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p-Guanidinoethyl calixarene and parent phenol derivatives exhibiting antibacterial activities. Synthesis and biological evaluation

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ABSTRACT

The tetra-*para*-guanidinoethyl-calix[4]arene, its distally-disubstituted ether derivatives involving 2,2′-bithiazolyl- or 2,2′-bipyridyl-methyl groups, as well as the *para*-guanidinoethylphenol and its analogous derivatives have been synthesized, fully characterized and evaluated as antibacterial agents towards both Gram positive and Gram negative reference bacteria. The simple phenolic species showed lower activity than their calixarene analogues, confirming the hypothesis that a synergistic effect should result from the spatial organization of guanidinium and heterocycles on a macrocyclic scaffold. Introduction of the bithiazole and bipyridine substituents enhanced the activity of simple phenol derivatives, reaching, for the two *Staphylococcus aureus* strains in particular, the values obtained for their calixarenic parents. MTT viability assays were carried out to determine selectivity indexes.

1. Introduction

The emergence of resistance in pathogenic microorganisms to commercial antibiotics requires the development of specific fields of research dedicated to the discovery of new drugs.[1] In this sense, we have focused on a promising approach based on a calixarene platform designed as a molecular drug dispenser with, at the lower rim, penicillin or quinolone moieties attached via a labile bound,[2] as well as on calixarene derivatives displaying intrinsic antimicrobial activity. For the latter, among the various approaches that are under current evaluation in our group, one is devoted to polycationic calixarene-based podands integrating, for example, guanidinium groups.[3] In recent years, the guanidinium derivatives have been but infrequently investigated as pharmaceutical antimicrobial agents.[4] Most compounds studied are poly-guanidinium species derived from the old antidiabetic and trypanocidal drugs Synthalin A or B,[5] in which the guanidiniums are found at the ends or along alkyl or polymeric chains, that could be regarded as flexible linear organizing templates.

More rigid, the calixarene species are oligomeric phenolic macrocycles that have demonstrated their excellent organizational behaviour for a multitude of active functionalities.[6] Some but very few reports, essentially in the form of patents, have focused on their intrinsic therapeutical properties; some of them, hydrophilic, have shown interesting levels of activity against bacteria,[7] fungi, cancerous cells and viruses,[8] enveloped viruses,[9] as well as against thrombosis[10] and fibrotic diseases.[11] In the mid 50s, the calixarene derivative 'Macrocyclon',[12] and more recently some parent species,[13] were studied in the treatment of tuberculosis and other mycobacterioses. Functionalized calixarene mimics of vancomycin has also been studied as antimicrobial agents.[14]

Given that most bacteria have a negatively charged surface, the introduction of positive charges on the calixarene core, that should lead to a constrained and highly organized oligomeric polycation, could give rise, with regards to the simple phenolic analogue, to an interesting synergistic effect in ionic interactions with the surface of bacteria, possibly resulting in an antibacterial activity.

Of the few simple organic cations available, guanidinium was first chosen for its stability over a large range of pH values. Only few calixarene guanidinium derivatives have been studied so far.[15] Biological studies related to plasmid DNA binding and cell transfection[16a–c] or endothelial cell proliferation inhibition[16d] properties of upper- or lower-rim guanidylated calixarene derivatives have recently been described.

Consistent with the anticipated synergistic effect described above, we have recently shown[3] that the tetra-*para*-guanidinoethyl-calix[4]arene **4** displayed very interesting antibacterial properties against various Gram positive and Gram negative reference strains

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of bacteria, at levels similar to those required with the well-known hexamidine **34**, but with a lower cytotoxicity. We have also shown[3a] that the constitutive monomer of **4**, the *para*-guanidinoethylphenol **18**, does not display any antibacterial activity, confirming our first hypothesis on the probable role of cyclotetramerization on the acquisition of activity.

In order to enlarge this new family, and as part of our centre of interest deals with metal chelation that might be involved in biological activity, we have prepared the chelating podands **14**, **15** and **16**, incorporating in alternate positions of the lower rim of **4**, two 2,2′-bipyridyl-methyl (α-methyl/**14**, β-methyl/**15**) or 2,2′-bithiazolyl-methyl subunits, respectively. In the cone conformation of the calixarene, these derivatives should display an amphiphilic character, strongly orienting the resulting tetracationic head towards the bacterium surface, and leaving the additional functionalities externally or internally oriented for further interactions. To evaluate the strength of the synergistic effect generated by the calixarene platform, we have also prepared the corresponding bipyridyl and bithiazolyl *para*-guanidinoethylphenyl ethers **29**, **30** and **31** (Scheme 1).

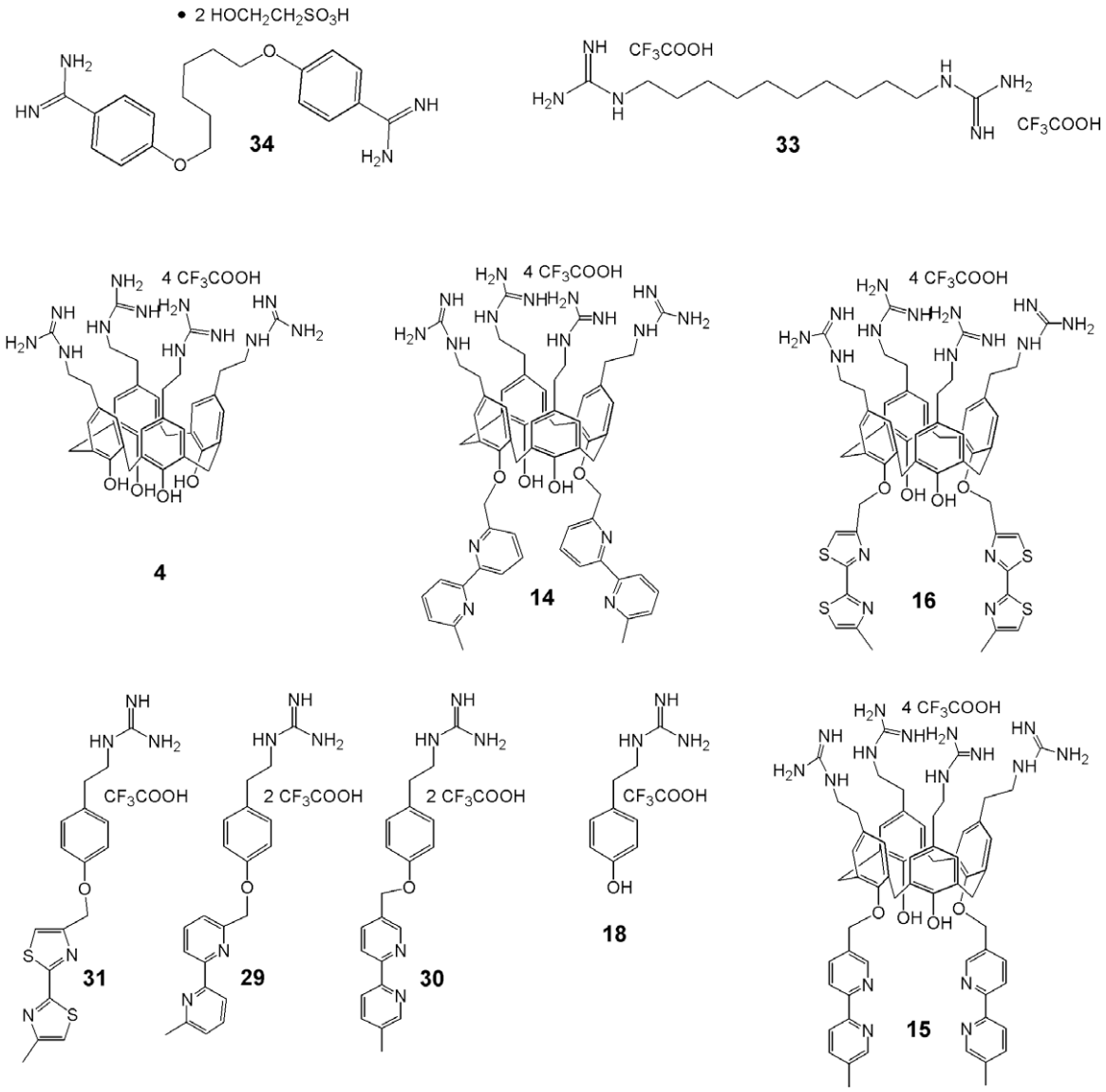
As reference compounds, we have chosen the old anthelminthic but toxic (pancreas) Synthalin A **33**,[3,5a] having two guanidino groups attached at the ends of a linear decyl chain, and the well-known and widely used cutaneous antiseptic 1,6-bis-(*para*-amidinophenoxy)hexane **34** (hexamidine). The latter, as commercially available antibacterial compound with the structurally closest ionic functionality, is described as an antibacterial for Gram positive bacteria.

We present here the syntheses of calixarenes species **4**, **14**, **15** and **16**, and of their constitutive monomers **18**, **29**, **30** and **31**, as well as Synthalin A **33**, accompanied by evaluation of their antibacterial activity and cellular viability.

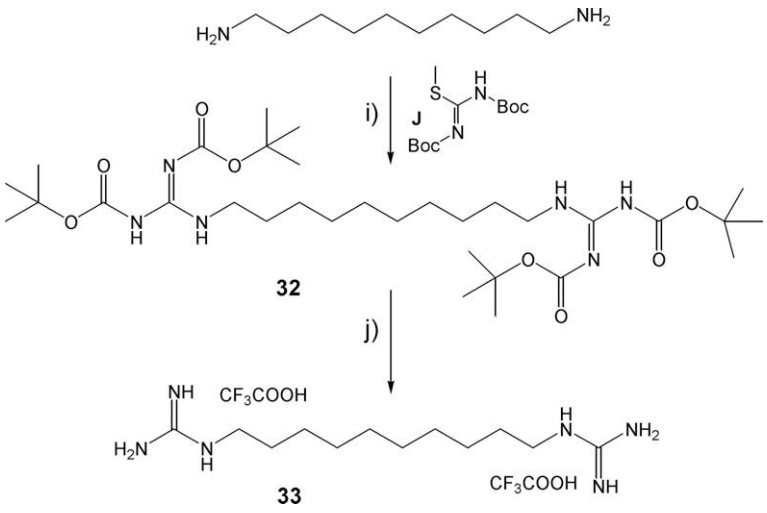
2. Chemistry

2.1. Synthalin A and hexamidine

Synthalin A **33** was prepared (Scheme 2) by an adaptation of the procedure of Elliot et al.,[17] based on the reaction of 1,12-diaminodecane and 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thio-



Scheme 1. Water-soluble guanidinium and amidinium compounds of this study.



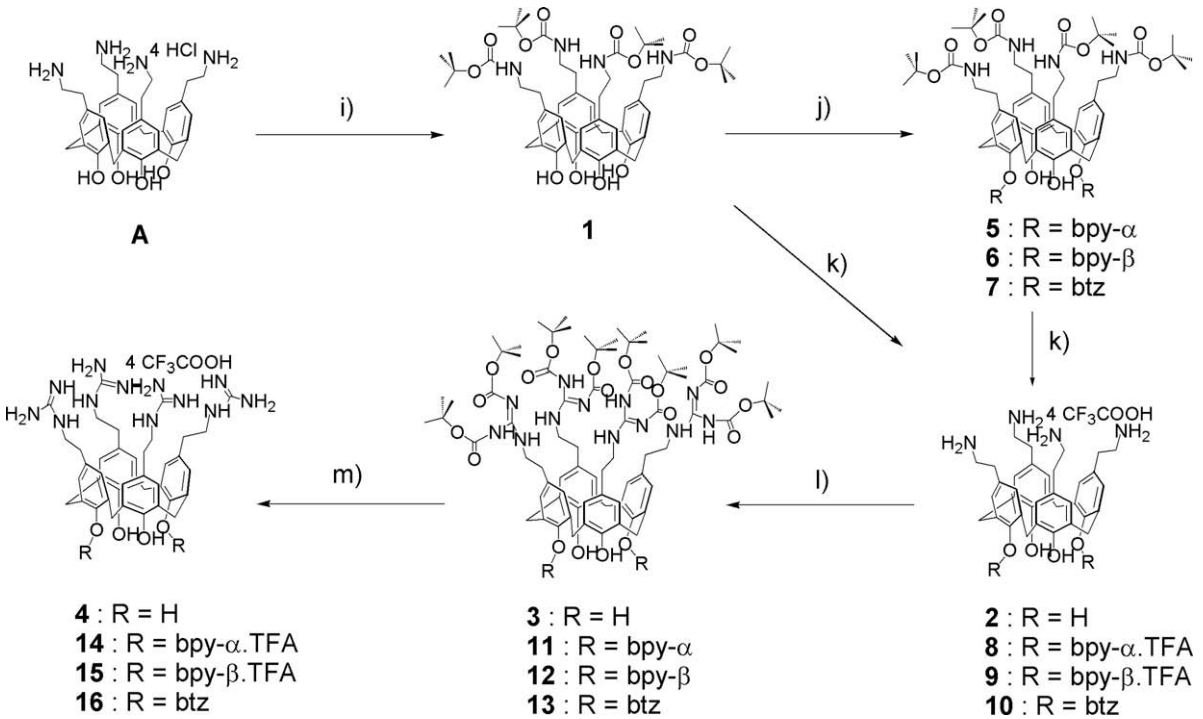
Scheme 2. Reagents and conditions: (i) **J**, NEt_3, $HgCl_2$, DMF, Ar, rt, 52%; (j) TFA, CH_2Cl_2, rt, dialysis, 70%.

pseudourea **J**, in the presence of $HgCl_2$; the resulting tetra-(Boc) species **32** was treated with TFA in CH_2Cl_2 at rt to give after dialysis and lyophilization the double trifluoroacetate salt **33**. All analyses were consistent with the proposed structure, and elemental analysis of each batch confirmed the presence of one molecule of associated water.

The hexamidine di-isetionate **34** was obtained by evaporation of solvents and lyophilization of the commercial solution, containing also 0.75 equiv of sodium acetate. The composition of each batch was monitored by ^1H NMR and elemental analysis.

2.2. Calixarenes

The tetra-*para*-guanidinoethylcalix[4]arene tetra-trifluoroacetate **4** was prepared via a four-step process from the tetra-*para*-aminoethyl-calix[4]arene **A**, obtained as tetra-hydrochloride salt according to Gutsche et al.[18] Following a standard procedure (Scheme 3), **A** was then transformed (90% yield) into its tetra-(*N*-*tert*-butoxycarbonyl)- analog **1**, easy to purify, and key compound for the development of lower-rim substituted derivatives. Compound **1** was then deprotected in acidic conditions (TFA, CH_2Cl_2) to give the tetra-*para*-aminoethyl-calix[4]arene, tetra-



Scheme 3. Reagents and conditions: (i) $(Boc)_2O$, NaOH, dioxane, H_2O, rt, ca. 12 h, 90%; (j) RCH_2Br, K_2CO_3, MeCN, reflux, Ar, 5–7 h, **5**: R = 6-yl-(6′-methyl-2,2′-bipyridine) (bpy-α), 70%; **6**: R = 5-yl-(5′-methyl-2,2′-bipyridine) (bpy-β), 61.4%; **7**: R = 4-yl-(4′-methyl-2,2′-bithiazole) (btz), 69%; (k) CH_2Cl_2, TFA, rt, **2**: R = H, 92%; **8**: R = bpy-α, 91%; **9**: R = bpy-β, 96%; **10**: R = btz, 93%; (l) **K**, NEt_3, CH_2Cl_2, MeOH, rt, 3–5 h, **3**: R = H, 65%; **11**: R = bpy-α, 76%; **12**: R = bpy-β, 84%; **13**: R = btz, 77%; (m) CH_2Cl_2, TFA, rt, **4**: R = H, 95%, **14**: R = bpy-α·TFA, 74%, **15**: R = bpy-β, 83%; **16**: R = btz, 73%. (bpy-α, bpy-β, btz: *see offset in* Scheme 4).

trifluoroacetate **2**, in 92% yield. The reaction of **2** with N_1,N_2-(di-Boc)-N_3-triflylguanidine **K** under the conditions defined by Baker et al.[19] afforded the *octa*-boc guanidino derivative **3** in 65% yield after chromatography. Attempts to prepare **3** with the *Elliot's* less expensive process resulted in the formation of multiple products not easy to separate, leading us to prefer *Baker's* procedure.

Acidic hydrolysis of **3** in TFA/CH_2Cl_2 gave the tetra-guanidino derivative **4**, in 95% yield after a quality normalization process involving multiple dialysis–lyophilization cycles.

The preparation of the bis-(2,2′-bipyridyl) and bis-(2,2′-bithiazolyl) guanidino podands **14**, **15** and **16** followed a similar strategy, starting from the tetra-*para*-(boc-aminoethyl)calix[4]arene **1**. Thus, the reaction of 6-bromomethyl-6′-methyl-2,2′-bipyridine[20a] or 5-bromomethyl-5′-methyl-2,2′-bipyridine[20b,c] or 4-bromomethyl-4′-methyl-2,2′-bithiazole[21] with **1** in dry MeCN, in the presence of K_2CO_3 as base, and following the usual stoichiometries proposed for base-strength regioselective functionalization of calix[4]arene,[22] afforded the podands **5**, **6** and **7**, respectively, with yields of 60–70%. Acidic hydrolysis of amine protecting groups was performed under the usual conditions (TFA, CH_2Cl_2), giving the tetra ammonium trifluoroacetates **8**, **9** and **10**, respectively, in almost quantitative yields.

The reaction of **8**, **9** and **10** with **K** under the conditions of Baker et al.[19] afforded in yields of ca. 75% after chromatography the *octa*-boc guanidino bipyridyl and bithiazolyl derivatives **11**, **12** and **13**, respectively. Attempts to synthesize **11**, **12** and **13** by reaction of bromomethyl heterocycles with the protected guanidino calixarene **3** gave unsatisfactory results, notably in terms of yield and ease of purification process. Acidic hydrolysis of the Boc-protective groups in TFA/CH_2Cl_2 mixture finally gave the corresponding tetraguanidinium trifluoroacetates **14**, **15** and **16**, respectively, in yields of 75–85%; a quality normalization process involving multiple dialysis–lyophilization cycles was applied in each case.

