3, 22.8, 24.4, 28.18, 28.19, 28.4, 29.5 (2 × C), 29.66 (3 × C), 29.72, 29.75, 29.8, 32.0, 32.5, 33.0, 40.0 (2 × C), 40.3, 42.2, 43.0, 44.2, 44.3, 47.8, 79.0, 79.8, 80.1, 81.6, 83.5, 83.8, 104.1, 153.17, 153.19, 157.0, 157.8, 162.7, 163.2, 172.0, 174.2. HRMS (ESI, *m/z*) for $C_{47}H_{82}N_8O_{12}$ $[M + H]^+$ calc. 951.6125; found 951.6131.

8-*endo*-9-*exo*-Di[2′-(2″,3″-bis-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]-4-[4′-(octyloxy)phenethyl]-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (9d). Compound **9d** was prepared from diacid **5d** (89 mg, 0.19 mmol) and amine **11** (180 mg, 0.57 mmol) according to general procedure D

and after purification by column chromatography (50–70% EtOAc in pet. spirits) was isolated as a clear oil (81 mg, 41%); R_f = 0.38 (70% EtOAc in pet. spirits). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 6.4 Hz, CH_3), 1.25–1.36 (8H, m, 4 × CH_2), 1.40–1.54 (39H, m, CH_2, 4 × *t*-Bu, H10*s*), 1.73–1.78 (2H, m, CH_2), 1.81 (1H, d, *J* = 10.2 Hz, H10*a*), 1.87–1.92 (2H, m, CH_2), 2.46 (1H, d, *J* = 5.5 Hz, H1), 2.60 (1H, s, H7), 2.64–2.68 (2H, m, $ArCH_2$), 2.74 (1H, d, *J* = 4.2 Hz, H9), 2.96 (1H, app. t, *J* = 5.4 Hz, H8), 3.35–3.45 (4H, m, 2 × CH_2), 3.55–3.61 (4H, m, 2 × CH_2), 3.91 (2H, t, *J* = 6.6 Hz, OCH_2), 3.98 (1H, d, *J* = 5.6 Hz, H6), 4.06 (1H, d, *J* = 5.7 Hz, H2), 4.64 (1H, t, *J* = 4.6 Hz, H4), 6.80 (2H, d, *J* = 8.5 Hz, ArH), 6.90 (1H, br s, NH), 7.07 (2H, d, *J* = 8.5 Hz, ArH), 8.03 (1H, t, *J* = 4.2 Hz, NH), 8.53 (1H, br s, NH), 8.65 (1H, t, *J* = 4.1 Hz, NH), 11.47–11.48 (2H, m, 2 × NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.8, 26.2, 28.2, 28.4, 29.4, 29.5 (3 × C), 32.0, 32.5, 34.8, 40.1 (2 × C), 40.3, 42.2, 43.0, 44.3, 44.4, 47.8, 68.2, 79.1, 79.9, 80.1, 81.8, 83.5, 83.8, 103.3, 114.6 (2 × C), 129.3 (2 × C), 133.5, 153.2 (2 × C), 157.0, 157.5, 157.9, 162.9, 163.3, 172.0, 174.1. HRMS (ESI, *m/z*) for $C_{52}H_{84}N_8O_{13}$ $[M + H]^+$ calc. 1029.6231; found 1029.6253.

9-*exo*-[2′-(2″,3″-Bis-*tert*-butoxycarbonylguanidino)ethylcarbamoyl]-8-*endo*-(heptylcarbamoyl)-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (24a). Compound **24a** was prepared from acid **23a** (43 mg, 0.10 mmol) and amine **11** (50 mg, 0.17 mmol) according to general procedure D and after purification by column chromatography (10–50% EtOAc in CH_2Cl_2) was isolated as a clear oil (21 mg, 30%); R_f = 0.48 (50% EtOAc in CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (6H, m, 2 × CH_3), 1.25–1.50 (39H, m, 10 × CH_2, 2 × *t*-Bu, H10*s*), 1.59–1.64 (2H, m, CH_2), 1.78–1.80 (1H, m, H10*a*), 2.39 (1H, d, *J* = 5.3 Hz, H9), 2.52 (1H, d, *J* = 4.1 Hz, H1), 2.56 (1H, br s, H7), 2.94 (1H, dd, *J* = 5.9, 4.6 Hz, H8), 3.15–3.26 (2H, m, CH_2), 3.40 (2H, dt, *J* = 8.3, 5.5 Hz, CH_2), 3.50–3.62 (2H, m, CH_2), 3.98 (1H, d, *J* = 5.6 Hz, H2), 4.19 (1H, d, *J* = 5.6 Hz, H6), 4.63 (1H, t, *J* = 4.9 Hz, H4), 6.17 (1H, t, *J* = 5.6 Hz, NH), 7.04 (1H, t, *J* = 4.9 Hz, NH), 8.53 (1H, t, *J* = 5.8 Hz, NH), 11.48 (1H, s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.20, 14.21, 22.7, 22.8, 24.4, 27.0, 28.2, 28.4, 29.0, 29.3, 29.6, 29.7, 31.8, 31.9, 32.3, 34.0, 39.9, 40.0, 40.7, 43.3, 44.5, 44.9, 47.5, 78.8, 79.8, 81.6, 83.6, 104.2, 153.3, 157.3, 163.4, 171.7, 174.1. HRMS (ESI, *m/z*) for $C_{37}H_{65}N_5O_8$ $[M + H]^+$ calc. 708.4906; found 708.4921.

General procedure E: deprotection of Boc groups

To a stirring solution of Boc-protected amine/guanidine and MeOH was added dropwise AcCl (10.0 equiv), and the reaction was stirred for 24 h at ambient temperature (in instances when ^{1}H NMR spectroscopy indicated the presence of Boc-groups the crude material was retreated using the aforementioned conditions). The reaction mixture was concentrated under vacuum and co-evaporated with MeOH (2 × 0.5 mL), to afford the title compound.

