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Guanidinium compounds with sub-micromolar activities against *Mycobacterium tuberculosis*. Synthesis, characterization and biological evaluations

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ABSTRACT

Seven polycharged species, incorporating 1, 2, 3, 4 and 6 guanidine arms organized around a benzene core were synthesized and assayed as anti-mycobacterial agents against *Mycobacterium tuberculosis*. They display MIC values comprised between 25 and 12.5 μM (close to ethambutol EMB) for the mono- and the hexa-substituted derivatives, and 0.8 μM (close to isoniazid and streptomycin) for the tri-substituted derivative. The three bi- and the tetra-substituted analogs displayed MIC values of ca. 6.5–3.0 μM. The latter were also evaluated against the isoniazid-resistant MYC5165 strain, resulting in highly interesting micromolar or sub-micromolar MIC, ca. 4–125 times more active than isoniazid. These preliminary results are attractive for the development of new anti-TB agents.

1. Introduction

Data from WHO concerning tuberculosis (TB), lead to an estimation of 9 million new cases in 2013 (6.1 million declared), with 1.5 million deaths (0.36 million HIV-positive people). Overall, emergence of multidrug-resistant tuberculosis resulted in an estimated population of 0.48 million people, the double of 2011' estimation, with 0.17 million deaths.[1] This airborne and contagious infectious disease that is preventable and curable is the second cause, after HIV, of human death due to a single etiologic agent, *Mycobacterium tuberculosis*. Approaches to fight TB are related to hygienic strategies, to detection, to vaccination and to drug discovery.[2] More particularly, a new diagnostic to detect rapidly *M. tuberculosis* sensitive or resistant to rifampicin has been launched very recently.

Some very recent reviews have focused on the past, present and future of anti-tuberculosis drugs. Currently, seven drugs are in Phase II, the oxazolidinones AZD5847, linezolid and sutezolid, the nitroimidazole-like PA-824, the 1,2-diamine SQ-109, the rifamycin derivative rifapentine and the diarylquinoline bedaquiline, the first novel TB drug approved in 2012, since ca. 40 years. In Phase III, are the nitroimidazole-like delamamid, the rifapentine and the fluoroquinolones gatifloxacin and moxifloxacin.[3–8]

Engaged in a research programme devoted to the discovery of new antibacterial agents targeting the bacterial membrane denaturation through expected supramolecular interactions between highly organized cations and anionic membrane constituents, we have recently shown that pre-organizing guanidinoethyl groups at the upper rim of calix[4]arene was a powerful way to generate an antibacterial activity while the constitutive phenolic monomer is inactive.[9] Such genesis of biological activity lead us to engage a wider programme devoted to evaluation of the antibacterial activity of the resulting tetra-*para*-guanidinoethylcalix[4]arene (**CX1**) against reference and resistant bacterial strains,[10–13] and development of physico-chemical investigations at the level of bacterial and model membrane.[14–17]

In parallel to Gram positive and Gram negative bacteria, we recently found that **CX1** and some of its derivatives were very interestingly active against reference and INH-resistant *M. tuberculosis* strains.[18]

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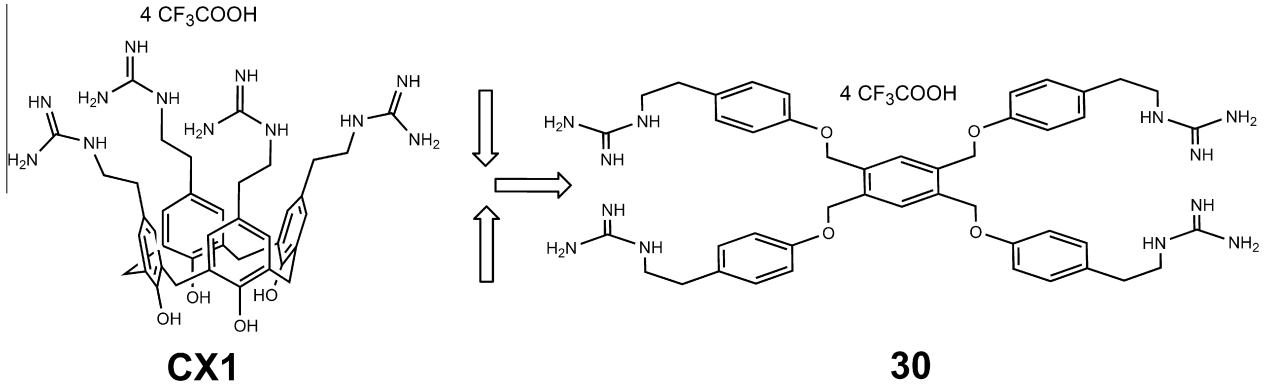