2.3. Phenol derivatives

The *para*-guanidinoethylphenol was prepared as its trifluoroacetate salt **18** by acidic hydrolysis of the *para*-N_1,N_2-di(Boc)-guanidinoethylphenol **17** in a mixture of TFA and CH_2Cl_2 at rt. Compound **17** was obtained from tyramine hydrochloride **F** in two different ways: the first one was an adaptation of the procedure of Elliot et al.[17] similar to that providing **33**, with an interesting yield of 90%; the second one consisted in the reaction of **K** with **F**, adapting the procedure of Baker et al.,[19] giving **17** with in 72% yield. As for the calixarenes, the introduction of the bipyridyl and bithiazolyl arms was done on an N-protected derivative of tyramine; for consistency in work-up procedures, the boc-protective group was chosen. The *N*-Boc-*para*-aminoethylphenol **19** was prepared by reaction of $(Boc)_2O$ and **F** under standard conditions, with a yield of ca. 70% after chromatography. The bipyridyl and bithiazolyl derivatives **20**, **21** and **22** were prepared in yields of ca. 80% after chromatography via nucleophilic attack of 6-bromomethyl-6-methyl-2,2′-bipyridine, 5-bromomethyl-5′-methyl-2,2′-bipyridine or 4-bromomethyl-4′-methyl-2,2′-bithiazole by **19** in MeCN, using K_2CO_3 as base. Standard acidic deprotection of amino groups of **20**, **21** and **22** afforded the trifluoroacetate salts **23**, **24** and **25**, in yields of 85%, 96% and 93%, respectively. The formation of the di(Boc)-guanidino derivatives **26** and **28** was carried out according to the two procedures mentioned above, with a clearly better yield when using *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine **K**. Only this procedure was employed to synthesize **27**. The acidic hydrolysis of Boc-protective groups afforded the bipyridyl derivatives **29** and **30** in yields of 80% and 95%, respectively, and the bithiazolyl derivative **31** in almost quantitative yield.

^1H, ^{13}C NMR, elemental analyses and mass spectrometry were consistent with the proposed formulas for all new compounds. According to the criterions established by de Mendoza and coworkers,[23] ^{13}C NMR spectrum of **4** (D_2O) indicated that the molecule adopts the cone conformation, showing an Ar-CH_2-Ar resonance signal at δ 30.96. Nonetheless, the corresponding protons appear as a broad singlet at 3.82 ppm, indicating that the conformation is quite mobile.[3a] The incorporation of bipyridyl and bithiazolyl arms resulted in rigidification of the calixarene platform in the cone conformation, characterized by the appearance of AB systems (J_{AB} = ca. 13 Hz) for the Ar-CH_2-Ar protons in the ^1H NMR spectra of **14**, **15** and **16**, and by corresponding ^{13}C NMR resonance signals at δ ca. 31 ppm. Elemental analysis of compounds **14** and **15** was consistent with the presence of 6 molecules of CF_3COOH, indicating the additional protonation of each bipyridyl group, while **16**, that includes the less basic bithiazolyl groups, contains 4 acid groups. Negative and/or positive mode electrospray was employed for mass spectrometric analysis of all compounds, giving in each case the expected information. As already observed for other poly-ionic calixarene species,[24] the amino- and guanidino-derivatives generally showed, here in the positive mode, a succession of groups of signals attributed to mono-, di- and tricharged species resulting from the loss of one to four CF_3COOH equilibrated by protonation.

3. Biological evaluation

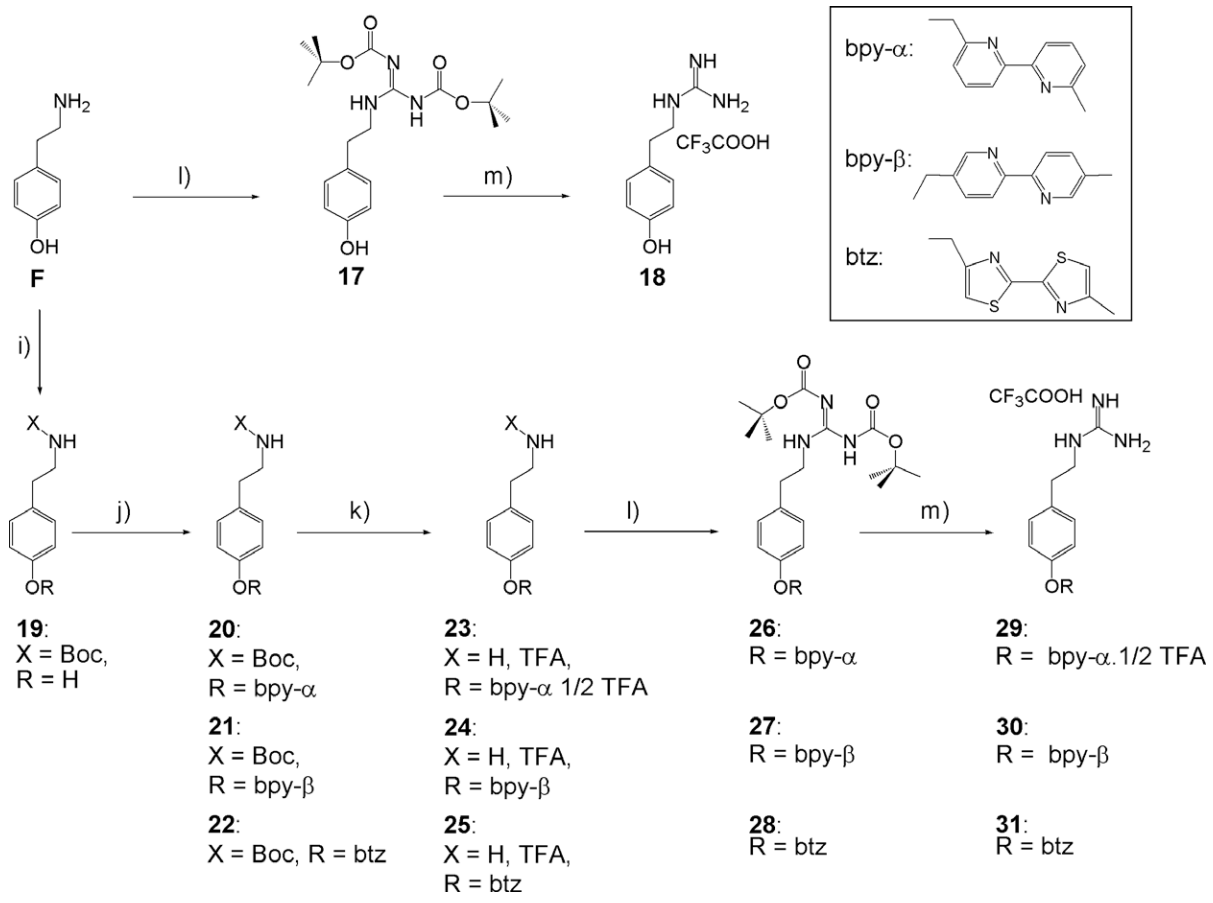
The *para*-guanidinoethylphenol **18**, monomer of **4**, has been evaluated as an uptake inhibitor of prazosin by transport-P system,[25] for treating mitochondria-associated diseases,[26] non-insulin-dependent diabetes mellitus and obesity,[27] and hypotension,[28] but no antibiotic activity evaluation had been described until our previous report.[3a]

In this study, in vitro antibacterial activity [e.g., minimum inhibition concentration (MIC) determination] of compounds **4**, **14**, **15** and **16** was evaluated and compared to their corresponding monomers **18**, **29**, **30** and **31**. Hexamidine **34** and 1,10-bis-guanidinodecane (Synthalin A) **33** were chosen as reference compounds. MICs were determined for two Gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and three Gram positive (*Staphylococcus aureus* ATCC 25923 and ATCC 29213, *Enterococcus faecalis* ATCC 29212) reference strains, by broth microdilution method recommended by the CLSI.[29] Due to its presence in high amounts in calixarenes species, the trifluoroacetate anion was also evaluated; in fact, sodium trifluoroacetate did not exhibit any activity at concentrations inferior to 256 μg mL^{-1}.

Drugs were also tested for their effect on eukariotic cell viability by MTT assays on MRC-5 cells (human pulmonary embryonic fibroblasts), or by LDH assays in order to determine IC_{50} (inhibitory concentration 50%) and deduce selectivity indexes (SI), at 24, 48 and 168 h exposure to drugs.

As shown in Table 1, the MIC values obtained for the calixarene species **14**, **15** and **16** remain in the micromolar range, close to the unsubstituted analog **4** and to hexamidine **34**, but with a better homogeneity with regards to the Gram character of the bacterial strains. The introduction of the bipyridyl and bithiazolyl arms at the lower rim of the calixarene platform does not result in a strong modification of the apparent antibacterial behaviour, with regards to **4**.

Passing to the monomeric species **29**, **30** and **31**, and depending on the bacterial strain, this behaviour changes drastically. These compounds display a variable but sometimes very interesting antibacterial activity, notably against the two *S. aureus* strains, and, for **30**, against *E. coli*. Compared to the unsubstituted analog **18**, the presence of the biheterocyclic arms appears to generate an important gain of activity against all of the tested strains.



Scheme 4. Reagents and conditions: (i) $(Boc)_2O$, NaOH, dioxane, H_2O, rt, ca. 12 h, 73%; (j) RCH_2Br, K_2CO_3, MeCN, reflux, Ar, 5 h, **20**: R = 6-yl-(6′-methyl-2,2′-bipyridine) (bpy-α), 82%; **21**: R = 5-yl-(5′-methyl-2,2′-bipyridine) (bpy-β), 80%; **22**: R = 4-yl-(4′-methyl-2,2′-bithiazole) (btz), 85%; (k) CH_2Cl_2, TFA, rt, **23**: R = bpy-α·1/2 TFA, 86%; **24**: R = bpy-β, 96%; **25**: R = btz, 95%; (l) way 1: **K**, NEt_3, CH_2Cl_2, MeOH, rt, 2–4 h, **17**: R = H, 72%; **26**: R = bpy-α, 85%; **27**: R = bpy-β, 84%; **28**: R = btz, 95%; way 2: **J**, NEt_3, $HgCl_2$, DMF, rt, Ar, **17**: R = H, 90%; **26**: R = bpy-α, 64%; **28**: R = btz, 60%; (m) CH_2Cl_2, TFA, rt, **18**: R = H, 90%; **29**: R = bpy-α·1/2 TFA, 82%; **30**: R = bpy-β, 95%; **31**: R = btz, 98%.

Table 1
MIC values (μg mL^{-1} and (10^{-6} mol L^{-1})) obtained by broth microdilution method, according to CLSI guideline

	E. coli ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213	*E. faecalis* ATCC 29212
Minimum inhibitory concentration (MIC) in μg mL^{-1} (10^{-6} mol L^{-1})					
4 (*1239.0*)	4 (3.2)	32 (26.0)	8 (6.5)	8 (6.5)	32 (26.0)
18 (*293.24*)	512 (1750.0)	>512 (1750.0)	512 (1750.0)	512 (1750.0)	>512 (1750.0)
14 (*1867.53*)	16 (8.6)	16 (8.6)	16 (8.6)	16 (8.6)	16 (8.6)
29 (*532.47*)	128 (240.0)	128 (240.0)	16 (30.0)	8 (15.0)	64 (120.2)
15 (*1885.56*)	32 (17)	32 (17)	8 (4.2)	8 (4.2)	8 (4.2)
30 (*475.46*)	8 (16.8)	128 (270)	8 (16.8)	4 (8.4)	64 (135)
16 (*1695.49*)	16 (9.5)	16 (9.5)	8 (4.7)	16 (9.5)	8 (4.7)
31 (*487.52*)	64 (131.3)	128 (262.5)	16 (33.0)	8 (16.4)	64 (131.3)
33 (*498.45*)	64 (128.4)	128 (256.8)	64 (128.4)	16 (32.0)	256 (513.6)
34 (*668.22*)	8 (12.0)	32 (47.9)	4 (6.0)	<1 (1.5)	2 (3.0)

Molecular weights in italic.

The analysis of molar or massic MIC ratios (Table 2; monomer vs calixarene) confirms the smaller impact of the calixarene structure on the antibacterial activity, for the group of the substituted species.

As shown in Table 3, the bipyridyl and bithiazolyl analogs **14**, **16**, **29**, **31** and, to a lesser extent, **15** and **30**, have an impact on MRC-5 cells viability stronger than **4** and **18**, reaching the IC_{50} of hexamidine **34** and Synthalin A **33** at 24 h.

The calculated selectivity indexes SI, that is, IC_{50} at 24 h/MIC reported in Table 4, thus appear less interesting for the substituted species in the calixarene family, and sometimes better for the

Table 2
Massic (mr) and molar (Mr) MIC ratios between calixarene species and their corresponding monomers

	18/4		29/14		30/15		31/16	
	mr	Mr	mr	Mr	mr	Mr	mr	Mr
E. coli ATCC 25922	128	547	8	28	0.25	1	4	14
P. aeruginosa ATCC 27853	>16	>67	8	28	4	16	8	28
S. aureus ATCC 25923	64	269	1	3.5	1	4	2	7
S. aureus ATCC 29213	64	269	0.5	1.7	0.5	2	0.5	1.7
E. faecalis ATCC 29212	>16	>67	4	14	8	32	8	28

Table 3
IC_{50} values obtained with viability assays (MTT; [a]:LDH)

	4	**18**	**14**	**29**	**15**[a]	**30**[a]	**16**	**31**	**33**	**34**
	1239.0[b]	293.2	1867.5	532.5	1885.5	475.5	1695.5	487.5	498.5	668.2
IC_{50} ($\mu g\ mL^{-1}$)										
24 h	256<	256<	16–32	32–64	>128	>128	16–32	64–128	128–256	32–64
48 h	256<	256<	16–32	16–32	>128	>128	16–32	32–64	8–16	16–32
168 h	128–256	32-64	16–32	2–4	nd	nd	16–32	8–16	<1	4–8

Compounds were added at concentrations from 256 to 1 $\mu g\ mL^{-1}$ for 24, 48 and 168 h. Each value is representative of three independent determinations. nd: not determined.
[b]Molecular weights in g mol^{-1}.

Table 4
Selectivity indexes calculated for the five reference strains, after 24 h exposure of MRC-5 cells to the compounds of the study

	E. coli ATCC 25922	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213	*E. faecalis* ATCC 29212
Selectivity indexes IC_{50} 24 h/MIC					
4	>64	>8	>32	>32	>8
18	>0.5	0.5	>0.5	>0.5	0.5
14	1–2	1–2	1–2	1–2	1–2
29	0.25–0.5	0.25–0.5	2–4	4–8	0.5–1
15	>4	>4	>16	>16	>16
30	16	>1	>16	>32	>2
16	1–2	1–2	2–4	1–2	2–4
31	1–2	0.5–1	4–8	8–16	1–2
33	2–4	1–2	2–4	8–16	0.5–1
34	4–8	1–2	8–16	32–64	16–32

monomers (cases of **30** and **31**). Nevertheless, the bipyridyl calixarene **15** exhibits a relatively pronounced selectivity towards Gram positive strains, with SI >16.

It is interesting to note (Table 3) that the toxicity of all calixarene species remains stable at 48 and 168 h, while the monomers, Synthalin A and hexamidine become more toxic, resulting for the latter in lower selectivity indexes. This fact can be of great importance when considering differences in eukaryotic and prokaryotic cell growth kinetics, or when considering long-lasting decontamination processes. This point is the subject of current specific investigations.

4. Conclusion

The results obtained in this study show that the introduction of two bipyridyl or bithiazolyl arms at the lower rim of the tetra-*para*-guanidinoethyl-calix[4]arene platform does not greatly modify the antibacterial properties of the lead compound **4**, maintaining the micromolar activity levels encountered for commercial products for both the reference Gram positive and negative strains.

These new calixarene compounds have a stronger impact on eukariotic cell viability at 24 h, thus diminishing their selectivity indexes; nevertheless, the fact that this toxicity remains stable for calixarenes **4**, **14**, **15** and **16** at 48 and 168 h, a contrario to reference compounds, reinforces the general interest of these structures for the building of a new family of antibacterial agents.

As for the initially-studied unsubstituted derivatives, but to a lesser extent, our results tend to confirm the organizational role of the calixarene core, that tethers close together and arrays at its upper rim four guanidinium groups, for the genesis of an antibacterial activity. At this stage of the study, and according to previous results, this seems indicative of a synergistic effect in ionic interactions with the membrane targets. Nevertheless, the introduction of the biheterocyclic substituents on the single phenol unit (monomers) generates an antibacterial activity unforeseen with the unsubstituted analog. This may be attributable to either the amphiphilic character of the new monomeric species, or to a specific activity of the water-solubilized biheterocyclic subunits. In this sense, such properties could be involved in the structure–activity relationships of the calixarene family, but seem only translated by a gain in cellular toxicity.

The development of new derivatives and investigations devoted to the mode of antibacterial action of these two new families of compounds are now under way.

5. Experimental

5.1. Chemistry

5.1.1. General remarks

Melting points (°C, uncorrected) were determined on an Electrothermal 9200 in Capillary apparatus. ^1H and ^{13}C spectra were recorded on a Bruker DRX 400 (chemical shifts in ppm). Mass spectra (electronic ionization—EI, and electrospray—ES) were recorded on a Micromass Platform II apparatus, at the Service Commun de Spectrométrie de Masse Organique, Nancy. Infrared measurements were performed on a Vector 22 Bruker FT apparatus (KBr, ν in cm^{-1}) and UV spectra were recorded a SAFAS *UV mc*^{2} apparatus, λ_{max} in nm, ε in $dm^3\ mol^{-1}\ cm^{-1}$. Elemental analyses were performed at the Service de Microanalyse, Nancy. Merck TLC plates were used for chromatography analysis (SiO_2, ref 1.05554; Al_2O_3, ref 1.05581). All commercially available products were used without further purification unless otherwise specified.