4-Undecanyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylamine hydrogen chloride (8b). Compound **8b** was synthesised from Boc-protected amine **7b** (459 mg, 0.69 mmol) according to general procedure E as a white solid (364 mg, 98%). m.p: 175.3–233.8 °C (slow decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (3H, t, J = 6.6 Hz, CH_3), 1.23–1.30 (19H, m, 9 × CH_2, H10*s*), 1.49–1.53 (3H, m, CHCH_2, H10*a*), 2.41 (1H, br s, H1), 2.50 (1H, m, H7), 2.61 (1H, d, J = 4.4 Hz, H9), 2.81–2.88 (4H, m, 2 × CH_2), 3.10 (1H, app. t, J = 4.9 Hz, H8), 3.21–3.36 (4H, m, 2 × CH_2), 3.85 (1H, d, J = 5.6 Hz, H6), 3.91 (1H, d, J = 5.6 Hz, H2), 4.59 (1H, t, J = 4.7 Hz, H4), 8.01 (6H, br s, 2 × NH_3), 8.24 (1H, t, J = 5.4 Hz, NH), 8.35 (1H, t, J = 5.1 Hz, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.0, 22.2, 23.8, 28.8, 28.99 (3 × C), 29.04, 29.1, 31.1, 31.3, 32.4, 36.7, 36.8, 38.48, 38.52, 42.8, 43.2, 44.5, 46.1, 78.3, 81.1, 103.0, 171.6, 173.5. HRMS (ESI, *m/z*) for $C_{25}H_{46}N_4O_4$ $[M + 2H]^{2+}$ calc. 234.1823; found 234.1826.

4-Pentadecyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylamine hydrogen chloride (8c). Compound **8c** was synthesised from Boc-protected amine **7c** (76 mg, 0.11 mmol) according to general procedure E as a white powder (58 mg, 94%). m.p: 156.3–200.1 °C (slow decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (3H, t, J = 7.1 Hz, CH_3), 1.23–1.31 (27H, m, 13 × CH_2, H10*s*), 1.49–1.53 (3H, m, CHCH_2, H10*a*), 2.41 (1H, br s, H9), 2.49–2.51 (1H, m, H7), 2.60 (1H, d, J = 4.4 Hz, H1), 2.81–2.86 (4H, m, 2 × CH_2), 3.10 (1H, app. t, J = 4.9 Hz, H8), 3.19–3.32 (4H, m, 2 × CH_2), 3.86 (1H, d, J = 5.6 Hz, H6), 3.91 (1H, d, J = 7.8 Hz, H2), 4.59 (1H, t, J = 4.7 Hz, H4), 7.96 (6H, br s, 2 × NH_3), 8.23 (1H, t, J = 5.4 Hz, NH), 8.33 (1H, t, J = 5.5 Hz, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.0, 22.1, 23.8, 28.7, 28.99 (3 × C), 29.04 (3 × C), 29.07 (3 × C), 31.1, 31.3, 32.4, 36.7, 36.8, 38.4, 38.5, 42.8, 43.1, 44.5, 46.1, 78.2, 81.0, 102.9, 171.5, 173.5. HRMS (ESI, *m/z*) for $C_{29}H_{54}N_4O_4$ $[M + 2H]^{2+}$ calc. 262.2145; found 262.2150.

4-[4′-(Octyloxy)phenethyl]-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylamine hydrogen chloride (8d). Compound **8d** was synthesised from Boc-protected amine **7d** (28 mg, 0.04 mmol) according to general procedure E as a white solid (23 mg, 99%). m.p: 159.7–197.8 °C (slow decomposition). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, J = 3.8 Hz, CH_3), 1.29–1.34 (8H, m, 4 × CH_2) 1.47–1.49 (3H, m, CH_2, H10*s*), 1.73–1.79 (3H, m, CH_2, H10*a*), 1.87–1.88 (2H, m, CH_2), 2.53 (1H, br s, H1), 2.64–2.67 (4H, m, ArCH_2, H9, H7), 3.06–3.09 (4H, m, 2 × CH_2), 3.24 (1H, app. t, J = 5.0 Hz, H8), 3.37–3.40 (2H, m, CH_2), 3.50–3.61 (2H, m, CH_2), 3.90–3.93 (2H, m, OCH_2), 4.02–4.03 (1H, m, H6), 4.06 (1H, m, H2), 4.67 (1H, t, J = 4.4 Hz, H4), 6.79–6.81 (2H, m, ArH), 7.05–7.08 (2H, m, ArH). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 23.7, 27.2, 30.4, 30.5 (2 × C), 32.8, 33.0, 36.0, 38.5 (2 × C), 40.9, 45.0, 45.1, 47.1, 47.3, 69.0, 80.1, 83.0, 104.3, 115.5 (2 × C), 130.2 (2 × C), 134.6, 158.9, 175.1, 177.0. HRMS (ESI, *m/z*) for $C_{30}H_{48}N_4O_5$ $[M + 2H]^{2+}$ calc. 273.1855; found 273.1893.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylguanidine hydrogen chloride (10a). Compound **10a** was synthesised from Boc-protected guanidine **9a** (156 mg, 0.17 mmol) according to general procedure E as a white solid (94 mg, 95%). ^{1}H NMR (270 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.5 Hz, CH_3), 1.29 (10H, m, 5 × CH_2), 1.45–1.61 (3H, m, CHC*H*$_2$, H10*s*), 1.74 (1H, d, *J* = 9.6 Hz, H10*a*), 2.45 (1H, br s, H9), 2.61–2.63 (2H, m, H1, H7), 3.22 (1H, app. t, *J* = 4.9 Hz, H8), 3.30–3.39 (8H, m, 4 × CH_2), 4.00 (1H, d, *J* = 5.6 Hz, H6), 4.05 (1H, d, *J* = 5.5 Hz, H2), 4.66 (1H, t, *J* = 4.7 Hz, H4), 7.43–7.46 (1H, m, NH). ^{13}C NMR (67.5 MHz, CD_3OD) δ 14.4, 23.7, 25.2, 28.2, 30.3, 30.6, 32.7, 32.9, 33.9, 39.6, 39.7, 41.9, 44.9, 45.1, 46.9, 47.7, 80.0, 82.9, 105.1, 153.6, 158.9, 174.7, 176.6. HRMS (ESI, *m/z*) for $C_{23}H_{42}N_8O_4$ $[M + 2H]^{2+}$ calc. 248.1737; found 248.1744.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylguanidine hydrogen 2,2,2-trifluoroacetate (12). To the stirring solution of Boc-protected guanidine **9a** (112 mg, 0.125 mmol) in CH_2Cl_2 (2.5 mL) was added trifluoroacetic acid (700 µL, 9.1 mmol) and the reaction was stirred at ambient temperature for 24 h. The solvent was removed under reduced pressure and the sample was co-evaporated with $CHCl_3$ (2 × 1 mL) and concentrated *in vacuo* to give the title compound (54 mg, 60%) as a yellow resin. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 0.85 (3H, t, *J* = 6.8 Hz, CH_3), 1.23–1.31 (11H, m, 5 × CH_2, H10*s*), 1.49–1.54 (3H, m, CHC*H*$_2$, H10*a*), 2.34 (1H, br s, H9), 2.53 (1H, d, *J* = 4.4 Hz, H7), 3.04–3.28 (10H, m, 4 × CH_2, H1, H8), 3.88 (1H, d, *J* = 5.6 Hz, H6), 3.90 (1H, d, *J* = 5.6 Hz, H2), 4.59 (1H, t, *J* = 4.8 H4), 7.21 (6H, br s, NH), 7.56–7.59 (2H, m, NH), 8.13 (1H, t, *J* = 5.7 Hz, NH), 8.20 (1H, t, *J* = 5.1 Hz, NH). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 13.6, 21.7, 23.4, 28.3, 28.6, 30.7, 32.0, 37.7, 37.8, 40.0, 40.1, 40.2, 42.4, 42.9, 44.0, 46.0, 77.8, 78.8, 102.6, 156.6 (2 × C), 171.3, 173.1. HRMS (ESI, *m/z*) for $C_{23}H_{42}N_8O_4$ $[M + 2H]^{2+}$ calc. 248.1737; found 248.1744.