Figure 1. From 3D-constrained **CX1** to 2D-constrained **30**.

For example, **CX1** showed Minimum Inhibitory Concentration (MIC) values at same low level of 1 μg mL^{-1} (0.8 μM) for both $H_{37}Rv$ reference and INH-resistant MYC5165 *M. tuberculosis* strains.

These results opened an interesting area of investigations, focusing on the nature of the organizing core. In this sense, as the previous active calixarenic guanidinium derivative displays a high 3D-constraint level, organizing the guanidinium arms as a tetrapod, we propose here to diminish this constraint level, through a higher degree of freedom given by a simple benzene ring. For this purpose, the synthesis of a flattened tetraguanidinium analog of **CX1** (Fig. 1), was obviously engaged. In the same time, in order to define preliminary structure activity relationships, other 2D-constrained benzene derivatives including from 1 to 6 guanidinium arms were prepared and evaluated, object of the present work.

The 2D-constrained systems that were prepared are shown in Scheme 1. The molecules are, as the seminal calixarene, conceived as poly-guanidine compounds, in which the guanidine functions are *para*-guanidinoethyl-phenoxy-methylene groups attached to the central phenyl core across its tolyl, xylyl, mesityl, durenyl and mellitenyl derivatives.

The compounds tested in this study are the mono-substituted (tolyl) species **5**, the 1,2-, 1,3- and 1,4-di-substituted (xylyl) species **10**, **15** and **20**, the 1,3,5-tri-substituted (mesityl) species **25**, the 1,2,4,5-tetra-substituted (durenyl) species **30** and the 1,2,3,4,5,6-hexa-substituted (mellitenyl) species **35**. All these compounds show interesting antibacterial activities against various Gram positive and Gram negatives bacterial strains, associated to low-to modest cytotoxicities.[19]

2. Results and discussion

2.1. Chemistry

The general synthetic pathway leading to final guanidylated compounds **5**, **10**, **15**, **20**, **25**, **30** and **35** involved fours steps from the starting bromomethylated phenyl species **1**, **6**, **11**, **16**, **21**, **26** and **31** (Scheme 2). The latter were commercially available, but the tri-substituted species **21** was synthesised according to a procedure adapted from Li et al.[20] by radical bromination of mesitylene with NBS in CCl_4.

The bromides were reacted with ca. stoichiometric amounts of *para*-[(Boc)aminoethyl]phenol **A** (Boc-tyramine) in refluxing acetonitrile, in the presence of K_2CO_3 as base. The corresponding Boc-amino derivatives **2**, **7**, **12**, **17**, **22**, **27** and **32** were obtained with yields of 90%, 62%, 93%, 97%, 70%, 98% and 98%, respectively, after chromatography. The latter were then treated with trifluoroacetic acid (TFA) in CH_2Cl_2, at rt, to afford, after evaporation of solvent and TFA, then multiple trituration/filtration sequences carried out in Et_2O, the corresponding ammonium salts **3**, **8**, **13**, **18**, **23**, **28** and **33** respectively, in 96%, 71%, 92%, 85%, 98%, 80%, and 88% yields.

The nucleophilic attack of the free amines issued from these salts onto N_1,N_2-(di-Boc)-N_3-triflylguanidine **B** afforded the (di-Boc)-guanidino derivatives **4**, **14**, **19**, **24**, **29** and **34**, with yields of 85%, 98%, 90%, 87%, 61% and 84%, respectively. As an example, **9** was also prepared from **7**, 1,3-bis-(*tert*-butoxycarbonyl)- 2-methy l-2-thiopseudourea and $HgCl_2$ in dry DMF with a yield of 94%.