5.1.2. 5,11,17,23-Tetra-(Boc-aminoethyl)-25,26,27,28-calix[4]arene tetrol 1

1 M aqueous NaOH (14 mL, 14 mmol) was added to a solution of **A** (0.83 g, 1.05 mmol) in a mixture of dioxane and H_2O (40 and 24 mL, respectively). After stirring during 10 min, di-*tert*-butyldicarbonate (Boc_2O) (0.97 g, 4.44 mmol) was added. The resulting solution was stirred overnight at rt and under Ar (TLC monitoring: Al_2O_3, CH_2Cl_2/MeOH, 98:2). The solution was concentrated under vacuum, and AcOEt (40 mL) was added; the pH was brought to 8 with 1 M HCl, and the organic phase was recovered. The aqueous phase was extracted with AcOEt (2 × 30 mL), and the combined organic phases were dried over Na_2SO_4, and evaporated to dryness. The resulting oily material was chromatographed (Al_2O_3, CH_2Cl_2/MeOH, 99:1) to give **1** (0.93 g, 90%). White solid. Mp: 131–131 °C. IR (KBr): 3351.6 (OH); 1701.3 (CO); 1509.6 (NH). UV–vis (CH_2Cl_2): 280 (31,186). ^1H NMR (400 MHz, $CDCl_3$): 1.27 (m, 36H, Me_3C); 2.59 (t, J = 6.6 Hz, 8H, CH_2CH_2N); 3.27 (br s, 8H, CH_2CH_2N); 3.44–4.21 (AB, J_{AB} = 13.6 Hz; 8H, $ArCH_2Ar$); 6.86 (s, 8H, Ar*H*); 10.26 (s, 4H, O*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.64 (Me_3C); 32.34 ($ArCH_2Ar$); 35.73 (CH_2CH_2N); 42.17 (CH_2CH_2N); 79.54 (Me_3C); 128.57 (C_o); 129.91 (C_m); 132.96 (C_p); 147.75 (C_{ipso}); 156.40 (*C*O). Anal. Calcd for $C_{56}H_{76}O_{12}N_4$ (997.31): C, 67.44; H, 7.68; N, 5.61. Found: C, 67.43; H, 7.35; N, 5.61. ES-MS (pos. mode): 1019.08 $[M+Na^+]^+$. ES-MS (neg. mode): 995.15 $[M-H^+]^-$.

5.1.3. Compounds 5, 6 and 7—general procedure

A mixture of **1** (1 equiv) and K_2CO_3 (1 equiv) in dry MeCN was refluxed under Ar during 0.5 h. The bromoalkyl reactant (2 equiv)

was then added, and reflux was maintained during × h (TLC monitoring, Al_2O_3, CH_2Cl_2/MeOH 98:2). After cooling to rt, the solvent was evaporated to dryness, and the solid residue was dissolved in CH_2Cl_2, filtered, then chromatographed to give the pure podand.

5.1.3.1. 5,11,17,23-Tetra-(Boc-aminoethyl)-25,27-bis(6-methyleneoxy-6′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene 5. From **1** (0.1 g, 0.1 mmol), K_2CO_3 (0.014 g, 0.1 mmol), MeCN (10 mL), 6-bromomethyl-6′-methyl-2,2′-bipyridine (0.053 g, 0.2 mmol); 5 h (TLC monitoring, Al_2O_3, CH_2Cl_2/MeOH 98:2); chromatography: Chromatotron, Al_2O_3, CH_2Cl_2; **5** (0.09 g, 70%). White powder. Mp: 114–115 °C. IR (KBr): 3367.5 (OH); 1707.9 (CO). UV–vis (CH_2Cl_2): 290 (47737). ^{1}H NMR (400 MHz, $CDCl_3$): 1.41–1.43 (m, 36H, *Me*$_3$C); 2.43 (m, 4H, C*H*$_2$CH$_2$N of **B**); 2.61 (s, 6H, *Me* bpy); 2.69 (m, 4H, C*H*$_2$CH$_2$N of **A**); 3.11 (m, 4H, CH$_2$C*H*$_2$N of **B**); 3.33 (m, 4H, CH$_2$C*H*$_2$N of **A**); 3.34–4.38 (AB, J_{AB} = 13.2 Hz, 8H, ArC*H*$_2$Ar); 4.50–4.62 (m, 4H, N*H*); 5.21 (s, 4H, OC*H*$_2$bpy); 6.72 (s, 4H, Ar*H* of **B**); 6.89 (s, 4H, Ar*H* of **A**); 7.04 (d, J = 7.5 Hz, 2H, $H_{5'}$ bpy); 7.54 (t, J = 7.5 Hz, 2H, $H_{4'}$ bpy); 7.61 (d, J = 7.5 Hz, 2H, H_4 bpy); 7.76 (br s, 2H, O*H*); 8.04 (d, J = 7.6 Hz, 2H, H_5 bpy); 8.16 (d, J = 7.8 Hz, 2H, $H_{3'}$ bpy); 8.35 (d, J = 7.8 Hz, 2H, H_3 bpy). ^{13}C NMR (100 MHz, $CDCl_3$): 25.00 (*Me* bpy); 28.89 (C*Me*$_3$); 31.98 (ArCH$_2$Ar); 35.69 (*C*H$_2$CH$_2$N of **A**); 36.13 (*C*H$_2$CH$_2$N of **B**); 42.24 (CH$_2$*C*H$_2$N of **B**); 42.63 (CH$_2$*C*H$_2$N of **B**); 79.16 (O*C*H$_2$bpy); 79.47 (*C*Me$_3$); 118.70 ($C_{3'}$ bpy); 120.51 (C_3 bpy); 121.51 (C_5 bpy); 123.75 ($C_{5'}$ bpy); 128.27 (C_o of **A**); 129.37 (C_m of **A**); 129.77 (C_p of **A**); 129.86 (C_m of **B**); 133.48 (C_o of **B**); 136.26 (C_p of **B**); 137.45 ($C_{4'}$ bpy); 128.50 (C_4 bpy); 151.19 (C_{ipso} of **B**); 152.26 (C_{ipso} of **A**); 155.68 (C_6 bpy); 156.22 ($C_{6'}$ bpy); 156.39 (*C*OO of **A** and **B**); 156.68 (C_2 bpy); 158.23 ($C_{2'}$ bpy). Anal. Calcd for $C_{80}H_{96}O_{12}N_8{\cdot}0.5H_2O$ (1370.68): C, 70.10; H, 7.13; N, 8.17. Found: C, 68.82; H, 6.93; N, 8.12. ES-MS (pos. mode): 681.5 $[M+2H]^{2+/2}$, 692.9 $[M+H+Na]^{2+/2}$, 703.5 $[M+2Na]^{2+/2}$, 711.4 $[M+Na+K]^{2+/2}$, 1361.9 $[M+H]^+$, 1384.0 $[M+Na]^+$, 1400.7 $[M+K]^{2+}$.

5.1.3.2. 5,11,17,23-Tetra-(Boc-aminoethyl)-25,27-bis(5-methyleneoxy-5′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene 6. From **1** (0.3 g, 0.3 mmol), K_2CO_3 (0.105 g, 0.8 mmol), MeCN (30 mL), 5-bromomethyl-5′-methyl-2-2′-bipyridine (0.158 g, 0.6 mmol); 4 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH 98:2); chromatography: Al_2O_3, CH_2Cl_2; **6** (0.251 g, 61.4%). White powder. Mp: 127–128 °C. IR (KBr): 3365.02 (OH); 1708.98 (CO). UV–vis (CH_2Cl_2): 290 (68,214). ^{1}H NMR (400 MHz, $CDCl_3$): 1.42 (br s, 36H, *Me*$_3$C); 3.13 (m, J = 5.8 Hz; 4H, CH$_2$C*H*$_2$N of **B**); 3.27 (m, 4H, C*H*$_2$C*H*$_2$N of **A**); 3.26–4.20 (AB, J_{AB} = 13.08 Hz, 8H, ArC*H*$_2$Ar); 5.14 (s, 4H, OC*H*$_2$bpy); 6.72 (s, 4H, Ar*H* of **B**); 6.83 (s, 4H, Ar*H* of **A**); 7.51 (dd, J = 7.2 Hz, 2H, $H_{4'}$ bpy); 7.61 (br s, 2H, O*H*); 8.12 (dd, J = 8.04 Hz, 2H, $H_{3'}$ bpy); 8.23 (d, J = 8.04 Hz, 2H, H_4 bpy); 8.38 (d, J = 7.6 Hz, 2H, H_3 bpy); 8.39 (s, 2H, $H_{6'}$ bpy); 8.87 (s, 2H, H_6 bpy). ^{13}C NMR (100 MHz, $CDCl_3$): 18.72 (*Me*bpy); 28.86 *Me*$_3$C; 32.14 (ArCH$_2$Ar); 35.64, 36.18 (*C*H$_2$CH$_2$N); 42.25, 42.52 (CH$_2$CH$_2$N); 76.09 (OCH$_2$bpy); 79.45 (Me$_3$*C*); 121.14, 121.27, 136.95, 137.72, 148.82, 149.83 (C_3, $C_{3'}$, C_4, $C_{4'}$, C_6, $C_{6'}$, of bpy); 129.27, 129.89 (C_m of Ar); 128.26, 132.20, 133.68, 136.39, 150.90 (C_o and C_p of Ar, C_5, $C_{5'}$, of bpy); 151.96, 153.56 (C_i of Ar); 156.25 (*C*OO); 156.40, 156.52 (C_2, $C_{2'}$, of bpy). Anal. Calcd for $C_{80}H_{96}N_8O_{12}{\cdot}0.25CH_2Cl_2$ (1444.66): C, 69.70; H, 7.03; N, 8.10. Found: C, 69.87; H, 6.98; N, 8.09. ES-MS (pos. mode): 1361.5 $[M+H]^+$, 1383.5 $[M+Na]^+$, 1389.2 $[3M+3H+CH_2Cl_2]^{3+/3}$.

5.1.3.3. 5,11,17,23-Tetra-(Boc-aminoethyl)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 7. From **1** (0.33 g, 0.33 mmol), K_2CO_3 (0.046 g, 0.33 mmol), MeCN (15 mL), 4-bromomethyl-4′-methyl-2,2′-bithiazole (0.182 g, 0.66 mmol); 7 h (TLC monitoring, Al_2O_3, CH_2Cl_2/MeOH 98:2); chromatography: Al_2O_3, CH_2Cl_2; **7** (0.28 g, 69%). Yellow powder. Mp: 128–129 °C. IR (KBr): 3370.9 (OH); 1704.7 (CO); 1509.8 (NH). UV–vis (CH_2Cl_2): 291 (23,731); 330 (34,366). ^{1}H NMR (400 MHz, $CDCl_3$): 1.46 (m, 36H, *Me*$_3$C); 2.48 (br t, J = 7.1 Hz, 4H, C*H*$_2$CH$_2$N of **B**); 2.54 (s, 6H, *Me*btz); 2.67 (br t, J = 6.9 Hz, 4H, C*H*$_2$CH$_2$N of **A**); 3.14 (br t, J = 6.5 Hz, 4H, CH$_2$C*H*$_2$N of **B**); 3.30 (m, 4H, CH$_2$C*H*$_2$N of **A**); 3.32–4.30 (AB, J_{AB} = 13.1 Hz, 8H, ArC*H*$_2$Ar); 4.55 (br s, 2H, N*H* of **A** or **B**); 4.66 (br s, 2H, N*H* of **A** or **B**); 5.23 (s, 4H, OC*H*$_2$ btz); 6.73 (s, 4H, Ar*H* of **B**); 6.87 (s, 4H, Ar*H* of **A**); 7.00 (s, 2H, $H_{5'}$ btz); 7.70 (br s, 2H, O*H*); 7.96 (s, 2H, H_5 btz). ^{13}C NMR (100 MHz, $CDCl_3$): 17.54 (*Me* btz); 28.89 (C*Me*$_3$); 31.91 (ArCH$_2$Ar); 35.67 (*C*H$_2$CH$_2$N of **A**); 36.17 (*C*H$_2$CH$_2$N of **B**); 42.21 (CH$_2$*C*H$_2$N of **B**); 42.56 (CH$_2$*C*H$_2$N of **A**); 74.57 (O*C*H$_2$btz); 79.50 (*C*Me$_3$); 116.40 ($C_{5'}$ btz); 118.28 (C_5 btz); 128.23 (C_p of **A**); 129.40 (C_m of **A**); 129.88 (C_m of **B**); 129.99 (C_o of **B**); 133.55 (C_o of **A**); 136.45 (C_p of **B**); 150.93 (C_{ipso} of **B**); 152.03 (C_{ipso} of **A**); 154.02 (C_2 btz); 154.75 ($C_{2'}$ btz); 156.24 (*C*OO of **B**); 156.39 (*C*OO of **A**); 160.76 ($C_{4'}$ btz); 162.27 (C_4 btz). Anal. Calcd for $C_{72}H_{88}O_{12}N_8S_4{\cdot}2CH_2Cl_2$ (1552.45): C, 57.13; H, 5.96; N, 7.20; S, 8.25. Found: C, 56.97; H, 5.49; N, 7.27; S, 8.53. ES-MS (pos. mode): 1406.6 $[M+Na^+]^+$.

5.1.4. Compounds 2, 8, 9 and 10—general procedure

A solution of tetra-boc aminoethyl calixarene derivative in dry CH_2Cl_2 and TFA was stirred at rt under Ar during ca. 3 h. The solvent were evaporated, and the residual TFA was eliminated by five dissolution in CH_2Cl_2–evaporation cycles. The semi-solid residual material was dried under high vacuum, then triturated in dry Et_2O to give the corresponding salt.

5.1.4.1. 5,11,17,23-Tetra-(aminoethyl)-25,26,27,28-calix[4]arene tetrol, tetra-trifluoroacetate salt 2. From **1** (0.53 g, 0.54 mmol), CH_2Cl_2 (40 mL), TFA (10 mL); **2** (0.53 g, 92%). White precipitate. Mp: >200 °C (dec). IR (KBr): 3144.7 (OH); 1686.6 (COO). UV–vis (H_2O): 284 (9675). ^{1}H NMR (400 MHz, DMSO-d_6): 2.59 (t, J = 7.9 Hz, 8H, C*H*$_2$CH$_2$N), 2.92 (br s, 8H, CH$_2$C*H*$_2$N); 3.67 (br s, 8H, ArC*H*$_2$Ar), 6.91 (s, 8H, Ar*H*), 7.84 (br s, 12H, N*H*$_3$). ^{1}H NMR (400 MHz, D_2O): 2.66 (t, J = 7.1 Hz, 8H, C*H*$_2$CH$_2$N); 3.04 (t, J = 7.3 Hz, 8H, CH$_2$C*H*$_2$N); 3.83 (br s, 8H, ArC*H*$_2$Ar); 6.97 (s, 8H, Ar*H*). ^{13}C NMR (100 MHz, D_2O): 31.01 (Ar-*C*H$_2$-Ar); 32.25 (ArCH$_2$CH$_2$N); 40.83 (ArCH$_2$CH$_2$N); 116.78 (q, J = 291.97 Hz, *C*F$_3$COOH); 128.98 (C_m); 129.32 (C_o); 130.62 (C_p); 147.80 (C_{ipso}); 163.07 (q, J = 35.21 Hz, *C*F$_3$COOH). Anal. Calcd for $C_{36}H_{44}O_4N_4{\cdot}4CF_3COOH{\cdot}H_2O$ (1070.86): C, 49.35; H, 4.70; N, 5.23. Found: C, 49.31; H, 4.85; N, 5.30. ES-MS (pos. mode): 711.00 $[M-3CF_3COOH+H^+]^+$. ES-MS (neg. mode): 1050.60 $[M-H^+]^-$, 936.76 $[M-CF_3COOH-H^+]^+$, 822.99 $[M-2CF_3COOH-H^+]^+$, 709.03 $[M-3CF_3COOH-H^+]^+$, 595.19 $[M-4CF_3COOH-H^+]^+$.

5.1.4.2. 5,11,17,23-Tetra-(aminoethyl)-25,27-bis(6-methyleneoxy-6′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene, hexa-trifluoroacetate salt 8. From **5** (0.15 g, 0.119 mmol), CH_2Cl_2 (15 mL), TFA (5 mL); **8** (0.17 g, 91%). White precipitate. Mp: 100–101 °C. IR (KBr): 3413.3 (OH); 2934.8 (NH_3^+); 1677.9 (COO). UV–vis (H_2O): 286 (28,048). ^{1}H NMR (400 MHz, D_2O): 2.53 (s, 6H, *Me*bpy); 2.62 (t, J = 7.3 Hz, 4H, C*H*$_2$CH$_2$N of **A**); 2.88 (t, J = 7.5 Hz, 4H, C*H*$_2$CH$_2$N of **B**); 3.03 (t, J = 7.5 Hz, 4H, CH$_2$C*H*$_2$N of **A**); 3.21 (t, J = 7.5 Hz, 4H, CH$_2$C*H*$_2$N of **B**); 3.56–4.33 (AB, J_{AB} = 13.1 Hz, 8H, ArC*H*$_2$Ar); 5.13 (s, 4H, OC*H*$_2$ bpy); 6.98 (s, 4H, Ar*H* of **B**); 7.19 (s, 4H, Ar*H* of **A**); 7.58 (d, J = 8.3 Hz, 2H, H_5 bpy); 7.85 (d, J = 8.8 Hz, 2H, $H_{5'}$ bpy); 7.91 (t, J = 7.8 Hz, 2H, H_4 bpy); 8.16 (d, J = 7.8 Hz, 2H, H_3 bpy); 8.27 (d, J = 8.1 Hz, 2H, $H_{3'}$ bpy); 8.48 (t, J = 7.8 Hz, 2H, $H_{4'}$ bpy). ^{1}H NMR (400 MHz, DMSO-d_6): 2.54 (s, 6H, C*H*$_3$ bpy); 2.60 (t, J = 8.3 Hz, 4H, C*H*$_2$CH$_2$N of **A** or of **B**); 2.68 (t, J = 8.0 Hz, 4H, C*H*$_2$CH$_2$N of **A** or **B**); 2.96 (m, 8H, CH$_2$C*H*$_2$N of **A** or **B**); 3.44–4.31 (AB, J_{AB} = 12.8 Hz, 8H, ArC*H*$_2$Ar); 5.14 (s, 4H, OC*H*$_2$ bpy); 6.99 (s, 4H, Ar*H* of **A** or **B**); 7.06 (s, 4H, Ar*H* of **A** or **B**); 7.26 (d, J = 7.6 Hz, 2H, *H* bpy); 7.66 (t, J = 7.8 Hz, 2H, *H* bpy); 7.70 (t, J = 7.8 Hz, 2H, *H* bpy); 7.77 (d, J = 7.5 Hz, 2H, *H* bpy); 7.88 (br s, 12H, N*H*); 8.19 (d, J = 7.8 Hz, 2H, *H* bpy); 8.32 (d, J = 7.8 Hz, 2H, *H* bpy); 8.40 (s, 2H,

O*H*). ^{13}C NMR (100 MHz, D_2O): 19.44 (*Me*bpy); 30.92 (ArCH_2Ar); 32.31 (CH_2CH_2N of **A**); 32.36 (CH_2CH_2N of **B**); 40.57 (CH_2CH_2N of **A**); 41.03 (CH_2CH_2N of **B**); 77.12 (OCH_2bpy); 116.71 (q, J = 291.8 Hz, CF_3COO^-); 122.37 ($C_{3'}$ bpy); 122.48 (C_3 bpy); 125.13 (C_5 bpy); 128.43 ($C_{5'}$ bpy); 129.02 (C_p of **B**); 129.11 (C_o of **A** or **B**); 129.68 (C_m of **A**); 129.85 (C_m of **B**); 134.57 (C_o of **A** or **B**); 134.75 (C_p of **A**); 140.13 (C_4 bpy); 146.55 (C_6 bpy); 147.18 ($C_{4'}$ bpy); 147.54 ($C_{6'}$ bpy); 150.75 (C_{ipso} of **B**); 151.20 (C_{ipso} of **A**); 155.19 ($C_{2'}$ bpy); 156.99 (C_2 bpy); 163.21 (q, J = 35.2 Hz, CF_3COO^-). Anal. Calcd for $C_{60}H_{64}O_4N_8{\cdot}6CF_3COOH{\cdot}4H_2O$ (1717.38): C, 50.35; H, 4.57; N, 6.52. Found: C, 50.39; H, 4.43; N, 6.76. ES-MS (pos. mode): 1189.97 $[M-2CF_3COOH+H^+]^+$, 1074.93 $[M-3CF_3COOH+H^+]^+$, 481.47 $[M-4CF_3COOH+2H^+]^{2+/2}$, 321.51 $[M-4CF_3COOH+3\ H^+]^{3+/3}$.