4-Undecanyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylguanidine hydrogen chloride (10b). Compound **10b** was synthesised from Boc-protected guanidine **9b** (93 mg, 0.10 mmol) according to general procedure E as a colourless residue (52 mg, 85%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.8 Hz, CH_3), 1.29–1.40 (18H, m, 9 × CH_2), 1.47 (1H, d, *J* = 10.4 Hz, H10*s*), 1.57–1.61 (2H, m, CHC*H*$_2$), 1.73 (1H, d, *J* = 10.0 Hz, H10*a*), 2.46 (1H, br s, H9), 2.62–2.63 (2H, m, H1, H7), 3.22 (1H, app. t, *J* = 4.9 Hz, H8), 3.30–3.39 (8H, m, 4 × CH_2), 4.00 (1H, d, *J* = 5.5 Hz, H6), 4.05 (1H, d, *J* = 5.6 Hz, H2), 4.66 (1H, t, *J* = 4.7 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.5, 23.7, 25.2, 30.5 (2 × C), 30.66 (2 × C), 30.74, 30.8, 32.7, 33.1, 33.9, 39.6, 39.7, 41.9, 42.0, 44.9, 45.1, 46.9, 47.7, 79.9, 82.8, 105.1, 158.8, 158.9, 174.6, 176.5. HRMS (ESI, *m/z*) for $C_{27}H_{50}N_8O_4$ $[M + 2H]^{2+}$ calc. 276.2050; found 276.2057.

4-Pentadecyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylguanidine hydrogen chloride (10c). Compound **10c** was synthesised from Boc-protected guanidine **9c** (58 mg, 0.06 mmol) according to general procedure E as an off-white solid (34 mg, 87%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.9 Hz, CH_3), 1.29–1.45 (26H, m, 13 × CH_2), 1.47 (1H, d, *J* = 10.4 Hz, H10*s*),

1.58–1.62 (2H, m, CHC*H*$_2$), 1.74 (1H, d, *J* = 9.8 Hz, H10*a*), 2.45 (1H, br s,, H1), 2.61–2.62 (2H, m, H7, H9), 3.22 (1H, app. t, *J* = 4.7 Hz, H8), 3.30–3.39 (8H, m, 4 × CH_2), 3.99 (1H, d, *J* = 5.6 Hz, H2), 4.04 (1H, d, *J* = 5.6 Hz, H6), 4.66 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 23.7, 25.2, 28.2, 30.5, 30.6, 30.70 (2 × C), 30.73, 30.74 (2 × C), 30.8 (2 × C), 32.7, 33.1, 33.9, 39.6, 39.7, 41.9, 42.0, 44.9, 45.1, 46.9, 47.7, 80.0, 82.9, 105.1, 158.9 (2 × C), 174.6, 176.6. HRMS (ESI, *m/z*) for $C_{31}H_{58}N_8O_4$ $[M + 2H]^{2+}$ calc. 304.2363; found 304.2374.

4-[4′-(Octyloxy)phenethyl]-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylguanidine hydrogen chloride (10d). Compound **10d** was synthesised from Boc-protected guanidine **9d** (78 mg, 0.08 mmol) according to general procedure E as a yellow oil (39 mg, 74%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.9 Hz, CH_3), 1.28–1.35 (8H, m, 4 × CH_2) 1.44–1.53 (3H, m, CH_2, H10*s*), 1.71–1.78 (3H, m, CH_2, H10*a*), 1.84–1.88 (2H, m, CH_2), 2.49 (1H, br s, H1), 2.62–2.65 (4H, m, ArCH_2, H9, H7), 3.23 (1H, app. t, *J* = 5.0 Hz, H8), 3.30–3.39 (8H, m, 4 × CH_2), 3.92 (2H, t, *J* = 6.5 Hz, OCH_2), 4.02 (1H, d, *J* = 5.5 Hz, H6), 4.07 (1H, d, *J* = 5.6 Hz, H2), 4.67 (1H, t, *J* = 4.4 Hz, H4), 6.79–6.81 (2H, m, ArH), 7.06–7.09 (2H, m, ArH). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 23.7, 27.2, 30.4, 30.46, 30.49, 30.7, 32.8, 33.0, 36.0, 39.6, 39.7, 41.9, 42.0, 44.9, 45.1, 47.0, 47.6, 69.0, 80.1, 82.9, 104.3, 115.5 (2 × C), 130.2 (2 × C), 134.6, 158.9 (3 × C), 174.6, 176.5. HRMS (ESI, *m/z*) for $C_{32}H_{52}N_8O_5$ $[M + 2H]^{2+}$ calc. 315.2103; found 315.2109.