The expected guanidiniums **5**, **10**, **15**, **20**, **25** and **35** were finally prepared by a treatment similar to previous ammonium salts **3**–**33**, and a final lyophilization from distilled water solutions. The guanidinium trifluoroacetate salts were obtained with a yield of 94%, 75%, 98%, 79%, 90%, 71%, and 79%, respectively.

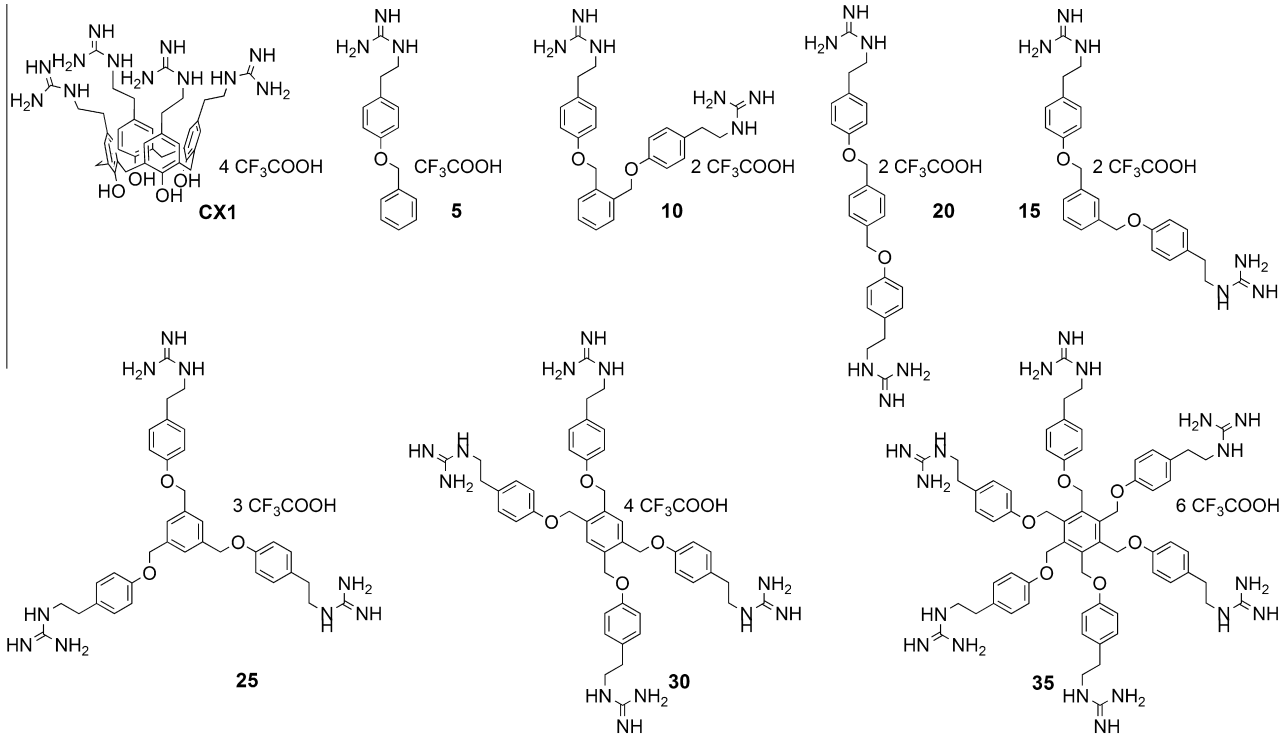
All compounds were fully characterized, notably by ^1H and ^{13}C NMR, mass spectrometry and elemental analyses. For the final guanidinium derivatives, elemental analyses were consistent with the expected salt structures, accompanied by some molecules of H_2O resulting from the lyophilization process.