5.1.4.3. 5,11,17,23-Tetra-(aminoethyl)-25,27-bis(5-methyleneoxy-5′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene, hexatrifluoroacetate salt 9. From **6** (0.2 g, 0.15 mmol), CH_2Cl_2 (20 mL), TFA (7 mL); **9** (0.199 g, 96%). Slightly pink solid. Mp: 129–130 °C. IR (KBr): 3400.2 (OH); 2900.8 (NH_3^+); 1660.1 (COO). UV–vis (H_2O): 286 (38,304). ^{1}H NMR (400 MHz, D_2O) 2.41 (s, 6H, *Me*bpy); 2.65 (t, J = 7.42 Hz, 4H, CH_2CH$_2$NH of **A**); 2.87 (t, J = 7.3 Hz, 4H, CH_2CH$_2$N of **B**); 3.04 (t, J = 7.54 Hz, 4H, CH_2CH$_2$N of **B**); 3.22 (t, J = 7.3 Hz, 4H, CH$_2$CH_2N of **B**); 3.55–4.21 (AB, J_{AB} = 13.6 Hz, 8H, ArCH_2Ar); 5.23 (s, 4H, OCH_2 bpy); 6.97 (s, 4H, Ar*H* of **B**); 7.16 (s, 4H, Ar*H* of **A**); 7.82–7.89 (m, 2$H_{4'}$ and 4$H_{3',3}$ of bpy); 8.26 (s, 2H, $H_{6'}$ bpy); 8.37 (d, J = 7.8 Hz, 2H, H_4 bpy); 9.03 (s, 2H, H_6 bpy). ^{13}C NMR (100 MHz, D_2O): 17.74 (*Me*bpy); 30.93 (ArCH$_2$Ar); 32.25, 32.29 (CH_2CH_2N); 40.47, 41.00 (CH_2CH_2N); 74.47 (OCH_2bpy); 116.71 (q, J = 291.8 Hz, CF_3COO^-); 122.00, 122.31, 129.55, 129.93, 137.78, 142.94, 145.55, 146.68 (C_3, $C_{3'}$, C_4, $C_{4'}$, C_6, $C_{6'}$, of bpy and C_m of Ar); 128.11, 128.70, 133.79, 135.00, 135.58, 137.69, 147.10, 149.05, 150.26, 151.30 (C_2, $C_{2'}$, C_5, $C_{5'}$ of bpy; C_p, C_o and C_i of Ar); 163.21 (q, J = 35.2 Hz, CF_3COO^-). Anal. Calcd for $C_{60}H_{64}O_4N_8{\cdot}6CF_3COOH{\cdot}1.5H_2O$ (1671.48): C, 51.71; H, 4.40; N, 6.70. Found: C, 51.69; H, 4.19; N, 6.87. ES-MS (pos. mode): 1075.3 $[M-3CF_3COOH+H^+]^+$, 961.3 $[M-4CF_3COOH+\ H^+]^+$, 481.29 $[M-4CF_3COOH+2H^+]^{2+/2}$.

5.1.4.4. 5,11,17,23-Tetra-(aminoethyl)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra-trifluoroacetate salt 10. From **7** (0.7 g, 0.56 mmol), CH_2Cl_2 (40 mL), TFA (15 mL); **10** (0.75 g, 93%). Pale yellow powder. Mp: 90–91 °C. IR (KBr): 3412.3 (OH); 2929.6 (NH_3^+); 1678.5 (NH_3^+). UV–vis (D_2O): 289 (8521); 330 (21,231). ^{1}H NMR (400 MHz, D_2O): 2.66 (s, 6H, CH_3 btz); 2.58 (t, J = 7.2 Hz, 4H, CH_2CH$_2$N of **A** or **B**); 2.88 (t, J = 7.4 Hz, 4H, CH_2CH$_2$N of **A** or **B**); 3.01 (t, J = 7.3 Hz, 4H, CH$_2$CH_2N of **A** or **B**); 3.25 (t, J = 7.3 Hz, 4H, CH$_2$CH_2N of **A** or **B**); 3.49–4.35 (AB, J_{AB} = 13.6 Hz, 8H, ArCH_2Ar); 4.98 (s, 4H, OCH_2btz); 6.85 (s, 4H, Ar*H* of **B**); 7.10 (s, 6H, Ar*H* of **A** and $H_{5'}$ btz); 7.69 (s, 2H, H_5 btz). ^{1}H NMR (400 MHz, DMSO-d_6): 2.46 (s, 6H, CH_3 btz); 2.62 (t, J = 7.7 Hz, 4H, CH_2CH$_2$N of **B**); 2.69 (t, J = 7.6 Hz, 4H, CH_2CH$_2$N of **A**); 2.97 (CH$_2$CH_2N of **A** and **B**) 3.42–4.27 (AB, J_{AB} = 12.8 Hz, 8H, ArCH_2Ar); 6.99 (s, 4H, Ar*H* of **B**); 7.06 (s, 4H, Ar*H* of **A**); 7.49 (s, 2H, $H_{5'}$ btz); 7.98 (m, 12H, H_5 btz and NH_3); 8.15 (s, 2H, O*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 17.02 (*Me* btz); 30.94 (ArCH$_2$Ar); 32.55 (CH$_2$CH$_2$N of **A**); 32.90 (CH$_2$CH$_2$N of **B**); 40.36, 40.42 (CH$_2$CH$_2$N of **A** and **B**); 117.90 ($C_{5'}$ Btz); 119.88 (C_5 btz); 127.89 (C_p of **A**); 128.08 (C_o of **B**); 129.09 (C_m of **A**); 129.58 (C_m of **B**); 134.21 (C_p of **B**); 134.84 (C_o of **A**); 151.16 (C_{ipso} of **B**); 151.84 (C_{ipso} of **A**); 153.36 (C_2 btz); 154.20 ($C_{2'}$ btz); 159.75 ($C_{4'}$ Btz); 161.51 (C_4 Btz); no visible TFA. Anal. Calcd for $C_{52}H_{56}O_4N_8S_4{\cdot}4CF_3COOH{\cdot}4H_2O$, (1512.45): C, 47.66; H, 4.53; N, 7.40; S, 8.48. Found: C, 47.64; H, 4.28; N, 7.27; S, 8.57. ES-MS (pos. mode): 1213.24 $[M-2CF_3COOH+H^+]^+$, 1098.44 $[M-3CF_3COOH+H^+]^+$, 984.61 $[M-4CF_3COOH+H^+]^+$, 493.23 $[M-4CF_3COOH+2H^+]^{2+/2}$, 329.35 $[M-4CF_3COOH+3H^+]^{3+/3}$. ES-MS (neg. mode): 1439.22 $[M-H^+]^-$, 1325.32 $[M-CF_3COOH-H^+]^-$, 1210.28 $[M-2CF_3COOH-H^+]^-$.

5.1.5. Compounds 3, 11, 12 and 13—general procedure

The calixarene tetra-ammonium salt, then Et_3N were added to a solution of *N,N′*-bis(*tert*-butoxycarbonyl)-*N″*-triflylguanidine **K** in dry CH_2Cl_2 and MeOH. The mixture was stirred at rt under Ar (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 99:1), and the solvent were evaporated to dryness. The solid residue was dissolved in CH_2Cl_2, washed with 2 M $NaHSO_4$ then with saturated $NaHCO_3$. The aqueous phases were washed with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4 or $MgSO_4$, filtered and concentrated. The residue was chromatographed to give the expected tetra-(di-boc-guanidinoethyl) calixarene derivative.

5.1.5.1. 5,11,17,23-Tetra-[(*N,N′*-di-Boc)guanidinoethyl]-25,26,27,28-calix[4]arene tetrol 3. From **A** (0.24 g, 0.32 mmol) or **2** (0.34 g; 0.32 mmol), Et_3N (0.53 mL, 3.81 mmol), **K** (0.50 g, 1.28 mmol) in CH_2Cl_2/MeOH (30 mL; 1:1). 3 h (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 99:1); washings: 2 M $NaHSO_4$ (30 mL), satd $NaHCO_3$ (30 mL), CH_2Cl_2 (10 mL). Drying: $MgSO_4$. Chromatography (SiO_2, from CH_2Cl_2 to CH_2Cl_2/MeOH 99:1). **3** (0.33 g, 65%). White solid. Mp: 134–135 °C. IR (KBr): 3336.8 (OH); 2978.8 (CH_3); 1721.2 (COO); 1638.7 (NH). UV–vis (CH_2Cl_2): 280 (11,703). ^{1}H NMR (400 MHz, $CDCl_3$): 1.50 (s, 9H, Me_3C); 1.52 (s, 9H, Me_3C); 2.69 (t, J = 7 Hz, 8H, CH_2CH$_2$N); 3.47–4.22 (AB, J_{AB} = 12.8 Hz, 8H, ArCH_2Ar); 3.63 (q, J = 6.5 Hz, 8H, CH$_2$CH_2N); 6.92 (s, 8H, Ar*H*); 8.41 (s, 4H, CH$_2$N*H*); 10.17 (s, 4H, O*H*); 11.50 (s, 4H, CN*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.47 (Me_3C); 28.72 (Me_3C); 32.12 (ArCH$_2$-Ar); 34.84 (ArCH$_2$CH$_2$NH); 42.59 (ArCH$_2$CH$_2$NH); 79.75 (Me_3C); 83.47 (Me_3C); 128.69 (C_o); 129.75 (C_m); 132.50 (C_p); 147.80 (C_{ipso}); 153.57 (CO); 156.46 (*C* guan); 163.89 (CO). Anal. Calcd for $C_{80}H_{116}O_{20}N_{12}$ (1565.85): C, 61.36; H, 7.46; N, 10.73. Found: C, 61.27; H, 7.35; N, 10.68. ES-MS (neg. mode): 1562.5 $[M-H^+]^-$, ES-MS (pos. mode): 1587.66 $[M+Na^+]^+$.

5.1.5.2. 5,11,17,23-Tetra-[(*N,N′*-di-Boc)guanidinoethyl]-25,27-bis(6-methyleneoxy-6′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene 11. From **8** (0.28 g, 0.163 mmol), CH_2Cl_2 (20 mL), MeOH (some drops), Et_3N (0.33 mL, 2.37 mmol), **K** (0.31 g, 0.79 mmol). 3 h (TLC monitoring: Al_2O_3, CH_2Cl_2). CH_2Cl_2 (30 mL); washings: 2 M $NaHSO_4$ (30 mL), satd $NaHCO_3$ (30 mL). Drying: Na_2SO_4. Chromatography (Al_2O_3, CH_2Cl_2). **11** (0.24 g, 76%). White powder. Mp: 138–139 °C. IR (KBr): 3336.5 (OH), 1721.7 (CO); 1639.77 (NCO, NHCO). UV–vis (CH_2Cl_2): 288 (54,477). ^{1}H NMR (400 MHz, $CDCl_3$): 1.42–1.50 (m, 72H, Me_3C); 2.59 (t, J = 7.2 Hz, 4H, CH_2CH$_2$N of **B**); 2.63 (s, 6H, CH_3 bpy); 2.77 (t, J = 7.0 Hz, 4H, CH_2CH$_2$N of **A**); 3.36, 4.37 (AB, J_{AB} = 13.2 Hz, 8H, ArCH_2Ar); 3.53 (q, J = 6.9 Hz; 4H, CH$_2$CH_2N of **B**); 3.64 (q, J = 6.8 Hz; 4H, CH$_2$CH_2N of **A**); 5.20 (s, 4H, OCH_2bpy); 6.79 (s, 4H, Ar*H* of **B**); 6.93 (s, 4H, Ar*H* of **A**); 7.12 (d, J = 7.6 Hz, 2H, $H_{5'}$ bpy); 7.60 (t, J = 7.8 Hz, 4H, $H_{4-4'}$ bpy); 7.95 (s, 2H, O*H*); 8.09 (d, J = 7.5 Hz, 2H, H_5 bpy); 8.18 (d, J = 7.8 Hz, 2H, $H_{3'}$ bpy); 8.33 (m, 4H, CH$_2$N*H* of **B** and H_3 bpy); 8.41 (t, J = 6.5 Hz, 2H, CH$_2$N*H* of **A**); 11.47 (s, 2H, N*H* of **A** or **B**); 11.48 (s, 2H, N*H* of **A** or **B**). ^{13}C NMR (100 MHz, $CDCl_3$): 25.03 (*Me* bpy); 28.43, 28.51; 28.73 (CMe_3); 31.93 (ArCH$_2$Ar); 34.90 (CH$_2$CH$_2$N of **A**); 35.19 (CH$_2$CH$_2$N of **B**); 42.65 (CH$_2$CH$_2$N of **B**); 43.01 (CH$_2$CH$_2$N of **A**); 79.14 (OCH$_2$bpy); 79.65, 83.35 (Me$_3$C); 118.73 ($C_{3'}$ bpy); 120.44 (C_3 bpy); 121.46 (C_5 bpy); 123.76 ($C_{5'}$ bpy); 128.18 (C_o of **A**); 129.17 (C_p of **A**); 129.42 (C_m of **B**); 130.13 (C_m of **A**); 133.42 (C_o of **B**); 135.78 (C_p of **B**); 137.52 ($C_{4'}$ bpy); 138.58 (C_4 bpy); 151.18 (C_{ipso} of **B**); 152.47 (C_{ipso} of **A**); 153.53 (CO); 156.43 (*C* guan of **B**); 156.50 (*C* guan of **A**); 153.54, 155.73, 156.83, 158.23 (C_2, $C_{2'}$, C_6, $C_{6'}$ of bpy); 163.98 (CO). Anal. Calcd for $C_{104}H_{136}O_{20}N_{16}$, (1930.3): C, 64.71; H, 7.10; N, 11.61. Found: C, 64.65; H, 6.86; N, 11.34. ES-MS (pos. mode): 1828.99 $[M-boc+2H^+]^+$, 1951.98 $[M+Na^+]^+$.

5.1.5.3. 5,11,17,23-Tetra-[(*N,N′*-di-Boc)guanidinoethyl]-25,27-bis(5-methyleneoxy-5′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene 12. From **9** (0.205 g, 0.144 mmol), CH_2Cl_2 (20 mL), MeOH (some drops), Et_3N (241 μL, 1.735 mmol), **K** (0,226 g,

0,578 mmol), 16 h (TLC monitoring, Al_2O_3, CH_2Cl_2). CH_2Cl_2 (50 mL); washings: H_2O (2 × 30 mL). Drying: Na_2SO_4. Chromatography (Al_2O_3, CH_2Cl_2); **12** (0,234 g, 84%). Slightly pink solid. Mp: 125–126 °C. IR (KBr): 3334.06 (OH); 1721.1 (COO); 1638.76 (NCO, NHCO). UV–vis (CH_2Cl_2): 289 (52,921). ^{1}H NMR (400 MHz, $CDCl_3$) 1.39–1.51 (m, 72H, *Me*$_3$C); 2.34 (s, 6H, C*H*$_3$ bpy); 2.57 (t, *J* = 7.17 Hz, 4H, C*H*$_2$CH$_2$N of **B**); 2.72 (t, *J* = 7.18 Hz, 4H, C*H*$_2$CH$_2$N of **A**); 3.27–4.22 (AB, J_{AB} = 13.1 Hz, 8H, ArC*H*$_2$Ar); 3.52 (q, *J* = 6.86 Hz, 4H, C*H*$_2$CH$_2$N of **B**); 3.6 (q, *J* = 6.79 Hz; 4H, CH$_2$C*H*$_2$N of **A**); 5.13 (s, 4H, OC*H*$_2$bpy); 6.78 (s, 4H, Ar*H* of **B**); 6.87 (s, 4H, Ar*H* of **A**); 7.47 (dd, *J* = 8,3 Hz, 2H, *H*$_{4'}$ bpy); 7.76 (s, 2H, O*H*); 8.14 (dd, *J* = 8.06 Hz, 2H, *H*$_4$ bpy); 8.21 (d, *J* = 8.06 Hz, 2H, *H*$_{3'}$ bpy); 8.29 (t, *J* = 6 Hz, 2H, CH$_2$N*H* of **B** or **A**); 8.35–8.41 (m, 6H, 2*H*$_3$, 2*H*$_6$ of bpy and 2 CH$_2$N*H* of **B** or **A**); 8.89 (dd, *J* = 1.51 Hz, 2H, *H*$_6$ of **A**); 11.46 (s, 4H, N*H* guan). ^{13}C NMR (100 MHz, $CDCl_3$): 18.74 (*Me* bpy); 28.41, 28.48, 28.72 (Me$_3$*C*); 32.06 (ArCH$_2$Ar); 34.85, 35.15 (*C*H$_2$CH$_2$N); 42.55, 42.89 (CH$_2$*C*H$_2$N); 76.17 (OCH$_2$ bpy); 79.58, 79.63, 83.29, 83.34 (*C*Me$_3$); 121.08, 121.25, 128.15, 130.16, 136.69, 137.55, 148.66, 149.88 (*C*$_3$, *C*$_{3'}$, *C*$_4$, *C*$_{4'}$, *C*$_6$, *C*$_{6'}$, of bpy and *C*$_m$ of Ar); 128.18, 129.33, 132.28, 133.50, 133.61, 135.92, 137.59, 150.96, 152.22, 153.52, 153.73, 156.43, 156.49, 156.59 (*C*$_2$, *C*$_{2'}$, *C*$_5$, *C*$_{5'}$ of bpy; *C*$_p$, *C*$_o$ and *C*$_i$ of Ar; *C* guan, *C*O); 153.57, 164.03 (*C*O). Anal. Calcd for $C_{104}H_{136}O_{20}N_{16}\cdot CH_2Cl_2$ (2013.94): C, 62.62; H, 6.90; N, 11.12. Found: C, 62.85; H, 7.03; N, 11.36. ES-MS (pos; mode): 1930.7 $[M+H^+]^+$, 1831.0 $[M-Boc+H+H^+]^+$; 965.5 $[M+2H^+]^{2+/2}$, 915.6 $[M-Boc+H+2H^+]^{2+/2}$; 644.5 $[M+3H^+]^{3+/3}$, 610.8 $[M-Boc+H+3H]^{3+/3}$, 577.4 $[M-2Boc+2H+3H]^{3+/3}$, 544.1 $[M-3Boc+3H+3H]^{3+/3}$, 510.51 $[M-4Boc+4H+3H]^{3+/3}$, 477.4 $[M-5Boc+5H+3H]^{3+/3}$, 444.0 $[M-6Boc+6H+3H]^{3+/3}$, 410.6 $[M-7Boc+7H+3H]^{3+/3}$, 377.4 $[M-8Boc+8H+3H]^{3+/3}$.