4-Ethylbenzene-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidoethylguanidine hydrogen chloride (10e). Compound **10e** was synthesised from Boc-protected guanidine **9e** (242 mg, 0.27 mmol) according to general procedure E as a white solid (145 mg, 93%). m.p: 133.1–149.1 °C (slow decomposition). ^{1}H NMR (500 MHz, CD_3OD) δ 1.50 (1H, d, *J* = 10.5 Hz, H10*s*), 1.78 (1H, d, *J* = 10.5 Hz, H10*a*), 1.88–1.92 (2H, m, CHC*H*$_2$), 2.49 (1H, br s, H9), 2.62–2.66 (2H, m, H1, H7), 2.69–2.72 (2H, m, CH_2), 3.23 (1H, app. t, *J* = 5.0 Hz, H8), 3.29–3.40 (8H, m, 4 × CH_2), 4.02 (1H, d, *J* = 5.6 Hz, H6), 4.08 (1H, d, *J* = 5.6 Hz, H2), 4.68 (1H, t, *J* = 4.8 Hz, H4), 7.14–7.18 (3H, m, ArH), 7.23–7.27 (2H, m, ArH). ^{13}C NMR (125 MHz, CD_3OD) δ 31.3, 32.8, 35.8, 39.6, 39.7, 41.9, 42.0, 44.9, 45.1, 47.0, 47.7, 80.1, 83.0, 101.3, 127.0, 129.3 (2 × C), 129.5 (2 × C), 142.7, 158.9 (2 × C), 174.6, 176.5. HRMS (ESI, *m/z*) for $C_{24}H_{36}N_8O_4$ $[M + 2H]^{2+}$ calc. 251.1503; found 251.1510.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidopropylguanidine hydrogen chloride (15). Compound **15** was synthesised from Boc-protected guanidine **13** (139 mg, 0.15 mmol) according to general procedure E as a white solid (66 mg, 74%). m.p: 142.4–146.4 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.8 Hz, CH_3), 1.29–1.42 (10H, m, 5 × CH_2), 1.48 (1H, d, *J* = 10.4 Hz, H10*s*), 1.58–1.62 (2H, m, CHCH_2), 1.71–1.79 (5H, m, 2 × CH_2, H10*a*), 2.42 (1H, br s, H9), 2.59–2.60 (2H, m, H1, H7), 3.18–3.29 (9H, m, 4 × CH_2, H8), 3.99 (1H, d, *J* = 5.6 Hz, H6), 4.05 (1H, d, *J* = 5.6 Hz, H2), 4.66 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 23.7, 25.2,

29.90, 29.93, 30.3, 30.6, 32.7, 32.9, 33.9, 37.69, 37.71, 39.99, 40.05, 44.9, 45.2, 46.9, 47.9, 80.0, 82.9, 105.1, 158.7 (2 × C), 174.1, 176.1. HRMS (ESI, *m/z*) for $C_{25}H_{46}N_8O_4$ $[M + 2H]^{2+}$ calc. 262.1894; found 262.1892.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-dicarboxamidobutylguanidine hydrogen chloride (16). Compound **16** was synthesised from Boc-protected guanidine **14** (78 mg, 0.08 mmol) according to general procedure E as a white solid (48 mg, 94%). m.p: 110.5–124.0 °C. ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.8 Hz, CH_3), 1.29–1.40 (10H, m, 5 × CH_2), 1.48 (1H, d, *J* = 10.3 Hz, H10*s*), 1.57–1.60 (10H, m, 5 × CH_2), 1.71 (1H, d, *J* = 10.2 Hz, H10*a*), 2.39 (1H, br s, H9), 2.58–2.59 (2H, m, H1, H7), 3.15–3.27 (9H, m, 4 × CH_2, H8), 3.99 (1H, d, *J* = 5.6 Hz, H6), 4.05 (1H, d, *J* = 5.6 Hz, H2), 4.65 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 23.7, 25.2, 27.17, 27.23, 27.7 (2 × C), 30.3, 30.6, 32.7, 32.9, 33.9, 39.9 (2 × C), 42.1 (2 × C), 44.9, 45.3, 46.9, 48.0, 80.0, 82.9, 105.1, 158.6 (2 × C), 173.9, 175.9. HRMS (ESI, *m/z*) for $C_{27}H_{50}N_8O_4$ $[M + 2H]^{2+}$ calc. 276.2050; found 276.2050.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-(heptylcarbamoyl)-9-*exo*-carboxamidoethylguanidine hydrogen chloride (25a). Compound **25a** was synthesised from Boc-protected guanidine **24a** (16 mg, 0.02 mmol) according to general procedure E as a colourless oil (10 mg, 99%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.88–0.91 (6H, m, 2 × CH_3), 1.29–1.40 (18H, m, 9 × CH_2), 1.47–1.52 (3H, m, CH_2, H10*s*), 1.57–1.61 (2H, m, CH_2), 1.72 (1H, d, *J* = 10.4 Hz, H10*a*), 2.42 (1H, br s, H9), 2.58–2.60 (2H, m, H1, H7), 3.07–3.13 (2H, m, CH_2), 3.17 (1H, app. t, *J* = 5.0 Hz, H8), 3.23–3.34 (4H, m, 2 × CH_2), 3.98 (1H, d, *J* = 5.6 Hz, H6), 4.04 (1H, d, *J* = 5.7 Hz, H2), 4.65 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.41, 14.44, 23.6, 23.7, 25.2, 27.9, 30.1, 30.3, 30.4, 30.6, 32.7, 32.91, 33.0, 33.9, 39.7, 40.5, 42.0, 44.8, 45.3, 46.9, 47.8, 80.0, 82.9, 105.1, 158.9, 173.5, 176.8. HRMS (ESI, *m/z*) for $C_{27}H_{49}N_5O_4$ $[M + H]^+$ calc. 508.3857; found 508.3861.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-(hexadecylcarbamoyl)-9-*exo*-carboxamidoethylguanidine hydrogen chloride (25b). Compound **25b** was synthesised from Boc-protected guanidine **24b** (21 mg, 0.03 mmol) according to general procedure E as an orange oil (16 mg, 80%). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (6H, t, *J* = 6.5 Hz, 2 × CH_3), 1.29–1.42 (36H, m, 18 × CH_2), 1.47–1.51 (3H, m, CH_2, H10*s*), 1.57–1.61 (2H, m, CH_2), 1.72 (1H, d, *J* = 10.2 Hz, H10*a*), 2.42 (1H, br s, H9), 2.59–2.61 (2H, m, H1, H7), 3.04–3.10 (2H, m, CH_2), 3.17 (1H, app. t, *J* = 5.1 Hz, H8), 3.26–3.35 (4H, m, 2 × CH_2), 3.99 (1H, d, *J* = 5.6 Hz, H6), 4.04 (1H, d, *J* = 5.6 Hz, H2), 4.65 (1H, t, *J* = 4.8 Hz, H4). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 14.5, 23.69, 23.73, 25.3, 28.0, 30.1, 30.3, 30.35, 30.39, 30.5, 30.6, 30.7 (2 × C), 30.76, 30.81 (4 × C), 32.7, 32.9, 33.1 (2 × C), 33.9 (2 × C), 39.7, 40.5, 42.0, 44.8, 45.3, 46.9, 47.8, 80.0, 82.9, 105.1, 158.9, 173.5, 176.8. HRMS (ESI, *m/z*) for $C_{36}H_{68}N_5O_4$ $[M + H]^+$ calc. 634.5266; found 634.5283.