2.2. Biological evaluations

In this study, in vitro antimycobacterial activity (e.g., Minimum Inhibitory Concentration (MIC) and 50% Inhibitory Concentration (IC_{50}) determination) of compounds **5**, **10**, **15**, **20**, **25**, **30** and **35** was evaluated against the reference strain *M. tuberculosis* $H_{37}Rv$ and the INH-resistant strain MYC5165 (*M. tuberculosis* strain mutated in InhA).[21,22] The results of these evaluations are condensed in Table 1. MIC and IC_{50} values are given in molar and mass dimensions.

The compound **5**, designed as monomeric species for comparison with poly-guanidinium species, is the less active of the series, with a MIC of 25 μM (9.8 μg/mL) and an IC_{50} of 5.9 μM (2.3 μg/mL). Similar results were obtained for the hexa-substituted species **35**. Even close to ethambutol (EMB, 9.8 μM), **5** and **35** were not evaluated against the INH-resistant strain. The *ortho*-di-substituted species **10** exhibits MIC value of 6.2 μM (4.2 μg/mL) and IC_{50} of 1.5 μM (1.0 μg/mL), also close to EMB, but at a level we found justified to select it for evaluation against the MYC5165 strain.

Nevertheless, **10** showed a moderate activity of 3.1 μM (2.2 μg/mL) against this strain, close to ciprofloxacin (2.5 μM), and 4 times as active as INH. The *meta*- (**15**) and *para*- (**20**) di-substituted species were judged identical in terms of MIC and IC_{50} against $H_{37}Rv$ strain with MIC values of 3.1 μM (ca. 2 μg/mL) close to ciprofloxacin, and IC_{50} around 0.8 μM (ca. 0.6 μg/mL); both compounds exhibit a submicromolar activity (0.8 μM) against the



Scheme 1. Compounds of the study.

MYC5165 strain, similar to **CX1** calixarene species,[18] and 16 times as active as INH.

The symmetric tri-substituted derivative **25** appears very active against both strains, with $H_{37}Rv$ MIC value of 0.8 μM (0.8 μg/mL), close to INH and streptomycin and a four-fold lower MIC against MYC5165 (0.2 μM; 0.2 μg/mL).

The tetra-substituted derivative **30** exhibits a modest activity against $H_{37}Rv$ strain at 3.2 μM (4.3 μg/mL), but appears ca. 16 times more active against the INH-resistant MYC5165 strain, with a sub-micromolar MIC (0.1 μM; 0.13 μg/mL), making it the leader of this family. Overall, **30** appears eight times more active than its tetra-guanidino-calixarene analog **CX1**.[18] The compounds bis-*meta* **15**, bis-*para* **20**, tris **25** and tetra **30** are active at sub-micromolar concentrations against MYC5165 strain, that makes them, respectively, 16, 60 and more than 125 times more active than INH against this strain.

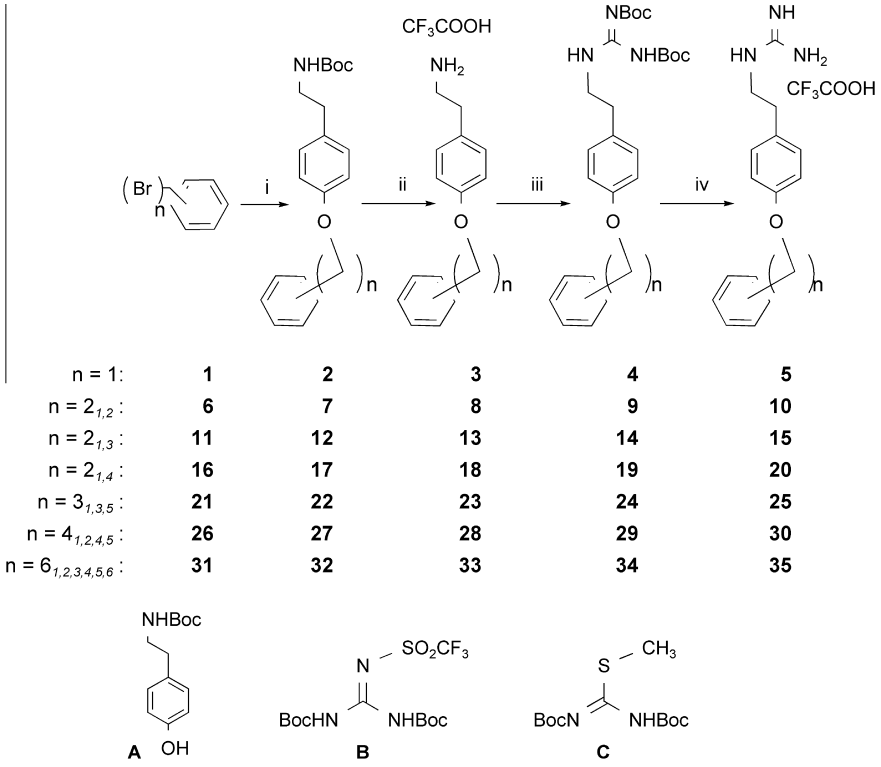
These results were compared to cellular cytotoxicity values previously obtained for these compounds on non-cancerous human pulmonary embryonic fibroblasts MRC5 cells,[19] in order to determine the corresponding Selectivity Indices (SI). The results given in Table 2 show that, with regards to the $H_{37}Rv$ strain, the SI are relatively low, comprised between 1 and 50, the higher value being obtained with the tri-substituted derivative **25**, which displays a sub-micromolar activity but a modest cytotoxicity (SI = 49).