5.1.5.4. 5,11,17,23-Tetra-[(*N*,*N*′-di-Boc)guanidinoethyl]-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 13. From **10** (0.45 g, 0.30 mmol), CH_2Cl_2 (40 mL), MeOH (some drops), Et_3N (522 μL, 3.75 mmol), **K** (0.49 g, 1.24 mmol). 5 h (TLC monitoring: Al_2O_3, CH_2Cl_2). CH_2Cl_2 (50 mL); washings: 2 M $NaHSO_4$ (40 mL), satd $NaHCO_3$ (40 mL). Drying: Na_2SO_4. Chromatography (Al_2O_3, CH_2Cl_2/Hex 90:10); **13** (0.48 g, 77%). White powder. Mp: 147–178 °C. IR (KBr): 3335.9 (OH), 1721.1 (CO); 1638.8 (NCO, NHCO). UV–vis (CH_2Cl_2): 291 (12,133); 330 (27,720). ^{1}H NMR (400 MHz, $CDCl_3$): 1.44–149 (m, 72H, *Me*$_3$C); 2.53 (s, 6H, C*H*$_3$ btz); 2.58 (t, *J* = 7.0 Hz, 4H, C*H*$_2$CH$_2$N of **A** or **B**); 2.75 (t, *J* = 7.0 Hz, 4H, C*H*$_2$CH$_2$N of **A** or **B**); 3.33–4.30 (AB, J_{AB} = 13.1 Hz, 8H, ArC*H*$_2$Ar); 3.52 (q, *J* = 6.5 Hz, 4H, CH$_2$CH$_2$N of **A** or **B**); 3.63 (q, *J* = 6.5 Hz, 4H, CH$_2$CH$_2$N of **A** or **B**); 5.22 (s, 4H, OC*H*$_2$btz); 6.78 (s, 4H, Ar*H* of **A** or **B**); 6.91 (s, 4H, Ar*H* of **A** or **B**); 6.98 (s, 2H, O*H*); 7.80 (s, 2H, H btz); 7.97 (s, 2H, H btz); 8.30 (t, *J* = 4.6 Hz, 2H, CH$_2$CH$_2$N*H* of **A** or **B**); 8.40 (t, *J* = 4.6 Hz, 2H, CH$_2$CH$_2$N*H* of **A** or **B**); 11.47 (s, 2H, N*H* of **A** or **B**); 11.48 (s, 2H, N*H* of **A** or **B**). ^{13}C NMR (100 MHz, $CDCl_3$): 17.59 (*Me* btz); 28.45, 28.51, 28.72 (*Me*$_3$C); 31.85 (ArCH$_2$Ar); 34.90 (*C*H$_2$CH$_2$N of **A**); 35.19 (*C*H$_2$CH$_2$N of **B**); 42.56 (CH$_2$*C*H$_2$N of **B**); 42.93 (CH$_2$*C*H$_2$N of **A**); 79.62 (OCH$_2$btz); 83.34 (Me$_3$*C*); 116.33 (*C*$_{5'}$ btz); 118.22 (*C*$_5$ btz); 128.17 (*C*$_o$ of **A**); 129.43 (*C*$_m$ of **A**); 130.13 (*C*$_m$ of **B**); 133.50 (*C*$_o$ of **B**); 135.99 (*C*$_p$ of **B**); 150.90 (*C*$_{ipso}$ of **B**); 152.23 (*C*$_{ipso}$ of **A**); 153.56 (*C*$_p$ of **A**); 154.11 (*C*$_2$ btz); 154.75 (*C*$_{2'}$ btz); 156.44 (*C* guan of **A**); 156.50 (*C* guan of **B**); 160.85 (*C*$_{4'}$ btz); 162.20 (*C*$_4$ btz); 164.00 (*C*O of **B**); 164.04 (*C*O of **A**). Anal. Calcd for $C_{96}H_{128}O_{20}N_{16}S_4\cdot 1.5CH_2Cl_2$ (2074.28): C, 56.17; H, 6.29; N, 10.80. Found: C, 56.32; H, 6.30; N, 10.82. ES-MS (pos. mode): 1952.34 $[M+H^+]^+$, 1852.50 $[M-COOC(CH_3)_3+H^+]^+$.

5.1.6. Compounds 4, 14, 15 and 16—general procedure

A solution of Boc-guanidino calixarene derivative in a mixture of CH_2Cl_2 and TFA was stirred at rt under Ar. The solvent were evaporated, and the residual TFA was eliminated by five dissolution in CH_2Cl_2–evaporation cycles. The semi-solid residual material was dried under high vacuum, then triturated in dry Et_2O to give a precipitate. The latter was dissolved in H_2O, dialyzed (Float-A-Lyser, cellulose acetate, MWCO 100D) then lyophilized to give the expected guanidinium salt.

5.1.6.1. 5,11,17,23-Tetra-(guanidinoethyl)-25,26,27,28-calix[4]-arene tetrol, tetra-trifluoroacetate salt 4. From **3** (0.19 g, 0.12 mmol), CH_2Cl_2 (11 mL), TFA (4 mL); 1 h. **4** (0.14 g, 95%). White cotton. Mp: 215 °C (dec). IR (KBr): 3365 (OH); 3185 (=NH); 1671 (NH_2). UV–vis (H_2O): 285 (3460). ^{1}H NMR (400 MHz, D_2O): 2.56 (t, *J* = 6.4 Hz; 8H, C*H*$_2$CH$_2$N); 3.23 (t, *J* = 6.4 Hz; 8H, CH$_2$C*H*$_2$N); 3.82 (br s, 8H, ArC*H*$_2$Ar), 6.94 (s, 8H, Ar*H*). ^{1}H NMR (400 MHz, DMSO-d_6): 2.51 (m, 8H, C*H*$_2$CH$_2$N); 3.22 (q, *J* = 6.5 Hz, 8H, CH$_2$C*H*$_2$N); 3.55 (br s, 8H, ArC*H*$_2$Ar); 6.94 (s, 8H, Ar*H*); 7.56 (t, *J* = 4.9 Hz, CH$_2$CH$_2$N*H*). ^{13}C NMR (100 MHz, D_2O): 30.96 (ArCH$_2$Ar); 33.65 (*C*H$_2$CH$_2$N); 42.77 (CH$_2$*C*H$_2$N); 129.12 (*C*$_o$); 129.44 (*C*$_m$); 132.34 (*C*$_p$); 147.73 (*C*$_{ipso}$); 156.99 (*C* guan); no visible TFA. Anal. Calcd for $C_{40}H_{52}O_4N_{12}\cdot 4CF_3COOH\cdot H_2O$ (1239.02): C, 46.53; H, 4.71; N, 13.56. Found: C, 46.50; H, 4.71; N, 13.13. ES-MS (ES^-): 1332.54 $[M+CF_3COO^-]^-$, 1218.68 $[M-H^+]^-$. ES-MS (pos. mode): 1106.72 $[M-CF_3COO^-]^+$, 992.86 $[M-2CF_3COO^-+H^+]^+$, 878.99 $[M-2CF_3COO^-+2H^+]^+$, 765.19 $[M-4CF_3COO^-+3H^+]^+$.

5.1.6.2. 5,11,17,23-Tetra-(guanidinoethyl)-25,27-bis(6-methyl-eneoxy-6′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene, hexa-trifluoroacetate salt 14. From **11** (0.35 g, 0.18 mmol), CH_2Cl_2 (20 mL), TFA (7.5 mL); 3 h. **14** (0.25 g, 74%). White cotton. Mp: 94–95 °C. IR (KBr): 3386.2 (OH); 1676.2 (NH). UV–vis (H_2O): 286 (26,644). ^{1}H NMR (400 MHz, D_2O): 2.47 (t, *J* = 4.8 Hz, 4H, C*H*$_2$CH$_2$N of **A**); 2.49 (s, 6H, *Me* bpy); 2.78 (t, *J* = 6.4 Hz, 4H, C*H*$_2$CH$_2$N of **B**); 3.17 (t, *J* = 6.0 Hz, 4H, CH$_2$C*H*$_2$N of **A**); 3.41 (t, *J* = 6.5 Hz, 4H, CH$_2$C*H*$_2$N of **B**); 3.51–4.30 (AB, J_{AB} = 13.3 Hz, 8H, ArC*H*$_2$Ar); 5.09 (s, 4H, OC*H*$_2$ bpy); 6.90 (s, 4H, Ar*H* of **B**); 7.16 (s, 4H, Ar*H* of **A**); 7.49 (d, *J* = 7.8 Hz, 4H, *H*$_5$ bpy); 7.68 (d, *J* = 7.8 Hz, 4H, *H*$_{5'}$ bpy); 7.83 (t, *J* = 7.8 Hz, 4H, *H*$_4$ bpy); 8.10 (d, *J* = 7.8 Hz, 4H, *H*$_3$ bpy); 8.16 (d, *J* = 8.1 Hz, 4H, *H*$_{3'}$ bpy); 8.30 (t, *J* = 7.5 Hz, 4H, *H*$_{4'}$ bpy). ^{1}H NMR (400 MHz, DMSO-d_6): 2.55 (s, 6H, *Me* bpy); 2.63 (t, *J* = 7.0 Hz, 8H, C*H*$_2$CH$_2$N); 3.26 (m, 8H, C*H*$_2$CH$_2$N); 3.41–4.37 (AB, J_{AB} = 13.0 Hz, 8H, ArC*H*$_2$Ar); 5.14 (s, 4H, OC*H*$_2$ bpy); 7.03 (s, 4H, Ar*H*); 7.08 (s, 4H, Ar*H*); 7.26 (d, *J* = 7.4 Hz, 4H, *H* bpy); 7.64 (t, *J* = 7.6 Hz, 4H, *H* bpy); 7.69 (t, *J* = 7.6 Hz, 4H, *H* bpy); 7.79 (d, *J* = 7.4 Hz, 4H, *H* bpy); 8.20 (d, *J* = 7.8 Hz, 4H, *H* bpy); 8.33 (d, *J* = 7.8 Hz, 4H, *H*bpy); 8.37 (s, 2H, O*H*). ^{13}C NMR (100 MHz, D_2O): 19.57 (*Me* bpy); 30.93 (ArCH$_2$Ar); 33.73 (*C*H$_2$CH$_2$N of **B**); 33.82 (*C*H$_2$CH$_2$N of **A**); 42.39 (CH$_2$*C*H$_2$N of **A**); 42.98 (CH$_2$*C*H$_2$N of **B**); 77.25 (OCH$_2$ bpy); 116.72 (q, *J* = 291.9 Hz, *C*F$_3$COO$^-$); 122.16 (*C*$_3$ bpy); 122.38 (*C*$_{3'}$ bpy); 125.07 (*C*$_5$ bpy); 128.22 (*C*$_{5'}$ bpy); 128.98 (*C*$_o$ of **A** or **B**); 129.73 (*C*$_m$ of **A**); 129.83 (*C*$_m$ of **B**); 130.72 (*C*$_p$ of **B**); 134.44 (*C*$_o$ of **A** or **B**); 136.38 (*C*$_p$ of **A**); 140.01 (*C*$_4$ bpy); 146.69 (*C*$_{4'}$ bpy); 146.87 (*C*$_6$ bpy); 147.78 (*C*$_{6'}$ bpy); 150.42 (*C*$_{ipso}$ of **B**); 150.93 (*C*$_{ipso}$ of **A**); 155.26 (*C*$_{2'}$ bpy); 156.84 (*C*$_2$ bpy and *C* guan of **A**); 157.12 (*C* guan of **B**); 163.17 (q, *J* = 35.5 Hz, CF$_3$*C*OO$^-$). Anal. Calcd for $C_{64}H_{72}O_4N_{16}\cdot 6CF_3COOH\cdot 4H_2O$ (1867.53): C, 48.87; H, 4.53; N, 12.00. Found: C, 48.71; H, 4.35; N, 11.86. ES-MS (neg. mode): 1697.56 $[M+CF_3COOH-H^+]^-$. ES-MS (pos. mode): 1470.74 $[M-CF_3COOH+H^+]^+$, 1356.85 $[M-2CF_3COOH+H^+]^+$, 1243.08 $[M-3CF_3COOH+H^+]^+$, 1129.00 $[M-4CF_3COOH+H^+]^+$, 565.39 $[M-4CF_3COOH+2H^+]^{2+/2}$, 377.46 $[M-4CF_3COOH+3H^+]^{3+/3}$.

5.1.6.3. 5,11,17,23-Tetra-(guanidinoethyl)-25,27-bis(5-methyl-eneoxy-5′-methyl-2,2′-bipyridyl)-26,28-dihydroxycalix[4]arene, hexa-trifluoroacetate salt 15. From **12** (0.2 g, 0.104 mmol), CH_2Cl_2 (20 mL), TFA (6 mL); **15** (0.163 g, 83%). Slightly pink solid. Mp: 120–121 °C. IR (KBr): 3357.88 (OH); 1673.88 (NH). UV–vis (H_2O): 287 (40,098). ^{1}H NMR (400 MHz, D_2O). 2.38 (s, 6H, *Me* bpy); 2.56 (t, *J* = 6.42 Hz, 4H, C*H*$_2$CH$_2$N of **A**); 2.75 (t, *J* = 6.5 Hz, 4H, C*H*$_2$CH$_2$N of **B**); 3.23 (t, *J* = 6.42 Hz, 4H, CH$_2$C*H*$_2$N of **A**); 3.39

(t, J = 6.67 Hz, 4H, CH_2CH_2N of **B**); 3.56–4.25 (AB, J_{AB} = 13.34 Hz, 8H, $ArCH_2Ar$); 5.24 (s, 4H, OCH_2 bpy); 6.96 (s, 4H, Ar*H* of **B**); 7.13 (s, 4H, Ar*H* of **A**); 7.80 (s, 4H, $H_{4'}$, $_4$ bpy); 7.84 (d, J = 8.31 Hz, 2H, $H_{3'}$ bpy); 8.24 (s, 2H, $H_{6'}$ bpy); 8.35 (d, J = 9.32 Hz, 2H, H_3 bpy); 8.99 (s, 2H, H_6 bpy). ^{13}C NMR (100 MHz, DMSO-d_6): 18.10 (*Me* bpy); 31.20 ($ArCH_2Ar$); 33.96 (CH_2CH_2N of **B**); 34.36 (CH_2CH_2N of **A**); 42.24 (CH_2CH_2N of **A**); 42.91 (CH_2CH_2N of **B**); 75.84 (OCH_2 bpy); 117.65 (q, J = 298.6 Hz, CF_3COO^-); 120.61, 120.65, 136.71, 138.40, 148.33, 148.98 (C_3, $C_{3'}$, C_4, $C_{4'}$, C_6, $C_{6'}$ of bpy); 127.66 (C_o of Ar **B**); 129.40 (C_m of Ar **A**); 129.51 (C_p of **B**); 129.84 (C_m of Ar **B**); 133.94 (C_o of Ar **A**); 136.08 (C_p of Ar **A**); 150.90, 151.37 (C_i of Ar **A** and Ar **B**); 133.02, 134.23, 151.81, 154.56 (C_2, $C_{2'}$, C_5, $C_{5'}$ of bpy); 157.25 (*C* guan); 163.17 (q, J = 35.5 Hz, CF_3COO^-). Anal. Calcd for $C_{64}H_{72}O_4N_{16}$·6CF_3COOH·4H_2O (1885.56): C, 48.41; H, 4.60; N, 11.89. Found: C, 48.82; H, 4.21; N, 11.98. ES-MS (pos. mode): 1471.4 [M−2CF_3-COOH−CF_3COO^-]$^+$, 1357.3 [M−3CF_3COOH−CF_3COO^-]$^+$, 1243.4 [M−4CF_3COOH−CF_3COO^-]$^+$, 1129.3 [M−5CF_3COOH−CF_3COO^-]$^+$, 565.3 [M−6CF_3COOH+2H$^+$]$^{2+/2}$, 377.3 [M−6CF_3COOH+3H$^+$]$^{3+/3}$.