8-*endo*-9-*exo*-Di[2′-(2″-bromoacetamide)ethylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (27). To the stirring solution of diamine **8a** (210 mg, 0.434 mmol), Et_3N (320 μL, 2.26 mmol) and CH_2Cl_2 (4.3 mL) at −78 °C under an inert atmosphere, was added bromoacetyl bromide (110 μL, 1.22 mmol) dropwise. The reaction was stirred for 5 h, before being warmed to −20 °C, quenched with H_2O (5 mL) and diluted with CH_2Cl_2 (20 mL). The combined organic phase was washed with H_2O (10 mL), sat. NH_4Cl (10 mL), sat. $NaHCO_3$ (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford a white solid that was purified by column chromatography (EtOAc–10% MeOH in CH_2Cl_2) to give the title compound (185 mg, 65%) as a white powder; R_f = 0.36 (10% MeOH in CH_2Cl_2). m.p: 65.6–70.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (3H, t, *J* = 6.7 Hz, CH_3), 1.23–1.31 (11H, m, 5 × CH_2, H10*s*), 1.50–1.52 (3H, m, CHCH_2, H10*a*), 2.32 (1H, br s, H7), 2.46 (1H, d, *J* = 4.6 Hz, H1), 2.49–2.50 (1H, m, H9), 3.01–3.19 (9H, m, 4 × CH_2, H8), 3.83 (2H, s, $BrCH_2$), 3.84 (2H, s, $BrCH_2$), 3.86 (1H, d, *J* = 5.7 Hz, H2), 3.90 (1H, d, *J* = 5.6 Hz, H6), 4.59 (1H, t, *J* = 4.7 Hz, H4), 8.00 (1H, t, *J* = 5.4 Hz, NH), 8.04 (1H, t, *J* = 5.0 Hz, NH), 8.29–8.30 (2H, m, 2 × NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.0, 22.1, 23.7, 28.6, 28.9, 29.5 (2 × C), 31.1, 31.2, 32.3, 38.1 (2 × C), 38.3 (2 × C), 42.7, 43.2, 44.5, 46.2, 78.2, 81.1, 102.9, 166.1, 166.2, 171.2, 173.1. HRMS (ESI, *m/z*) for $C_{25}H_{40}N_4O_6$ $[M + H]^+$ calc. 651.1387; found 651.1406.

8-*endo*-9-*exo*-Di[2′-([2″-benzylaminocyclobut-1″-ene-3″,4″-dione]amino)ethylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (26a). Treatment of salt **8a** (99 mg, 0.204 mmol) in MeOH (730 μL) with Et_3N (85 μL, 0.612 mmol) was followed by addition of squaric ester **S2** (90 mg, 0.408 mmol) and the reaction was stirred for 2.5 h at ambient temperature. The white slurry was concentrated under vacuum before being triturated in 2M HCl (3 mL) for 40 min at ambient temperature. The white solid was collected by vacuum filtration before being diluted with MeOH (600 μL), treated with 1,2-ethylenediamine (20 μL) and stirred at ambient temperature for a further 2 h. Again the resulting slurry was triturated in 2M HCl (3 mL) for 20 min at ambient temperature and the off white solid was collected and dried *in vacuo* to afford the title compound (63 mg, 40%). m.p: 166.4–204.9 °C (slow decomposition). ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.84 (3H, t, *J* = 6.7 Hz, CH_3), 1.22–1.35 (11H, m, 5 × CH_2, H10*s*), 1.48–1.49 (3H, CHCH_2, H10*a*), 2.29 (1H, br s, H1), 2.46 (1H, d, *J* = 5.0 Hz, H7), 2.48–2.50 (1H, m, H9), 3.06 (1H, app. t, *J* = 3.8 Hz, H8), 3.10–3.27 (8H, m, 4 × NHCH_2), 3.83 (1H, d, *J* = 5.6 Hz, H6), 3.87 (1H, d, *J* = 5.6 Hz, H2), 4.54 (1H, t, *J* = 4.6 Hz, H4), 4.70 (4H, br s, 2 × ArCH_2), 7.27–7.38 (10H, m, ArH), 7.49 (2H, br s, 2 × NH), 7.89 (2H, br s, 2 × NH), 8.11 (1H, t, *J* = 5.0 Hz, NH), 8.16 (1H, br s, NH). ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.9, 22.1, 23.7, 28.6, 28.9, 31.1, 31.2, 32.4, 39.52 (4 × C), 43.0, 43.1, 44.5, 46.2, 46.8 (2 × C), 78.2, 81.0, 102.9, 127.4 (2 × C), 127.5 (4 × C), 128.6 (4 × C), 139.0 (2 × C), 167.5 (2 × C), 168.0 (2 × C), 171.3, 173.2, 182.5 (4 × C). HRMS (ESI, *m/z*) for $C_{43}H_{52}N_6O_8$ $[M + H]^+$ calc. 781.3919; found 781.3904.