With regards to the INH-resistant strain MYC5165, the Table 2 reveals that the SI become highly advantageous for two species, **25** (SI: 198) and, above all, the tetra-substituted derivative **30** which acquires the leader character of this series, with a SI around 380.

As mentioned at the beginning of this study, the tetra-substituted analog **30** has been designed to feature a flattened analogy of the 3D-tetra-*para*-guanidinocalix[4]arene **CX1**. The representation of **30** in flat conformation and **CX1** in conical conformation (Fig. 2) shows that **30** is approximately twice as wide as **CX1**, corresponding to a surface occupancy four to five times superior to that of the latter. Such differences should result, if we consider that **30** keeps this flat conformation, in strong differences of activity. The biological results given in Table 1 show that **30** is three-times less active than **CX1** against $H_{37}Rv$, but, very interestingly, is ca. eight times more active against MYC5165. This difference of activity, to the benefit of compound **30**, is reinforced by the selectivity indices. Compound **30** appears in fact more cytotoxic than **CX1**, making it less interesting for $H_{37}Rv$ strain (SI_{30} = 11.5 vs SI_{CX1} >256), but much more pertinent when evaluated against the INH resistant one, with a SI_{30} around 380 (vs SI_{CX1} >256). These results show that it is possible to maintain a high anti-mycobacterial activity by keeping close together four guanidinium ions, whatever nature of the organizing platform, calixarenic or benzenic.

3. Conclusion

In this investigation, seven guanidinium-containing compounds based on a benzene organizing platform and displaying between one and six arms, have been prepared and characterized, then subjected to antibacterial evaluation against INH-sensitive and resistant *M. tuberculosis* strains. Except the mono- and the hexa-substituted derivatives, all compounds displayed Minimum Inhibitory Concentrations close to 1 μM, notably the tri-substituted derivative which exhibits a sub-micromolar MIC against the INH-sensitive strain $H_{37}Rv$. More particularly, four of these compounds, the bis-*meta*-, the bis-*para*, the tri- and the tetra-substituted species **15**, **20**, **25** and **30**, respectively, showed a sub-micromolar activity accompanied for **15**, **25** and **30** by a high selectivity index of ca. 62, 200 and 380, respectively when tested on the INH resistant MYC5165 strain. In the present case, the fact that a positive discrimination between the standard $H_{37}Rv$ and the INH-resistant MYC5165 *M. tuberculosis* strains is observed for the compounds **15**, **25** and **30** calls for deeper investigations within this new family of polycationic substances. These results make this



Scheme 2. Reagents and conditions: (i) **A**, K_2CO_3, MeCN, reflux, TLC monitoring; (ii) CH_2Cl_2, TFA, 85:15, rt, TLC monitoring; (iii) **B**, NEt_3, CH_2Cl_2, MeOH or **C**, $HgCl_2$, DMF, TLC monitoring; (iv) CH_2Cl_2, TFA, 85:15, rt, TLC monitoring.