5.1.6.4. 5,11,17,23-Tetra-(guanidinoethyl)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra-trifluoroacetate salt 16. From **13** (0.23 g, 0.111 mmol), CH_2Cl_2 (20 mL), TFA (7 mL); 2 h. **16** (0.15 g, 73%). White cotton. Mp: 110–111 °C. IR (KBr): 3366.1 (OH); 2179.5 (NH_3^+); 1671.1 (NH_3^+). UV–vis (H_2O): 289 (10,595); 330 (24414). ^{1}H NMR (400 MHz, D_2O): 2.29 (s, 6H, CH_3 btz); 2.44 (t, J = 5.6 Hz, 4H, CH_2CH_2N of **A** or **B**); 2.76 (t, J = 6.4 Hz, 4H, CH_2CH_2N of **A** or **B**); 3.17 (t, J = 6.4 Hz, 4H, CH_2CH_2N of **A** or **B**); 3.39 (t, J = 7.0 Hz, 4H, CH_2CH_2N of **A** or **B**); 3.40–4.25 (AB, J_{AB} = 13.0 Hz, 8H, $ArCH_2Ar$); 5.03 (s, 4H, OCH_2 btz); 6.80 (s, 4H, Ar*H* of **A** or **B**); 7.00 (s, 2H, *H* btz); 7.05 (s, 4H, Ar*H* of **A** or **B**); 7.63 (s, 2H, *H* btz). ^{1}H NMR (400 MHz, DMSO-d_6): 2.46 (s, 6H, CH_3 btz); 2.55 (m, 4H, CH_2CH_2N of **B**); 2.62 (t, J = 6.1 Hz, 4H, CH_2CH_2N of **A**); 3.26 (m, 8H, CH_2CH_2N of **A** and **B**); 3.38–4.29 (AB, J_{AB} = 13.1 Hz, 8H, $ArCH_2Ar$); 5.17 (s, 4H, OCH_2 btz); 6.60–7.60 (br signal, 16H, N*H* guanidine); 7.01 (s, 4H, Ar*H* of **B**); 7.06 (s, 4H, Ar*H* of **A**); 7.49 (s, 2H, $H_{5'}$ btz); 7.61 (m, CH_2CH_2NH of **A** or **B**); 7.69 (m, CH_2CH_2NH of **A** or **B**); 7.97 (s, 2H, H_5 btz); 8.10 (s, 2H, O*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 17.03 (*Me* btz); 31.00 ($ArCH_2Ar$); 33.98 (CH_2CH_2N of **A**); 34.37 (CH_2CH_2N of **B**); 42.24, 42.92 (CH_2CH_2N of **A** and **B**); 73.74 (OCH_2btz); 117.45 (q, J = 298.5 Hz, CF_3COO^-); 117.92 ($C_{5'}$ btz); 119.73 (C_5 btz); 127.72 (C_o of **A**); 129.19 (C_p of **A**); 129.30 (C_m of **A**); 129.69 (C_m of **B**);134.04 (C_o of **B**);135.84 (C_p of **B**); 150.92 (C_{ipso} of **B**); 151.56 (C_{ipso} of **A**); 153.43 (C_2 btz); 154.22 ($C_{2'}$ btz); 157.14, 157.19 (*C* guan); 159.28 (q, J = 30.8 Hz, CF_3COO^-); 159.75 ($C_{4'}$ btz); 161.51 (C_4 btz). Anal. Calcd for $C_{56}H_{64}O_4N_{16}S_4$·4CF_3COOH·3H_2O, (1695.49): C, 45.35; H, 4.39; N, 13.20. Found: C, 45.33; H, 4.39; N, 13.21. ES-MS (pos. mode): 1494.22 [M−CF_3COOH+H$^+$]$^+$, 1380.29 [M−2 CF_3 COOH+H$^+$]$^+$, 1266.54 [M−3CF_3COOH+H$^+$]$^+$, 1152.49 [M−4 CF_3COOH+H$^+$]$^+$, 577.20 [M−4CF_3COOH+2H$^+$]$^{2+/2}$, 385.29 [M−4 CF_3COOH+3H$^+$]$^{3+/3}$.

5.1.7. *N*-Boc-tyramine 19

A solution of NaOH (0.46 g, 11,5 mmol) in H_2O (15 mL) was added to a solution of tyramine hydrochloride **F** (1.00 g, 5.76 mmol) in dioxane/H_2O (25/12 mL). The mixture was stirred at rt under Ar during 10 min, and di-*tert*-*butyl*-dicarbonate (Boc_2O) (1.26 g, 5.76 mmol) was added. The solution was stirred overnight at rt, then was concentrated. AcOEt (40 mL) was added and pH was brought to 7–8 with 1 M HCl. The aqueous phase was collected and washed with AcOEt (2 × 15 mL). The combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness. The raw material was chromatographed (Al_2O_3, CH_2Cl_2/Hex, 85:15) to give **19** (0.98 g, 73%). Mp: 61–62 °C. IR (KBr): 3378.9 (–OH; –CONH–); 1686.6 (CO). UV–vis (CH_2Cl_2): 277 (1803). ^{1}H NMR (400 MHz, $CDCl_3$): 1.441 (s, 9H, Me_3C); 2.703 (t, J = 7.9 Hz; 2H, CH_2CH_2N); 3.329 (m, 2H, CH_2CH_2N); 4.602 (br s, 1H, N*H* or OH); 6.023 (br s, 1H, N*H* or O*H*); 6.774 (d, J = 8.3 Hz; 2H; Ar*H*); 7.013 (d, J = 8.3 Hz; 2H; Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.84 (Me_3C); 35.64 (CH_2CH_2N); 42.49 (CH_2CH_2N); 80.15 (Me_3C); 115.97 (C_m); 130.18 (C_o); 130.50 (C_p); 155.38 (C_{ipso}); 156.84 (CO). Anal. Calcd for $C_{13}H_{19}O_3N$ (237.29): C, 65.80; H, 8.07; N, 5.90. Found: C, 65.77; H, 8.09; N, 5.90. EI-MS: 181 [M−$(Me)_3$-CO−H+Na]$^{\cdot+}$; 107 [M−$(Me)_3COC(O)NHCH_2$]$^+$.

5.1.8. Compounds 20, 21 and 22—general procedure

K_2CO_3 (1 equiv) was added to a solution of **19** (1.1 equiv) in anhydrous MeCN. The mixture was refluxed under Ar during 30 min, and the bromomethyl reactant (1 equiv) was added in one portion. Reflux was continued during 5 h (TLC monitoring), and the solvent was evaporated to dryness. The residue was triturated in CH_2Cl_2, then filtered. The filtrate was concentrated and chromatographed to give the expected ether.

5.1.8.1. *N*-Boc-Aminoethyl-4-[6-methyleneoxy-6′-methyl-2,2′-bipyridyl]-benzene 20. From K_2CO_3 (0.04 g, 0.33 mmol), **19** (0.09 g, 0.38 mmol), MeCN (20 mL), 6-bromomethyl-6′-methyl-2,2′-bipyridine (0.09 g, 0.33 mmol). TLC monitoring: Al_2O_3; CH_2Cl_2. Chromatography: Al_2O_3; CH_2Cl_2/Hex 90:10. **20** (0.12 g, 85%). White solid. Mp: 81–82 °C. IR (KBr): 3353.5 (–CONH–); 1685.6 (CO). UV–vis (CH_2Cl_2): 289 (19,189). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 9H, Me_3C); 2.64 (s, 3H, *Me*bpy); 2.73 (t, J = 6.8 Hz, 2H, CH_2CH_2NH); 3.34 (m, 2H, CH_2CH_2NH); 4.52 (br s, 1H, N*H*); 5.27 (s, 2H, OCH_2bpy); 6.96 (d, J = 8.3 Hz, 2H, Ar*H*); 7.11 (d, J = 8.3 Hz, 2H, Ar*H*); 7.18 (d, J = 7.5 Hz, 2H, *H* bpy); 7.52 (d, J = 7.8 Hz, 2H, *H* bpy); 7.71 (t, J = 7.7 Hz, 2H, *H* bpy); 7.82 (t, J = 7.7 Hz, 2H, *H* bpy); 8.20 (d, J = 7.5 Hz, 2H, *H* bpy); 8.32 (d, J = 7.5 Hz, 2H, *H* bpy). ^{13}C NMR (100 MHz, $CDCl_3$): 25.09 (*Me*bpy); 28.83 (Me_3C); 35.70, 42.34 (CH_2CH_2NH and CH_2CH_2NH); 71.34 (Me_3C); 79.54 (OCH_2bpy); 115.37 (C_o or C_m); 118.63, 120.40, 121.35, 123.72 (C_3, $C_{3'}$, C_5 and $C_{5'}$ of bpy); 130.22 (C_o or C_m); 131.90 (C_p); 137.46, 138.01 (C_4, $C_{4'}$ of bpy); 155.88, 156.30, 156.36, 157.27, 157.53,158.37 (C_2, $C_{2'}$, C_6, $C_{6'}$ of bpy, C_{ipso}, *C*O). Anal. Calcd for $C_{25}H_{29}O_3N_3$ (419.52): C, 71.57; H, 6.96; N, 10.01. Found: C, 71.52; H, 6.84; N, 9.94. EI-MS: 421 [M+H$^+$]$^+$.

5.1.8.2. *N*-Boc-Aminoethyl-4-[5-methyleneoxy-5′-methyl-2,2′-bipyridyl]-benzene 21. From K_2CO_3 (0.12 g, 0.843 mmol), MeCN (30 mL), **19** (0.2 g, 0.843 mmol), 5-bromomethyl-5′-methyl-2-2′-bipyridine (0.222 g, 0.843 mmol); 23 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH 98:2) addition of NEt_3 (2 drops); chromatography: SiO_2, CH_2Cl_2/MeOH 99.4:0.6. **21** (0.281 g, 80%). White solid. Mp: 134–135 °C. IR (KBr): 3378.32 (–CONH–); 1689.06 (CO). UV–vis (CH_2Cl_2): 289 (26,079). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 9H, Me_3C); 2.39 (s, 3H, *Me*bpy); 2.73 (t, J = 6.8 Hz, 2H, CH_2CH_2NH); 3.34 (br s, 2H, CH_2CH_2NH); 4.53 (br s, 1H, N*H*); 5.11 (s, 2H, OCH_2b-py); 6.93 (d, J = 8.56 Hz, 2H, Ar*H*); 7.12 (d, J = 8.6 Hz, 2H, Ar*H*); 7.26 (dd, J = 8.06 Hz, 1H, *H* bpy); 7.87 (dd, J = 8.31 Hz, 1H, *H* bpy); 8.28 (d, J = 8.06 Hz, 1H, *H* bpy); 8.38 (d, J = 8.06 Hz, 2H, *H* bpy); 8.50 (s, 1H, *H* bpy); 8.71 (dd, J = 1.26 Hz, 1H, *H* bpy). ^{13}C NMR (100 MHz, $CDCl_3$): 18.78 (*Me*bpy); 28.82 (Me_3C); 35.71 (CH_2CH_2NH); 42.33 (CH_2CH_2NH); 68.00 (Me_3C); 79.59 (OCH_2bpy); 115.40, 130.27 (C_o and C_m of Ar); 121.00, 121.04 ($C_{3'}$, $_3$ of bpy); 132.18 (C_p of Ar); 136.64, 137.89, 148.73, 150.09 (C_4, $C_{4'}$, C_6, $C_{6'}$ of bpy); 132.66, 133.95, 156.28, 156.50 (C_2, $C_{2'}$, C_5, $C_{5'}$ of bpy);153.72 (C_i of Ar);157.46 (*C*OO). Anal. Calcd for $C_{25}H_{29}O_3N_3$ (419.52): C, 71.57; H, 6.97; N, 10.02. Found: C, 71.65; H, 6.88; N, 9.93. ES-MS (pos. mode): 420.1 [M+H$^+$]$^+$, 442.2 [M+Na$^+$]$^+$.

5.1.8.3. *N*-Boc-Aminoethyl-4-[4-methyleneoxy-4′-methyl-2,2′-bithiazolyl]-benzene 22. From K_2CO_3 (0.23 g, 1.64 mmol), **19** (0.45 g, 1.89 mmol), MeCN (40 mL), 4-bromomethyl-4′-methyl-2,

2′-bithiazole (0.45 g, 1.64 mmol). TLC monitoring: Al_2O_3; CH_2Cl_2. Chromatography: Al_2O_3, CH_2Cl_2/Hex 85:15. **22** (0.60 g, 85%). White solid. Mp: 126–127 °C. IR (KBr): 3333.4 (–CONH–); 1684.2 (CO). UV–vis (CH_2Cl_2): 330 (6979). ^{1}H NMR (400 MHz, $CDCl_3$): 1.46 (s, 9H, Me_3C); 2.54 (s, 3H, *Me*btz); 2.77 (t, J = 6.8 Hz, 2H, CH_2CH$_2$NH); 3.37 (m, 2H, CH$_2$CH_2NH); 4.54 (br s, 1H, N*H*); 5.26 (s, 2H, OCH_2btz); 6.97 (d, J = 8.5 Hz, 2H, Ar*H*); 7.02 (s, 1H, *H* btz); 7.15 (d, J = 8.3 Hz, 2H, Ar*H*); 7.42 (s, 1H, *H* btz). ^{13}C NMR (100 MHz, $CDCl_3$): 17.54 (*Me*btz); 28.82 (Me_3C); 35.72 (CH_2CH$_2$NH); 42.30 (CH$_2$CH$_2$NH); 66.70 (Me$_3$*C*); 79.57 (OCH$_2$btz); 115.31 (C_m); 116.27, 118.04 (C_5 and $C_{5'}$ of btz) 130.24 (C_o); 132.14 (C_p); 154.31 (C_{ipso}); 154.70, 156.27, 157.37, 160.79 (C_2, $C_{2'}$, C_4, $C_{4'}$ of btz); 162.19 (*C*O). Anal. Calcd for $C_{21}H_{25}O_3N_3S_2$ (431.57): C, 58.44; H, 5.83; N, 9.73. Found: C, 57.90; H, 5.73; N, 9.52. EI-MS: 431 [M+H$^+$]$^+$.

5.1.9. Compounds 23, 24 and 25—general procedure

TFA was added to a solution of boc-aminoethyl derivative in CH_2Cl_2. The mixture was stirred at rt under Ar during ca. 2 h (TLC monitoring). The solvents were evaporated, and the residual TFA was eliminated by successive dissolution in CH_2Cl_2–evaporation cycles, until a white solid was obtained (ca. four cycles). The latter was dried under high vacuum, triturated in Et_2O, filtered and rinsed with Et_2O to give the expected ammonium salt.

5.1.9.1. Aminoethyl-4-[6-methyleneoxy-6′-methyl-2,2′-bipyridyl]-benzene, sesqui-trifluoroacetate salt 23. From TFA (10 mL), **20** (0.52 g, 1.24 mmol), CH_2Cl_2 (30 mL). TLC monitoring: SiO_2, CH_2Cl_2/MeOH 99:1. **23** (0.52 g, 86%). White powder. Mp: 130–131 °C. IR (KBr): 3029.6 (–NH_3^+); 1674.2 (COO); 1515.88 (–NH_3^+). UV–vis (H_2O): 287 (11,399). ^{1}H NMR (400 MHz, D_2O): 2.73 (s, 3H, *Me*bpy); 2.93 (t, J = 7.5 Hz, 2H, CH_2CH$_2$N); 3.22 (t, J = 7.3 Hz, 2H, CH$_2$CH_2N); 5.34 (s, 2H, OCH_2bpy); 7.08 (d, J = 8.6 Hz, 2H, Ar*H*); 7.27 (d, J = 8.6 Hz, 2H, Ar*H*); 7.63 (d, J = 7.5 Hz, 1H, *H* bpy); 7.69 (d, J = 6.8 Hz, 1H, *H* bpy); 8.03 (m, 3H, *H* bpy); 8.18 (m, 1H, *H* bpy). ^{1}H NMR (400 MHz, DMSO-d_6): 2.57 (s, 3H, *Me*bpy); 2.79 (t, J = 8.0 Hz, 2H, CH_2CH$_2$N); 3.01 (m, 2H, CH$_2$CH_2N); 5.27 (s, 2H, OCH_2bpy); 7.04 (d, J = 8.3 Hz, 2H, Ar*H*); 7.20 (d, J = 8.6 Hz, 2H, Ar*H*); 7.34 (d, J = 7.5 Hz, 1H, *H* bpy); 7.57 (d, J = 7.5 Hz, 1H, *H* bpy); 7.77 (br s, 3H, NH_3); 7.85 (t, J = 7.7 Hz, 1H, *H* bpy); 7.98 (t, J = 7.8 Hz, 1H, *H* bpy); 8.19 (d, J = 7.8 Hz, 1H, *H* bpy); 8.32 (d, J = 7.8 Hz, 1H, *H* bpy). ^{13}C NMR (100 MHz, DMSO-d_6): 24.57 (*Me*bpy); 32.57 (CH_2CH$_2$N); 40.51 (CH$_2$CH$_2$N); 70.78 (OCH$_2$bpy); 115.35 (C_o or C_m); 118.07, 119.86, 122.08, 124.05 (C_3, $C_{3'}$, C_5, $C_{5'}$ of bpy); 129.99 (C_p); 130.17 (C_o or C_m); 137.91, 138.43 (C_4, $C_{4'}$ of bpy); 154.64, 155.28, 156.89, 157.42, 157.95 (C_2, $C_{2'}$, C_6, $C_{6'}$ of bpy; C_{ipso}); no visible TFA. Anal. Calcd for $C_{20}H_{21}ON_3{\cdot}1.5CF_3COOH$ (490.43): C, 56.32; H, 4.92; N, 8.56. Found: C, 56.09; H, 4.25; N, 8.56. ES-MS (pos. mode): 320.35 [M−CF_3COOH+H$^+$]$^+$, 184.31 [CH_3−bpy−CH_2^+]$^+$.

5.1.9.2. Aminoethyl-4-[5-methyleneoxy-5′-methyl-2,2′-bipyridyl]-benzene, trifluoroacetate salt 24. From TFA (6 mL), **21** (0.25 g, 0.6 mmol), CH_2Cl_2 (20 mL), TLC monitoring: Al_2O_3, CH_2Cl_2. **24** (0.255 g, 96%). Slightly pink solid. Mp: 169–170 °C. IR (KBr): 3007 (NH_3^+); 1676 (COO); 1554 (C=N). UV–vis (H_2O): 288 (27,679), 240 (22,009), 228 (20,464). ^{1}H NMR (400 MHz, D_2O) 2.36 (s, 3H, *Me*bpy); 2.92 (t, J = 7.17 Hz, 2H, CH_2CH$_2$N); 3.22 (t, J = 7.17 Hz, 2H, CH$_2$CH_2N); 5.19 (s, 2H, OCH_2bpy); 7.04 (d, J = 8.6 Hz, 2H, Ar*H*); 7.25 (d, J = 8.31 Hz, 2H, Ar*H*); 7.78 (d, J = 8.56 Hz, 1H, *H* bpy); 7.89 (d, J = 7.81 Hz, 1H, *H* bpy); 7.98 (s, 2H, *H* bpy); 8.41 (s, 1H, *H* bpy); 8.61 (s, 1H, *H* bpy). ^{13}C NMR (100 MHz, DMSO-d_6): 18.18 (*Me*bpy); 32.56 (CH_2CH$_2$N); 40.52 (CH$_2$CH$_2$N); 67.08 (OCH$_2$bpy); 115.38, 130.16 (C_o andC_m of Ar); 120.24, 120.38, 137.03, 137.99, 148.93, 149.92 (C_3, $C_{3'}$, C_4, $C_{4'}$, C_6, $C_{6'}$ of bpy); 133.10, 134.10, 152.82, 155.36 (C_2, C_2', C_5, $C_{5'}$ of bpy); 130.02 (C_p of Ar); 157.30 (C_i of Ar); no visible TFA. Anal. Calcd for $C_{20}H_{21}ON_3{\cdot}CF_3COOH{\cdot}0.5H_2O$ (442.17): C, 59.72; H, 5.24; N, 9.50. Found: C, 59.77; H, 5.20; N, 9.57. ES-MS (pos. mode): 320.0 [M−CF_3COO^-]$^+$.