8-*endo*-9-*exo*-Di[2′-(phenylthioureido)ethylcarbamoyl]-4-heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane (26b). Treatment of salt **8a** (52 mg, 0.108 mmol) in CH_2Cl_2 (500 μL)

with Et_3N (50 μL, 0.109 mmol) was followed by addition of phenylisothiocyanate (13 μL, 0.109 mmol) and the reaction was stirred for 22 h at ambient temperature. The reaction was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phase was washed with 2M HCl (10 mL), brine (10 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a white waxy solid which was purified by column chromatography (50% EtOAc in pet. spirits–EtOAc) to afford the title compound (35 mg, 95%) as a white paste; R_f = 0.37 (EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, t, *J* = 6.8 Hz, CH_3), 1.25–1.42 (11H, m, 5 × CH_2, H10*s*), 1.59–1.63 (2H, m, CHC*H*$_2$) 1.75 (1H, d, *J* = 9.9 Hz, H10*a*), 2.35 (1H, d, *J* = 5.8 Hz, H7), 2.52 (1H, br s, H1), 2.59 (1H, d, *J* = 3.9 Hz, H9), 2.85 (1H, app. t, J_{app} = 4.8 Hz, H8), 3.26–3.28 (2H, m, NHC*H*$_2$) 3.45–3.51 (2H, m, NHC*H*$_2$), 3.68–3.87 (4H, m, 2 × NHC*H*$_2$), 3.89 (1H, d, *J* = 5.7 Hz, H6), 3.99 (1H, d, *J* = 5.6 Hz, H2), 4.60 (1H, t, *J* = 4.9 Hz, H4), 6.68 (2H, br s, 2 × NH), 6.81 (1H, t, *J* = 5.6 Hz, NH), 7.06 (1H, t, *J* = 5.3 Hz, NH), 7.21–7.31 (6H, m, ArH), 7.41 (4H, t, *J* = 7.7 Hz, ArH), 8.06 (1H, br s, NH), 8.12 (1H, br s, NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2, 22.8, 24.4, 29.3, 29.7, 31.9, 32.6, 33.0, 39.4, 40.4, 43.4, 44.3, 44.4, 44.9, 45.6, 48.0, 78.7, 81.5, 104.2, 125.5 (2 × C), 125.6 (2 × C), 127.5 (2 × C), 130.3 (4 × C), 136.2 (2 × C), 172.9 (2 × C), 174.8 (2 × C). HRMS (ESI, *m/z*) for $C_{35}H_{48}N_6O_4S_2$ $[M + H]^+$ calc. 681.3251; found 681.3251.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-di[2′-(ethylcarbamoyl)amidomethyl]di-1-methyl-1*H*-imidazol-3-ium bromide (26c). To the stirring solution of 1-methylimidazole (16 μL, 0.19 mmol) in anhydrous THF (500 μL) at −78 °C under an inert atmosphere, was added alpha bromoamide **27** (63 mg, 0.10 mmol) in THF (500 μL) slowly. The reaction was warmed to −15 °C and stirred for 1 h, before being warmed to ambient temperature and stirred for a further 2 d. The THF was decanted off and the material was washed and decanted with Et_2O (3 × 1 mL). The material was diluted with H_2O (5 mL) and extracted with CH_2Cl_2 (4 mL). The aqueous portion was lyophilised for 48 h to give the title compound (59 mg, 74%) as light yellow residue. ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 0.85 (3H, t, *J* = 6.8 Hz, CH_3), 1.23–1.29 (11H, m, 5 × CH_2, H10*s*), 1.49–1.51 (3H, m, CHC*H*$_2$, H10*a*), 2.32 (1H, br s, H7), 2.48–2.55 (2H, m, H1, H9), 3.01–3.23 (9H, m, 4 × CH_2, H8), 3.85 (1H, d, *J* = 5.6 Hz, H2), 3.89 (6H, s, 2 × NCH_3), 3.92 (1H, d, *J* = 5.5 Hz, H6), 4.60 (1H, t, *J* = 4.7 Hz, H4), 4.97 (4H, s, 2 × CH_2N), 7.68–7.71 (4H, m, 4 × ArH), 8.08–8.12 (2H, m, 2 × NH), 8.48–8.51 (2H, m, 2 × NH), 9.08 (2H, s, NCHN). ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ 14.0, 22.1, 23.8, 28.7, 28.9, 31.1, 31.2, 32.4, 35.9 (2 × C), 38.2, 38.4, 38.6, 38.7, 42.7, 43.4, 44.4, 46.2, 50.5, 50.6, 78.2, 81.1, 102.9, 123.1 (2 × C), 123.8 (2 × C), 137.7 (2 × C), 165.1 (2 × C), 171.2, 173.1. HRMS (ESI, *m/z*) for $C_{33}H_{50}N_8O_6$ $[M + 2H]^{2+}$ calc. 328.1999; found 328.1996.

4-Heptyl-3,5-dioxatricyclo[5.2.1.0^{2,6}]decane-8-*endo*-9-*exo*-di[2′-(ethylcarbamoyl)amidomethyl]dipyridin-1-ium bromide (26d). To the stirring solution of alpha bromoamide **27** (31 mg, 0.05 mmol) in anhydrous THF (480 μL), under inert conditions was added pyridine (100 μL, 1.24 mmol) and the reaction was stirred at 66 °C for 16 h. The reaction was concentrated under vacuum and the crude material was rinsed and decanted with Et_2O (5 × 1 mL).

Excess solvent was removed under vacuum before the sample was diluted in H_2O (5 mL) and extracted with CH_2Cl_2 (3 × 4 mL). The aqueous portion was lyophilised for 48 h to give the title compound (25 mg, 64%) as an orange spongy material. ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 6.9 Hz, CH_3), 1.29–1.45 (11H, m, 5 × CH_2, H10*s*), 1.59–1.63 (2H, m, CHC*H*$_2$) 1.70 (1H, dd, *J* = 10.3, 1.2 Hz, H10*a*), 2.44 (1H, br s, H1), 2.58–2.59 (2H, m, H9, H7), 3.18 (1H, app. t, *J* = 4.8 Hz, H8), 3.33–3.43 (8H, m, 4 × CH_2), 4.01 (1H, d, *J* = 5.6 Hz, H6), 4.04 (1H, d, *J* = 5.6 Hz, H2), 4.66 (1H, t, *J* = 4.8 Hz, H4), 5.45 (2H, s, CH_2), 5.46 (2H, s, CH_2), 8.14–8.17 (4H, m, ArH), 8.65–8.68 (2H, m, ArH), 8.93–8.94 (4H, m, ArH). ^{13}C NMR (125 MHz, CD_3OD) δ 14.4, 23.7, 25.3, 30.4, 30.6, 32.8, 32.9, 33.9, 39.9, 40.0, 40.6, 40.7, 45.1 (2 × C), 47.2, 47.5, 63.0 (2 × C), 78.0, 82.9, 105.1, 129.0 (4 × C), 147.5 (2 × C), 147.55 (2 × C), 147.60 (2 × C), 166.3 (2 × C), 174.3, 176.2. HRMS (ESI, *m/z*) for $C_{35}H_{48}N_6O_6$ $[M + 2H]^{2+}$ calc. 325.1890; found 325.1898.