Table 1
Anti-mycobacterial activities of guanidinium derivatives **5**, **10**, **15**, **20**, **25**, **30** and **35** against reference $H_{37}Rv$ and INH-resistant MYC5165 *M. tuberculosis* strains

Compounds (MW)	$H_{37}Rv$		MYC5165	
	MIC (μM) (μg/mL)	IC_{50} (μM) (μg/mL)	MIC (μM) (μg/mL)	IC_{50} (μM) (μg/mL)
5 (392.37)	25 (9.8)	5.9 (2.3)	ND	ND
10 (706.60)	6.2 (4.2)	1.5 (1.0)	3.1 (2.2)	0.9 (0.63)
15 (697.6)	3.1 (2.2)	0.7 (0.5)	0.8 (0.56)	0.3 (0.21)
20 (688.62)	3.1 (2.13)	0.8 (0.55)	0.8 (0.55)	0.2 (0.14)
25 (1002.88)	0.8 (0.8)	0.35 (0.35)	0.2 (0.2)	ND (ND)
30 (1326.15)	3.2 (4.3)	0.5 (0.7)	0.1 (0.13)	0.03 (0.04)
35 (1954.60)	12.5 (24.4)	3.8 (7.4)	ND (ND)	ND (ND)
Reference compounds				
CX1 (1239.00)[a]	0.8 (1)	0.25 (0.31)	0.8 (1)	0.1 (0.12)
INH (137.14)	0.6 (0.08)	0.22 (0.03)	12.5 (1.7)	0.65 (0.9)
EMB (277.15)	9.8 (2)	ND		
Ciprofloxacin (331.40)	2.5 (0.8)	0.6 (0.2)	2.5 (0.8)	1.8 (0.6)
Streptomycin (581.57)	0.7 (0.4)		2.7 (1.6)	

ND: not determined.
[a] See Ref. 18.

family highly attractive for deepening investigations related to the mode of action notably with regards to the bis-guanidinium streptomycin, their synergistic behavior and in vivo assays.

Table 2
Selectivity indices calculated (CC_{50} 24 h[19]/MIC) for the reference $H_{37}Rv$ and INH-resistant MYC5165 *M. tuberculosis* strains, after 24 h exposure of MRC-5 cells to the compounds of the study

	$H_{37}Rv$	SI	MYC5165	SI
5	26.0:9.8	2.6	ND	
10	100.0:4.2	24	100:2.2	45
15	34.5:2.2	16	34.5:0.56	62
20	21:2.13	10	21:0.55	38
25	39.5:0.8	49	39.5:0.2	198
30	49:4.3	11.5	49:0.13	377
35	26.0:24.4	1	ND	
CX1	>256:1	>256	>256:1	>256
INH[a]	>10:0.08	>125	>10:1.7	>6
EMB[a]	>10:2	>5		

Concentrations in μg/mL; ND: not determined.
[a] From Ref. 23.

4. Experimental section

4.1. Chemistry

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 (electrospray—ES) were recorded on a Bruker Daltonics micrOTOF-Q apparatus, at the Service Commun de Spectrométrie de Masse Organique, Nancy. Infrared measurements were performed on a Vector 22 Bruker FT apparatus (KBr, γ in cm^{-1}) and UV spectra were recorded on a SAFAS UV mc2 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.