5.1.9.3. Aminoethyl-4-[4-methyleneoxy-4′-methyl-2,2′-bithiazolyl]-benzene, trifluoroacetate salt 25. From TFA (10 mL), **22** (0.55 g, 1.27 mmol), CH_2Cl_2 (40 mL). TLC monitoring: Al_2O_3, CH_2Cl_2. **25** (0.55 g, 95%). White powder. Mp: 184–185 °C. IR (KBr): 3093.2, 1675.4, 1515.15 (NH_3^+). UV–vis (H_2O): 331 (13649). ^{1}H NMR (400 MHz, D_2O): 2.41 (s, 3H, *Me*btz); 2.94 (t, J = 7.3 Hz, 2H, CH_2CH$_2$N); 3.24 (t, J = 7.3 Hz, 2H, CH$_2$CH_2N); 5.17 (s, 2H, OCH_2btz); 7.05 (d, J = 8.3 Hz, 2H, Ar*H*); 7.27 (d, J = 7.3 Hz, 2H, Ar*H*); 7.27 (s, 1H, *H* btz); 7.66 (s, 1H, *H* btz). ^{1}H NMR (400 MHz, DMSO-d_6): 2.44 (s, 3H, *Me*btz); 2.80 (t, J = 7.7 Hz, 2H, CH_2CH$_2$N); 3.02 (br s, 2H, CH$_2$CH_2N); 5.21 (s, 2H, OCH_2btz); 7.04 (d, J = 7.55 Hz, 2H, Ar*H*); 7.20 (d, J = 7.55 Hz, 2H, Ar*H*); 7.52 (s, 1H, *H* btz); 7.80 (br s, 2H, NH_2); 7.89 (s, 1H, *H* btz). ^{13}C NMR (100 MHz, D_2O): 17.03 (*Me*btz); 32.58 (CH_2CH$_2$N); 40.52 (CH$_2$CH$_2$N); 65.49 (OCH$_2$btz); 115.29 (C_o or C_m); 117.66, 120.72 (C_5, $C_{5'}$ of btz); 130.02 (C_p); 130.13 (C_o or C_m); 153.56, 154.11, 157.30 (C_2, $C_{2'}$, C_4, $C_{4'}$ of btz; C_{ipso}); no visible TFA. Anal. Calcd for $C_{16}H_{17}ON_3S_2{\cdot}CF_3COOH{\cdot}0.5H_2O$ (454.48): C, 47.57; H, 4.21; N, 9.24; S, 14.11. Found: C, 47.48; H, 4.13; N, 9.13; S, 13.66. ES-MS (pos. mode): 332.33 [M−CF_3COOH+H$^+$]$^+$, 195.28 [CH_3−btz−CH_2]$^+$.

5.1.10. Compounds 17, 26, 27 and 28—general procedure way 1

A suspension of the ammonium salt in CH_2Cl_2 was solubilized by addition of the minimum amount of MeOH. NEt_3, then **K** were added, and the solution was stirred at rt under Ar. The reaction was followed by TLC. When all the amine was consumed, the solvent were evaporated to dryness. The residue was solubilized in CH_2Cl_2, and the solution was washed with 2 M aqueous $NaHSO_4$, satd aqueous $NaHCO_3$, then satd aqueous NaCl. After drying over Na_2SO_4 or $MgSO_4$, filtration and evaporation, the resulting material was chromatographed to give the expected di-boc guanidinoethyl derivative. *General procedure way 2:* A solution of ammonium salt (1 equiv), NEt_3 (ca. 4 equiv)), 1,3-bis(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea **J** (1.1−1.2 equiv)) in dry DMF (dstd over CaH_2, or conserved over anhydrous $CaSO_4$) was cooled at 4 °C under Ar. $HgCl_2$ was added in one portion, and stirring was continued at rt under Ar to give a white suspension. The solvent was evaporated to dryness under high vacuum, and the solid residue was triturated in AcOEt or CH_2Cl_2, and filtered over Celite. The filtrate was evaporated to dryness, and residual **J** was eliminated by trituration of the residue with heptane, and filtration. The resulting solid was finally chromatographed to give the expected di-boc guanidinoethyl derivative.

5.1.10.1. 4-[(*N*,*N*′-Di-Boc)guanidinoethyl]-phenol 17. *Way 1*: From tyramine hydrochloride **F** (0.21 g, 1.20 mmol), **K** (0.47 g, 1.20 mmol), NEt_3 (0.66 mL, 0.48 g, 4.80 mmol), CH_2Cl_2 (10 mL), MeOH (10 mL). 2 h, TLC monitoring, SiO_2, CH_2Cl_2, and the solvent were evaporated to dryness. Washings: CH_2Cl_2 (20 mL), 2 M $NaHSO_4$ (30 mL), satd $NaHCO_3$ (30 mL), satd NaCl (40 mL). Chromatography: SiO_2 deactivated with NEt_3; CH_2Cl_2). **17** (0.32 g, 72%). White powder. *Way 2:* from tyramine hydrochloride **F** (0.50 g, 2.87 mmol), NEt_3 (0.74 mL, 10.07 mmol), DMF (15 mL), **J** (0.92 g, 3.16 mmol), $HgCl_2$ (0.86 g, 3.16 mmol). 18 h. Trituration in AcOEt. Chromatography: SiO_2, CH_2Cl_2/MeOH, 99:1. **17** (0.98 g; 90%). White solid. Mp: 142–143 °C. IR (KBr): 3447.9 (OH); 1724.7 (COO); 1624.5 (C=N). UV–vis (CH_2Cl_2): 276 (1762). ^{1}H NMR (400 MHz, $CDCl_3$): 1.48 (s, 9H, Me_3C); 1.49 (s, 9H, Me_3C); 2.77 (t, J = 7.4 Hz, 2H, CH_2CH$_2$NH); 3.60 (q, J = 7.0 Hz, 6H, CH$_2$CH_2NH); 5.82 (br s, 1H, N*H*); 6.75 (d, J = 8.5 Hz, 2H, Ar*H*); 7.00 (d, J = 8.3 Hz, 2H, Ar*H*); 8.43 (s, 1H, O*H*); 11.44 (s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.42, 28.59 (Me_3C); 34.75 (CH_2CH$_2$N); 43.13

(CH_2CH_2N); 80.18, 83.70 (Me_3C); 115.98 (C_o); 129.76 (C_p); 129.95 (C_m); 153.50 (*C*O); 155.42 (C_{ipso}); 156.59 (*C* guan). Anal. Calcd for $C_{19}H_{29}O_5N_3$ (379.45): C, 60.14; H, 7.70; N, 11.07. Found: C, 60.13; H, 7.65; N, 11.04. ES-MS (pos. mode): 380.37 $[M+H^+]^+$.

5.1.10.2. [(*N,N′*-Di-Boc)-guanidinoethyl]-4-[6-methyleneoxy-6′-methyl-2,2′-bipyridyl]-benzene 26. *Way 1: From* **23** (0.25 g, 0.51 mmol), NEt_3 (0.24 mL, 1.73 mmol), **K** (0.23 g, 0.58 mmol), CH_2Cl_2 (20 mL), EtOH. 2 h, TLC monitoring: Al_2O_3, CH_2Cl_2. Washings: CH_2Cl_2 (40 mL), 2 M $NaHSO_4$ (30 mL), satd $NaHCO_3$ (30 mL). Drying: Na_2SO_4. Chromatography: Al_2O_3, CH_2Cl_2. **26** (0.28 g, 85%). White powder. *Way 2:* from **23** (0.64 g, 1.30 mmol), NEt_3 (0.72 mL, 5.14 mmol), **J** (0.47 g, 1.61 mmol), DMF (30 mL). $HgCl_2$ (0.44 g, 1.61 mmol). 5 h. Trituration in CH_2Cl_2. Chromatography: Al_2O_3, CH_2Cl_2. **26** (0.47 g, 64%). Mp: 132–133 °C. IR (KBr): 3328.4 (CONH); 1734.3 (CO); 1654.9, 1619.0 (NCO); 1511.2 (NH). UV–vis (CH_2Cl_2): 290 (17,595). ^{1}H NMR (400 MHz, $CDCl_3$): 1.47 (s, 9H, Me_3C); 1.50 (s, 9H, Me_3C); 2.64 (s, 3H, *Me*bpy); 2.81 (t, *J* = 7.3 Hz, 2H, CH_2CH_2N); 3.63 (q, *J* = 7.0 Hz, 2H, CH_2CH_2N); 5.27 (s, 2H, OCH_2bpy); 6.95 (d, *J* = 8.6 Hz, 2H, Ar*H*); 7.14 (d, *J* = 8.6 Hz, 2H, Ar*H*); 7.18 (d, *J* = 7.8 Hz, 1H, *H* bpy); 7.51 (d, *J* = 7.5 Hz, 1H, *H* bpy); 7.71 (t, *J* = 7.8 Hz, 1H, *H* bpy); 7.82 (t, *J* = 7.8 Hz, 1H, *H* bpy); 8.20 (d, *J* = 7.8 Hz, 1H, *H* bpy); 8.32 (d, *J* = 7.8 Hz, 1H, *H* bpy); 8.35 (m, 1H, CH_2CH_2NH); 11.46 (s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 25.02 (*Me*bpy); 28.46, 28.72 (Me_3C); 34.81 (CH_2CH_2N); 42.85 (CH_2CH_2N); 71.31 (Me_3C); 79.66 (OCH_2bpy); 83.42 (Me_3C); 115.36 (C_m); 118.69, 120.44, 121.38, 123.74 (C_3, $C_{3'}$, C_5, $C_{5'}$ of bpy); 130.25 (C_o); 131.46 (C_p); 137.55, 138.01 (C_4, $C_{4'}$ of bpy); 153.57 (C_{ipso}); 156.23 (*C*O); 156.51 (*C* guan); 155.85, 157.30, 157.61, 158.34 (C_2, $C_{2'}$, C_6, $C_{6'}$ of bpy); 163.97 (*C*O). Anal. Calcd for $C_{31}H_{39}O_5N_5$ (561.67): C, 66.29; H, 6.99; N, 12.47. Found: C, 66.34; H, 7.02; N, 12.38. EI-MS: 345 $[M-(Me)_3COC(O)NH-(Me)_3COC(O)]^+$; 302 $[M-((Me)_3COC(O))_2guan]^+$; 289 $[M-((Me)_3COC(O))_2guanCH_2]^+$.

5.1.10.3. [(*N,N′*-Di-Boc)-guanidinoethyl]-4-[5-methyleneoxy-5′-methyl-2,2′-bipyridyl]-benzene 27. From **24** (0.233 g, 0.54 mmol), NEt_3 (0.3 mL, 2.15 mmol), **K** (0,21 g, 0,54 mmol), CH_2Cl_2 (25 mL), MeOH. 23 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH 98:2 with 2 drops of NEt_3). CH_2Cl_2 (50 mL). Washings: H_2O (2 × 30 mL). Drying: Na_2SO_4. Chromatography: SiO_2, deactivated by CH_2Cl_2/NEt_3 90:10 then CH_2Cl_2; **12** (0.234 g, 84%) slightly pink powder. Mp: 148–149 °C. IR (KBr): 3331.55 (CONH); 1709.01(CO); 1636.81 (NCO); 1512.12 (NH). UV–vis (CH_2Cl_2): 289 (28,230). ^{1}H NMR (400 MHz, $CDCl_3$) 1.47 (s, 9H, Me_3C); 1.50 (s, 9H, Me_3C); 2.41 (s, 3H, *Me*bpy); 2.82 (t, *J* = 7.16 Hz, 2H, CH_2CH_2N); 3.64 (q, *J* = 7.0 Hz, 2H, CH_2CH_2N); 5.29 (s, 2H, OCH_2bpy); 6.92 (d, *J* = 8.56 Hz, 2H, Ar*H*); 7.15 (d, *J* = 8.56 Hz, 2H, Ar*H*); 7.67 (d, *J* = 7.8 Hz, 1H, *H* bpy); 7.9 (dd, *J* = 8.2 Hz, 1H, *H* bpy); 8.33 (d, *J* = 8.08 Hz, 1H, *H* bpy); 8.37 (br s, 1H, N*H*); 8.43 (d, *J* = 8.04 Hz, 1H, *H* bpy); 8.52 (s, 1H, *H* bpy); 8.72 (s, 1H, *H* bpy); 11.46 (s, 1H, N*H* guan). ^{13}C NMR (100 MHz, $CDCl_3$): 18.78 (*Me*bpy); 28.44, 28.71 (Me_3C); 34.81 (CH_2CH_2N); 42.79 (CH_2CH_2N); 67.99 (Me_3C); 79.63 (OCH_2bpy); 83.41 (Me_3C); 115.37, 130.30 (C_o and C_m of Ar); 120.99, 121.05, 136.64, 137.87, 148.73, 150.07 (C_3, $C_{3'}$, C_4, $C_{4'}$, C_6, $C_{6'}$ of bpy); 131.73 (C_p of Ar); 132.67, 133.91, 156.53, 157.55 (C_2, $C_{2'}$, C_5, $C_{5'}$ of bpy); 153.57, 153.74 (C_{ipso} of Ar, *C*O); 157.55 (C_2 of bpy); 156.47 (*C* guan); 164.02 (*C*O). Anal. Calcd for $C_{31}H_{39}N_5O_5{\cdot}0.33CH_2Cl_2$ (589.98): C, 63.79; H, 6.78; N, 11.87. Found: C, 63.70; H, 5.57; N, 11.96. EI-MS (pos. mode): 320.1 [M−(BocNH–C=NBoc)]$^+$; 342.0 [M−(BocNH–C=NBoc)+Na$^+$]$^+$; 639.3 [2(M−(BocNH–C=NBoc))+H$^+$]$^+$.

5.1.10.4. [(*N,N′*-Di-Boc)-guanidinoethyl]-4-[4-methyleneoxy-4′-methyl-2,2′-bithiazolyl]-benzene 28. *Way 1: From* **25** (0.25 g, 0.55 mmol), NEt_3 (0.235 mL, 1.68 mmol), **K** (0.22 g, 0.56 mmol), CH_2Cl_2 (20 mL), MeOH. 4 h, TLC monitoring: Al_2O_3, CH_2Cl_2. Washings: CH_2Cl_2 (30 mL), 2 M $NaHSO_4$ (20 mL), satd $NaHCO_3$ (20 mL). Drying: Na_2SO_4. Chromatography: Al_2O_3, CH_2Cl_2. **28** (0.31 g, 95%). White powder. *Way 2:* from **25** (0.74 g, 1.63 mmol), NEt_3 (0.80 mL, 5.81 mmol), **J** (0.53 g, 1.82 mmol), DMF (30 mL), $HgCl_2$ (0.49 g, 1.82 mmol). 5 h. Trituration in CH_2Cl_2. Chromatography: Al_2O_3, CH_2Cl_2/Hex, 1:1. **28** (0.58 g, 60%). White powder. Mp: 126–127 °C. IR (KBr): 3347.0 (CONH); 1720.9 (CO); 1647,2, 1617.7 (NCO); 1510.5 (NH). UV–vis (CH_2Cl_2): 286 (2387). ^{1}H NMR (400 MHz, $CDCl_3$): 1.48 (s, 9H, Me_3C); 1.51 (s, 9H, Me_3C); 2.52 (s, 3H, *Me*btz); 2.84 (t, *J* = 6.9 Hz, 2H, CH_2CH_2N); 3.63 (q, *J* = 6.5 Hz, 2H, CH_2CH_2N); 5.24 (s, 2H, OCH_2btz); 6.94 (d, *J* = 8.1 Hz, 2H, Ar*H*); 6.99 (s, 1H, *H* btz); 7.15 (d, *J* = 8.3 Hz, 2H, Ar*H*); 3.39 (s, 1H, *H* btz); 8.48 (br s, 1H, N*H*); 11.47 (s, 1H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 17.55 (*Me*btz); 28.46, 28.72 (Me_3C); 34.80 (CH_2CH_2N); 42.82 (CH_2CH_2N); 66.69 (Me_3C); 79.66 (OCH_2btz); 83.43 (Me_3C); 115.29 (C_o or C_m); 116.25, 118.01 (C_5, $C_{5'}$ btz); 130.27 (C_o or C_m) 131.69 (C_p); 153.56 (C_{ipso}); 154.30, 154.70 (C_2, $C_{2'}$ btz); 156.50 (*C* guan); 157.43 (*C*O); 160.80, 162.19 (C_4, $C_{4'}$ btz); 163.91 (*C*O). Anal. Calcd for $C_{27}H_{35}O_5N_5S_2{\cdot}0.2CH_2Cl_2$ (590.7): C, 54.30; H, 6.04; N, 11.86; S, 10.86. Found: C, 55.41; H, 6.09; N, 11.98; S, 10.85. ES-MS (pos. mode): 374.2 $[M-2Boc+3H^+]^+$; 474.3 $[M-Boc+2H^+]^+$; 574.2 $[M+H^+]^+$.

5.1.11. Compounds 18, 29, 30 and 31—general procedure

TFA was added to a solution of di-boc-guanidinoethyl derivatives in CH_2Cl_2. The mixture was stirred at rt under Ar during ca. 2–3 h (TLC monitoring). The solvents were evaporated, and the residual TFA was eliminated by successive dissolution in CH_2Cl_2–evaporation cycles, until a white solid was obtained (ca. four cycles). The latter was dried under high vacuum, triturated in Et_2O, filtered and rinsed with Et_2O to give the expected guanidinium salt.

5.1.11.1. 4-(Guanidinoethyl)-phenol, trifluoroacetate salt 18. From **17** (0.5 g, 1.31 mmol), CH_2Cl_2 (30 mL), TFA (10 mL). 2 h, TLC monitoring: SiO_2, CH_2Cl_2/MeOH 99:1. **18** (0.35 g, 90%). White solid. Mp: 156–157 °C. IR (KBr): 3408.8 (OH); 1686.3 (NH). UV–vis (H_2O): 274 (1916). ^{1}H NMR (400 MHz, D_2O): 2.80 (t, *J* = 6.6 Hz, 2H, CH_2CH_2NH); 3.41 (t, *J* = 6.6 Hz, 2H, CH_2CH_2NH); 6.86 (d, *J* = 7.5 Hz, 2H, Ar*H*); 7.17 (d, *J* = 7.8 Hz, 2H, Ar*H*). ^{1}H NMR (400 MHz, DMSO-d_6): 2.66 (t, *J* = 7.3 Hz, 2H, CH_2CH_2NH); 3.29 (q, *J* = 6.7 Hz, 2H, CH_2CH_2NH); 6.70 (d, *J* = 8.6 Hz, 2H, Ar*H*); 6.90 (br s, 1H, N*H*), 7.05 (d, *J* = 8.3 Hz, 2H, Ar*H*); 7.34 (br s, 2H, NH_2); 7.5 (s, 1H, O*H*); 9.27 (s, 1H, N*H*). ^{13}C NMR (400 MHz, D_2O): 33.68 (CH_2CH_2NH); 42.82 (CH_2CH_2NH); 115.82 (C_o); 116.71 (q, *J* = 291.9 Hz, CF_3COOH); 130.59 (C_m); 130.64 (C_p); 154.49 (C_{ipso}); 157.06 (*C* guan); 163.35 (q, *J* = 35.9 Hz, CF_3COOH). Anal. Calcd for $C_9H_{13}ON_3{\cdot}CF_3COOH$ (293.24): C, 45.05; H, 4.81; N, 14.33. Found: C, 44.85; H, 4.90; N 14.03. ES-MS (pos. mode): 180.38 $[M-CF_3COOH+H^+]^+$. ES-MS (neg. mode): 406.17 $[M+CF_3COOH-H^+]^-$.