Crystallography. Intensity data were collected with an Oxford Diffraction SuperNova CCD diffractometer using Cu- Kα radiation, the temperature during data collection was maintained at 130.0(1) using an Oxford Cryosystems cooling device. The structure was solved by direct methods and difference Fourier synthesis.[27] Thermal ellipsoid plots were generated using the program ORTEP-3[28] integrated within the WINGX[29] suite of programs. Disordered solvent, assumed to be ethanol was removed using the Squeeze procedure.[30]

Disk Diffusion Assay. A stock solution of 10 mg/mL was made for each compound under observation using DMSO as a solvent. Each of these stock solutions was then diluted by a factor of 1:2 to bring the concentration to 5 mg/mL. The diluted solutions were then filter-sterilised using a 0.2-µm nylon filter, and 10 µL of the 5 mg/mL stock was pipetted onto a blank disk (i.e. 50 µg/disk; Oxoid Limited, Hampshire, UK). Suspensions of all bacterial isolates were adjusted to a 0.5 McFarland standard (in 0.9% NaCl) before they were swabbed onto nutrient agar plates. The controls used were a 10 µg colistin disk (sulphate, Oxoid), 10 µL of DMSO and a plate swabbed with saline from the dispenser used.

Minimum Inhibitory Concentration (MIC) determination. Bacteria were obtained either from American Type Culture Collection (ATCC; Manassas, VA, USA), Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) or from a clinical isolate library at The University of Queensland, Centre for Clinical Research (UQCCR) as listed in Table S1 (see ESI). Bacteria were cultured in Nutrient broth (NB; Bacto Laboratories, catalogue No. 234000) or Muller-Hinton broth (MHB; Bacto Laboratories, catalogue No. 211443) at 37 °C overnight with shaking (~180 RPM). A sample of each culture was diluted 50-fold in fresh MHB and incubated at 37 °C for 1.5–3 h with shaking (~180 RPM). Compound stock solutions were prepared as 10 mg/mL in DMSO and colistin was dissolved in milli-Q water at 5.12 mg/mL. The compounds, at twice the final desired concentration, were serially diluted 2-fold across the wells of 96–well plates (Non-Binding Surface, Corning, catalogue No. 3641). Mid-log phase bacterial cultures (after 1.5–3 h incubation) were diluted to a final

concentration of $5x10^5$ colony forming units (CFU)/mL, and 50 μL was added to each well giving a final compound concentration range of 32 μg/mL to 0.015 μg/mL (DMSO ≤ 1%). MICs were determined visually after 20 h of incubation at 37 °C, with the MIC defined as the lowest compound concentration at which no bacterial growth was visible. Determined MIC values are the result of two independent experiments of n = 2, giving a final dataset of n = 4.

Cytotoxicity evaluation. HEK293 (ATCC CRL-1573) and HepG2 (ATCC HB-8065) cells were seeded as 3000 cells per well in a 384-well plate in in DMEM medium (GIBCO-Invitrogen #11995-073), in which 10% of FBS was added. Cells were incubated for 24 h at 37 °C, 5% CO_2 to allow cells to attach to the plates. A concentration series of compounds was then added into each well. The cells were incubated with the compounds for 24 h at 37 °C, 5% CO_2. After the incubation, 10 μM resazurin (dissolved in PBS) was added to each well. The plates were then incubated for 2 h at 37 °C, 5% CO_2. The fluorescence intensity was read using Polarstar Omega with excitation/emission 560/590. The data was analysed by Prism software. Results are presented as the average percentage of control ± SD for each set of duplicate wells using the following equation: Percentage Viability = (FITEST – $FI_{Negative}$/$FI_{UNTREATED}$ –$FI_{Negative}$) × 100.

Acknowledgements

F.M.P., S.M.H., S.K.K., R.N.R. & T.D.A. thank the ARC (DP140100227) and the Strategic Research Centre for Chemistry and Biotechnology (Deakin University) for financial support and a top-up scholarship for S.M.H. The authors would also like to thank Dr Damien Callahan for his assistance with the collection of HRMS and the Australian Research Council for funding Deakin University's Magnetic Resonance Facility through LIEF grant LE110100141. J.L. & R.L.N. are supported by a research grant from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (R01 AI098771). J.L. is an Australian NHMRC Senior Research Fellow, while M.A.C. is an Australian NHMRC Principal Research Fellow. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Allergy and Infectious Diseases or the National Institutes of Health. The MIC screening was done in collaboration with CO-ADD (Community for Open Antimicrobial Drug Discovery, co-add.org) and we thank David L. Paterson (UQCCR, University of Queensland) for his kind donation of clinical Gram-positive isolates used for testing.

Supporting Information Available: Crystal structure of compound **4a** (CIF), 2D NMR spectra for compounds **4a**, and *exo*/*endo* isomers from the reaction products of diol 3 and a selected benzaldehye, synthetic procedures for all known compounds and copies of NMR spectra (^{1}H and ^{13}C) for all new compounds. All bacterial strains tested, and all cytotoxicity data. This material is available free of charge *via* the Internet at http://

References

1. (a) H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg and J. Bartlett, *Clin. Infect. Dis.*, 2009, **48**, 1-12; (b) G. H. Talbot, J. Bradley, J. E. Edwards, D. Gilbert, M. Scheld and J. G. Bartlett, *Clin. Infect. Dis.*, 2006, **42**, 657-668; (c) W. Yau, R. J. Owen, A. Poudyal, J. M. Bell, J. D. Turnidge, H. H. Yu, R. L. Nation and J. Li, *J. infect.*, 2009, **58**, 138-144.
2. (a) E. Breukink and B. de Kruijff, *Nature Reviews Drug Discovery*, 2006, **5**, 321-323; (b) G. Taubes, *Science*, 2008, **321**, 356-361.
3. M. A. Cooper and D. Shlaes, *Nature*, 2011, **472**, 32-32.
4. (a) J. Harder, R. Gläser and J. M. Schröder, *J. Endotoxin Res.*, 2007, **13**, 317; (b) L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schaberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen and K. Lewis, *Nature*, 2015, **517**, 455-459.
5. (a) M. S. Butler, M. A. Blaskovich and M. A. Cooper, *J. Antibiot.*, 2013, **66**, 571-591; (b) C. T. Walsh and T. A. Wencewicz, *J. Antibiot.*, 2014, **67**, 7-22; (c) R. E. W. Hancock, *Nat. Biotech.*, 2014, **32**, 66-68; (d) A. Ivankin, L. Livne, A. Mor, G. A. Caputo, W. F. DeGrado, M. Meron, B. Lin and D. Gidalevitz, *Angew. Chem.*, 2010, **122**, 8640-8643.
6. (a) Y. Shai, *Biochim. Biophys. Acta*, 1999, **1462**, 55–70; (b) E. Guan -Guerra, T. Santos-Mendoza and S. Lugo-Reyes, *Clin. Immuno.*, 2010, **135**, 1-11; (c) G. Baumann and P. Mueller, *J. Supramol. Struct.*, 1974, **2**, 538-557; (d) D. R. Laver, *J. Biophys.*, 1994, **66**, 355-359.
7. (a) L. S. McCoy, K. D. Roberts, R. L. Nation, P. E. Thompson, T. Velkov, J. Li and Y. Tor, *ChemBioChem*, 2013, **14**, 2083-2086; (b) T. Velkov, P. E. Thompson, R. L. Nation and J. Li, *J. Med. Chem.*, 2010, **53**, 1898; (c) T. M. Arnold, G. N. Forrest and K. J. Messmer, *Am. J. Health-Sys. Pharm.*, 2007, **64**; (d) J. Li, R. L. Nation, R. W. Milne, J. D. Turnidge and K. Coulthard, *Int. J. Antimicrob. Ag.*, 2005, **25**, 11-25; (e) D. R. Storm, K. S. Rosenthal and P. E. Swanson, *Annu. Rev. Biochem*, 1977, **46**, 723-763.
8. (a) A. J. Lowe, G. A. Dyson and F. M. Pfeffer, *Org. Biomol. Chem.*, 2007, **5**, 1343-1346; (b) A. J. Lowe, G. A. Dyson and F. M. Pfeffer, *Eur. J. Org. Chem.*, 2008, **2008**, 1559-1567; (c) A. J. Lowe, B. M. Long and F. M. Pfeffer, *Chem. Commun.*, 2013, **49**, 3376-3388.
9. L. C. Henderson, J. Li, R. L. Nation, T. Velkov and F. M. Pfeffer, *Chem. Commun.*, 2010, **46**, 3197-3199.
10. S. M. Hickey, T. D. Ashton, J. M. White, J. Li, R. L. Nation, H. Y. Yu, A. G. Elliott, M. S. Butler, J. X. Huang, M. A. Cooper and F. M. Pfeffer, *RSC Adv.*, 2015, **5**, 28582-28596.
11. L. D. Van Vliet, T. Ellis, P. J. Foley, L. Liu, F. M. Pfeffer, R. A. Russell, R. N. Warrener, F. Hollfelder and M. J. Waring, *J. Med. Chem.*, 2007, **50**, 2326-2340.
12. G. Pandey, N. R. Gupta and T. M. Pimpalpalle, *Org. Lett.*, 2009, **11**, 2547-2550.
13. A 4-fluorophenyl acetal analogue was converted to the intended diBoc guanidine and the acetal was indeed unstable to the acidic conditions used during the final Boc-deprotection step (as evidenced by ^{1}H NMR spectroscopy, see ESI).
14. (a) S. M. Hickey, T. D. Ashton, S. K. Khosa and F. M. Pfeffer, *Synlett*, 2012, **23**, 1779-1782; (b) S. M. Hickey, T. D. Ashton and F. M. Pfeffer, *Asian J. Org. Chem.*, 2015, **4**, 320-326.
15. A. J. Lowe and F. M. Pfeffer, *Chem. Commun.*, 2008, 1871-1873.
16. (a) N. Hamanaka, T. Seko, T. Miyazaki, M. Naka, K. Furuta and H. Yamamoto, *Tetrahedron Lett.*, 1989, **30**, 2399-2402; (b) S. M. Hickey, S. K. Tripcony, R. Li, R. J. Williams and F. M. Pfeffer, *Supramol. Chem.*, 2015, **27**, 425-435.
17. D. Coleman, M. Spulak, M. T. Garcia and N. Gathergood, *Green Chemistry*, 2012, **14**, 1350-1356.
18. R. Ian Storer, C. Aciro and L. H. Jones, *Chem. Soc. Rev.*, 2011, **40**, 2330-2346.
19. (a) M. Mohamed, T. P. Gonçalves, R. J. Whitby, H. F. Sneddon and D. C. Harrowven, *Chem. Euro. J.*, 2011, **17**, 13698-13705; (b) N. C. Lim, M. D. Morton, H. A. Jenkins and C. Brückner, *J. Org. Chem.*, 2003, **68**, 9233-9241.

20. L. B. Reller, M. Weinstein, J. H. Jorgensen and M. J. Ferraro, *Clin. Infect. Dis.*, 2009, **49**, 1749-1755.
21. D. J. Payne, M. N. Gwynn, D. J. Holmes and D. L. Pompliano, *Nat. Rev. Drug Discov.*, 2007, **6**, 29-40.
22. X. Chen, R. P. M. Dings, I. Nesmelova, S. Debbert, J. R. Haseman, J. Maxwell, T. R. Hoye and K. H. Mayo, *J. Med. Chem.*, 2006, **49**, 7754-7765.
23. L. He, H.-S. Byun and R. Bittman, *J. Org. Chem.*, 2000, **65**, 7618-7626.
24. V. Rauniyar and D. G. Hall, *J. Org. Chem.*, 2009, **74**, 4236-4241.
25. G. D. Vilela, R. R. da Rosa, P. H. Schneider, I. H. Bechtold, J. Eccher and A. A. Merlo, *Tetrahedron Lett.*, 2011, **52**, 6569-6572.
26. D. E. Rajsfus, S. Alter-Zilberfarb and A. A. Frimer, *J. Fluorine Chem.*, 2013, **148**, 49-58.
27. G. M. Sheldrick, *Acta Cryst.*, 2007, **64**, 112-122.
28. L. J. Farrugia, *J. Appl. Cryst.*, 1997, **30**, 565-565.
29. L. J. Farrugia, *J. Appl. Cryst.*, 1999, **32**, 837-838.
30. P. Van der Sluis and A. Spek, *Acta Cryst.*, 1990, **46**, 194-201.