5.1.11.2. Guanidinoethyl-4-[6-methyleneoxy-6′-methyl-2,2′-bipyridyl]-benzene, sesqui-trifluoroacetate salt 29. From **26** (0.23 g, 0.41 mmol), CH_2Cl_2 (20 mL), TFA (5 mL). 2 h, TLC monitoring: SiO_2, CH_2Cl_2/MeOH 99:1. **29** (0.18 g, 82%). White solid. Mp: 142–143 °C. IR (KBr): 3422.2 (NH_2); 3182.0 (NH_3^+); 1694.0 (C=O); 1663.5, 1514.7 (NH_3^+). UV–vis (H_2O): 286 (15,506). ^{1}H NMR (400 MHz, D_2O): 2.66 (s, 3H, *Me*bpy); 2.84 (t, *J* = 6.5 Hz, 2H, CH_2CH_2N); 3.43 (t, *J* = 6.5 Hz, 2H, CH_2CH_2N); 5.34 (s, 2H, OCH_2btz); 7.07 (d, *J* = 8.8 Hz, 2H, Ar*H*); 7.26 (d, *J* = 8.6 Hz, 2H, Ar*H*); 7.50 (d, *J* = 7.5 Hz, 1H, *H* bpy); 7.65 (d, *J* = 7.5 Hz, 1H, *H* bpy); 7.89 (d, *J* = 7.8 Hz, 1H, *H* bpy); 7.97 (t, *J* = 7.0 Hz, 1H, *H* bpy); 8.01 (d, *J* = 7.8 Hz, 1H, *H* bpy); 8.02 (t, *J* = 7.8 Hz, 1H, *H* bpy). ^{1}H NMR (400 MHz, DMSO-d_6): 2.58 (s, 3H, *Me*bpy); 2.73 (t, *J* = 7.0 Hz, 2H, CH_2CH_2N); 3.42 (q, *J* = 5.8 Hz, 2H, CH_2CH_2N); 5.26 (s, 2H, OCH_2btz); 6.70–7.80 (broad signal, 3H, N*H* guan); 7.03 (d, *J* = 7.6 Hz, 2H, Ar*H*); 7.21 (d, *J* = 7.6 Hz, 2H, Ar*H*); 7.35 (d, *J* = 7.6 Hz, 1H, *H* bpy); 7.49 (br s, 1H, CH_2CH_2NH); 7.55 (d, *J* = 7.6 Hz, 1H, *H* bpy); 7.86 (d, *J* = 7.5 Hz, 1H, *H* bpy); 7.98 (t, *J* = 7.3 Hz, 1H, *H* bpy); 8.49 (d, *J* = 7.8 Hz, 1H, *H* bpy); 8.32 (t, *J* = 7.8 Hz, 1H, *H* bpy). ^{13}C NMR (100 MHz, DMSO-d_6):

24.54 (*Me*bpy); 33.93 (*C*H_2CH_2N); 42.59 (CH_2CH_2N); 70.77 (O*C*H_2bpy); 115.12 (C_o or C_m); 118.10, 119.87, 122.09, 124.08 (C_3, $C_{3'}$, C_5, $C_{5'}$ bpy); 130.27 (C_o or C_m); 131.06 (Cp); 137.97, 138.43 (C_4, $C_{4'}$ bpy); 156.94 (*C* guan); 157.14 (C_{ipso}); 154.60, 155.21, 157.20, 157.94 (C_2 and $C_{2'}$, C_6, $C_{6'}$ bpy). Anal. Calcd for $C_{21}H_{23}ON_5.1.5CF_3COOH$ (532.47): C, 54.13; H, 4.63; N, 13.15. Found: C, 54.25; H, 4.76; N, 13.03. ES-MS (pos. mode): 365.35 $[M-CF_3COOH+H^+]^+$. ES-MS (neg. mode): 588.12 $[M+CF_3COO^-]^-$.

5.1.11.3. Guanidinoethyl-4-[5-methyleneoxy-5′-methyl-2,2′-bipyridyl]-benzene, trifluoroacetate salt 30. From **27** (0.244 g, 0.434 mmol), CH_2Cl_2 (25 mL), TFA (6 mL). 22 h, TLC monitoring: SiO_2, CH_2Cl_2/MeOH 98:2. **30** (0.195 g, 95%). Slightly pink solid. Mp: 162–163 °C. IR (KBr): 3392.73 (NH_2); 3116.38 (NH_3^+); 1673.98 (C=O); 1626.79, 1513.25 (NH_3^+). UV–vis (H_2O): 288 (19,072), 240 (15,422), 228 (14,836). ^{1}H NMR (400 MHz, D_2O): 2.35 (s, 3H, *Me*bpy); 2.79 (t, *J* = 6.28 Hz, 2H, C*H*$_2$CH$_2$N); 3.43 (t, *J* = 6.54 Hz, 2H, CH$_2$C*H*$_2$N); 5.16 (s, 2H, OC*H*$_2$bpy); 7.00 (d, *J* = 8.04 Hz, 2H, Ar*H*); 7.20 (d, *J* = 8.28 Hz, 2H, Ar*H*); 7.76 (d, *J* = 7.04. Hz, 1H, *H* bpy); 7.87 (d, *J* = 7.28 Hz, 1H, *H* bpy); 7.96 (s, 2H, *H* bpy); 8.40 (s, 1H, *H* bpy); 8.58 (s, 1H, *H* bpy). ^{1}H NMR (400 MHz, DMSO-d_6): 2.64 (s, 3H, *Me*bpy); 2.86 (t, *J* = 7.16 Hz, 2H, C*H*$_2$CH$_2$N); 3.45 (q, *J* = 4.78H, CH$_2$C*H*$_2$N); 5.33 (s, 2H, OC*H*$_2$bpy); 7.14 (d, *J* = 8.32 Hz, 2H, Ar*H*); 7.34 (d, *J* = 8.32 Hz, 2H, Ar*H*); 6.9–7.77 (broad signal, 3H, N*H* guan); 7.79 (br s, 1H, CH$_2$CH$_2$N*H*);); 7.89; (d, *J* = 7.8 Hz, 1H, *H* bpy); 8.12 (d, *J* = 8.28 Hz, 1H, *H* bpy); 8.42 (t, *J* = 8.04 Hz, 1H, *H* bpy); 8.51 (d, *J* = 8.08 Hz, 1H, *H* bpy); 8.66 (s, 1H, *H* bpy); 8.87 (s, 1H, *H* bpy). ^{13}C NMR (100 MHz, DMSO-d_6): 18.19 (*Me*bpy); 33.93 (*C*H_2CH_2N); 42.59 (CH_2CH_2N); 67.08 (OCH_2bpy); 115.16, 130.24 (C_o or C_m of Ar); 120.22, 120.37, 137.02, 137.98, 148.95, 149.94 (C_3, C_3', C_4, $C_{4'}$, C_6, $C_{6'}$ of bpy); 131.09 (C_p of Ar); 133.13, 134.09, 152.84, 155.37 (C_2, $C_{2'}$, C_5, $C_{5'}$ of bpy); 157.10, 157.14 (*C* guan; C_{ipso} of Ar); 158.85 (q, *J* = 30.9 Hz, CF_3COO^-); *C*F_3COO^- not observed. Anal. Calcd for $C_{21}H_{23}ON_5$·CF_3COOH (475.46): C, 58.10; H, 5.09; N, 14.73. Found: C, 57.96; H, 5.20; N, 14.63. ES-MS (pos; mode): 362.1 $[M-CF_3COO^-]^+$.

5.1.11.4. Guanidinoethyl-4-[4-methyleneoxy-4′-methyl-2,2′-bithiazolyl]-benzene, trifluoroacetate salt 31. From **28** (0.25 g, 0.42 mmol), CH_2Cl_2 (30 mL), TFA (10 mL). 3 h, TLC monitoring: Al_2O_3, CH_2Cl_2. **31** (0.2 g, 98%). White solid. Mp: 179–180 °C. IR (KBr): 3367.2 (NH_2); 3166.7 (NH_3^+); 1674.9 (C=O); 1624.2, 1515.3 (NH_3^+). UV–vis (H_2O): 331 (12,553). ^{1}H NMR (400 MHz, D_2O): 2.44 (s, 3H, *Me*btz); 2.85 (t, *J* = 6.5 Hz, 2H, C*H*$_2$CH$_2$N); 3.43 (t, *J* = 6.5 Hz, 2H, CH$_2$C*H*$_2$N); 5.22 (s, 2H, OC*H*$_2$btz); 7.06 (d, *J* = 8.1 Hz, 2H, Ar*H*); 7.26 (d, *J* = 8.1 Hz, 2H, Ar*H*); 7.30 (s, 1H, *H* btz); 7.68 (s, 1H, *H* btz). ^{1}H NMR (400 MHz, DMSO-d_6): 2.44 (s, 3H, *Me*btz); 2.74 (t, *J* = 7.3 Hz, 2H, C*H*$_2$CH$_2$N); 3.33 (q, *J* = 6.8 Hz, 2H, CH$_2$C*H*$_2$N); 5.20 (s, 2H, OC*H*$_2$btz); 6.50–7.80 (broad signal, 4H, NH_3^+, N*H*); 7.02 (d, *J* = 8.4 Hz, 2H, Ar*H*); 7.21 (d, *J* = 8.5 Hz, 2H, Ar*H*); 7.51 (s, 1H, *H* btz); 7.63 (m, 1H, CH$_2$CH$_2$N*H*); 7.88 (s, 1H, *H* btz). ^{13}C NMR (100 MHz, DMSO-d_6): 17.03 (*Me*btz); 33.94 (*C*H_2CH_2N); 42.60 (CH_2CH_2N); 65.48 (OCH_2btz); 115.06 (C_o or C_m); 117.67, 120.69 (C_5, $C_{5'}$ btz); 130.22 (C_o or C_m); 131.08 (C_p); 153.60, 154.12 (C_2, $C_{2'}$ btz); 157.09, 157.14 (C_{ipso} and *C* guan); 159.85, 161.34 (C_4, $C_{4'}$ btz); no visible TFA. Anal. Calcd for $C_{17}H_{19}ON_5S_2$·CF_3COOH (487.52): C, 46.81; H, 4.13; N, 14.36; S, 13.15. Found: C, 46.61; H, 4.42; N, 14.39; S, 12.79. ES-MS (pos. mode): 374.24 $[M-CF_3COOH+H^+]^+$. ES-MS (neg. mode): 600.04 $[M+CF_3COO^-]^-$.

5.1.12. 1,10-Bis-[(*N*,*N*′-di-Boc)-guanidino]-decane 32

The 1,10-diaminodecane (0.25 g, 1.45 mmol) was added under Ar to a solution of NEt_3 (1.41 mL, 10.15 mmol) and **J** (0.93 g, 3.19 mmol) in dry DMF (15 mL). The mixture was cooled to 4 °C, and $HgCl_2$ (0.86 g, 3.19 mmol) was added in one portion. Stirring was continued at rt during 20 h, giving a light brown precipitate. The solvent was evaporated under high vacuum, to give a solid that was triturated in CH_2Cl_2, filtered over Celite, concentrated and chromatographed (Al_2O_3, CH_2Cl_2/Hex, 70:30) to give **32** (0.50 g, 52%). Mp: 101–102 °C. IR (KBr): 3336.8 (–NH, –NH_2); 1739.3 (–C=N); 1654.5 (–N–CO). ^{1}H NMR (400 MHz, $CDCl_3$): 1.26 (m, 12H, $H_{3,4,5}$); 1.50 (m, 40H, H_2, Me_3C); 3.39 (q, *J* = 7.2 Hz, 4H; H_1), 8.29 (s, 2H, N*H*); 11.50 (s, 2H, N*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 27.21, 29.33, 29.73 (C_3, C_4, C_5); 28.45, 28.69 (Me_3C); 29.59 (C_2); 41.34 (C_1); 79.51, 83.30 (Me_3C); 153.70 (*C*O); 156.78 (*C* guan); 164.04 (*C*O). Anal. Calcd for $C_{32}H_{60}O_8N_6$·$0.33C_6H_{12}$ (685.58): C, 59.56; H, 9.51; N, 12.26. Found: C, 59.42; H, 9.17; N, 12.63. EI-MS: 577 $[M-(Me)_3COC(O)+Na]^+$; 577 $[M-(Me)_3COC(O)-(Me)_3C+Na]^+$.

5.1.13. 1,10-Bis-(guanidino]-decane, bis-trifluoroacetate salt 33; Synthalin A

TFA (15 mL) was added to a solution of **32** (1.14 g, 1.7 mmol) in anhydrous CH_2Cl_2 (40 mL). The mixture was stirred at rt under Ar during 3 h, and the solvents were evaporated. The residual TFA was eliminated by successive dissolution in CH_2Cl_2–evaporation cycles, until a white solid was obtained (ca. four cycles). The latter was dried under high vacuum, triturated in Et_2O, filtered and rinsed with Et_2O (0.85 g). This last solid was dialyzed (Float-A-Lyser, cellulose acetate, MWCO 100 D) and lyophilized to give **33** (0.6 g; 70%). White cotton. Mp: 109–110 °C. IR (KBr): 3376.7 (NH, NH_2); 1675.9 (C=N). ^{1}H NMR (400 MHz, D_2O): 1.28 (m, 12H, $H_{3,4,5}$); 1.56 (m, *J* = 6.4 Hz, 4H, H_2); 3.15 (t, *J* = 7.0 Hz, 4H, H_1). ^{1}H NMR (400 MHz, DMSO-d_6): 1.26 (m, 12H, $H_{3,4,5}$); 1.45 (m, 4H, H_2); 3.09 (m, *J* = 6.4 Hz, 4H, H_1). ^{13}C NMR (100 MHz, D_2O): 26.17, 28.65, 28.89 (C_3, C_4, C_5); 28.20 (C_2); 41.53 (C_1); 116.67 (q, *J* = 291.4 Hz, *C*F_3COO^-); 157.05 (*C* guan); 163.20 (q, *J* = 35.2 Hz, CF_3COO^-). Anal. Calcd for $C_{12}H_{28}N_6$·$2CF_3COOH$·H_2O (498.45): C, 38.55; H, 6.47; N, 16.86. Found: C, 38.55; H, 6.50; N, 16.99. ES-MS (pos. mode): 371.41 $[M-CF_3COOH+H^+]^+$; 257.54 $[M-2CF_3COOH+H^+]^+$. ES-MS (neg. mode): 597.25 $[M+CF_3COOH-H^+]^-$; 483.38 $[M+CF_3COOH-H^+]^-$; 369.45 $[M-CF_3COOH-H^+]^-$.

5.2. Biology

5.2.1. Bacteriology

Bacteria were grown in Mueller-Hinton broth (Difco, 275730), or Mueller Hinton agar (Difco, 225250); purity of isolates was checked at the time of every test by examination of colony morphology and Gram staining. For MIC determination experiments, bacteria were inoculated in 96-well U shape microtiter plates to yield a final inoculum of 1×10^5 colony-forming units (CFU)/mL. Then various concentrations of the drugs were added. The cultures were grown for 18–24 h at 35 °C. The resulting bacterial growth was measured with an ELISA plate reader (Multiskan EX, Thermo Electron Corporation, France) at a wavelength of 540 nm.

5.2.2. Cell viability

MRC-5 cells (human pulmonary embryonic fibroblasts) were obtained from BioMerieux (France). The cells were maintained in modified Eagle's medium (MEM, Invitrogen 41090) supplemented with 10% decomplemented fetal bovine serum (FBS, Invitrogen 10270, batch 40Q5150K) without antibiotics at 37 °C, 5% CO_2, under a humid atmosphere. Cells were plated at 10^4 cells/well in 96-well plates (Sarstedt 831835). Forty eight hours after plating, the growth medium was removed and replaced with the test solutions (100 μL). Viability tests were performed after 24, 48 and 168 h exposure to drugs; they were carried out using a commercially available cell proliferation reagent MTT [3-(4,5-dimethylthiazolyl-2-yl-2,5-diphenyltetrazolium bromide] (Sigma 1350380) as described previously.[3,30] The assay is based on cleavage of the tetrazolium salt MTT by active mitochondria to produce an

insoluble purple formazan salt. Since this conversion only occurs with viable cells, it directly correlates with cell count. After 24, 48 and 168 h exposure, 10 μL of a 5 mg/mL MTT solution were added to each well and plates were incubated at 37 °C for 4 h. Then, insoluble purple formazan was dissolved by adding 100 μL SDS to each well. The absorbance $A_{540\ nm}$ was measured with a reference wavelength of $A_{690\ nm}$, using an ELISA reader (Multiskan EX, Thermo Electron Corporation, France). The results were expressed as the percent absorbance of treated versus untreated control cultures. Eight wells per dose and time point were counted in 3 different experiments. Lactate dehydrogenase (LDH) release experiments: The detection procedures of LDH release were in accordance with the manufacturer's instructions (CytoTox 96® Non-Radioactive Cytotoxicity Assay, Promega Corp, Madison, WI). Briefly, all reagents stocked at 4 °C were slowly warmed up. Then, on appropriate microtiter plate (MTP), 10 μL of lysis solution were added to 'control cell' wells in order to determine 100% release of LDH; and MTP was centrifuged at 2500 g for 4 min to have 'cell-free' supernatants. Subsequently, 25 μL of each sample were transferred to a new MTP, and 25 μL of freshly prepared Substrate Mix were added to each well and incubated up to 30 min in the dark at room temperature. Finally, 50 μL of stop solution were added to each well. The absorbance $A_{490\ nm}$ was measured using an ELISA reader (Multiskan EX, Thermo Electron Corporation, France). The results were expressed as the percent absorbance of treated versus untreated control cultures. Eight wells per dose and time point were counted in 3 different experiments.

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