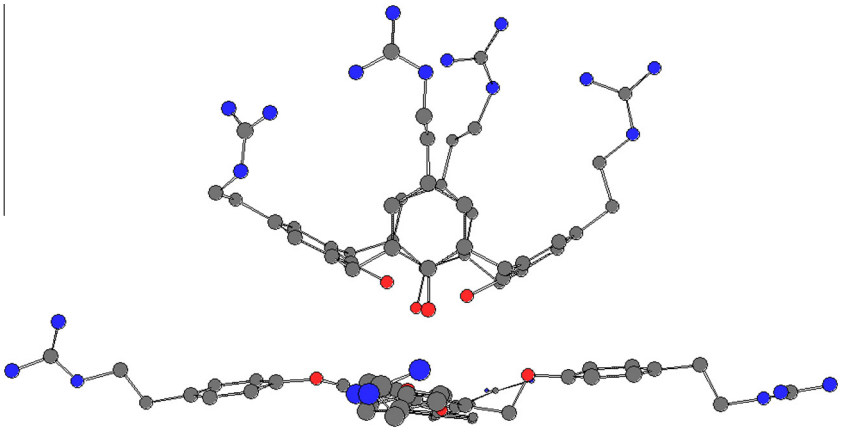


Figure 2. Chem3D side-representation of **30** and **CX1** (not minimized).

4.1.1. Compounds 2, 7, 12, 17, 22, 27, 32—general procedure

A mixture of *N*-Boc-tyramine **A** and K_2CO_3 in dry MeCN was refluxed under Ar during 30 min. The bromomethylbenzene derivative was added and reflux was continued during 24 h (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 99.8:0.2 to 98:2). After cooling, the solvent was evaporated to dryness, and the solid residue was dissolved in CH_2Cl_2. The insoluble materials were filtered off, and the concentrated filtrate was chromatographed (SiO_2; CH_2Cl_2/MeOH X:Y%) to give the desired *n*-[*para*-(Boc-aminoethyl)-phenoxymethyl] benzene.

4.1.1.1. α-[*para*-(Boc-aminoethyl)-phenoxy]toluene (2). From **A** (0.10 g, 0.42 10^{-3} mol), K_2CO_3 (0.06 g, 0.42 10^{-3} mol), dry MeCN (15 mL), benzyl bromide **1** (50 μL, 0.42 10^{-3} mol) (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 2%). Chromatography: SiO_2; CH_2Cl_2/MeOH 100:0 to 98:2. **2** (0.13 g, 90%). White solid. Mp: 81–83 °C. IR (KBr): 3360.66 (NH); 1681.61 (CO). UV–vis ($CHCl_3$): 277 (2960.3). ^{1}H NMR (400 MHz, $CDCl_3$): 1.46 (s, 9H, Me_3C); 2.74 (t, *J* = 7.04 Hz, 2H, ArCH_2CH$_2$NH); 3.34 (br s, 2H, ArCH$_2$$CH_2$NH); 4.62 (br s, 1 H, N*H*); 5.05 (s, 2H, ArOCH_2); 6.93 (d, *J* = 8.56 Hz, 2H, Ar*H*); 7.12 (d, *J* = 8.56 Hz, 2H, Ar*H*); 7.33–7.44 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.53; 35.60; 42.21; 70.70; 79.52; 114.20; 127.21; 128,32; 128.80; 129.00; 131.80; 141.20; 151.02; 157.90. Anal. calcd for $C_{20}H_{25}NO_3$ (327.42): C, 73.37; H, 7.70; N, 4.28; found: C, 73.45; H, 7.64; N, 4.34. ES-MS (pos. mode): 350.20 $[M+Na]^+$, 228.05 $[M-COOCMe_3+2H]^+$, 211.02 $[M-COOCMe_3-NH]^+$.

4.1.1.2. Bis-[α,α′-[*para*-(Boc-aminoethyl)-phenoxy]]-*o*-xylene (7). From **A** (0.36 g, 1.52 10^{-3} mol), K_2CO_3 (0.21 g, 1.52 10^{-3} mol), dry MeCN (20 mL) and *o*-dibromomethylbenzene **6** (0.20 g, 0.76 10^{-3} mol). (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 98:2). Chromatography (SiO_2; CH_2Cl_2/MeOH 100:0 to 98:2). **7** (0.27 g, 62%). White powder. Mp: 110–112 °C. IR (KBr): 3375.0 (–CONH–); 1685.1 (CO). UV–vis ($CHCl_3$): 276 (3067.0). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 18H, Me_3C); 2.73 (t, *J* = 6.8 Hz, 4H, CH_2CH_2NH); 3.33 (br t, *J* = 8.0 Hz, 4H, CH_2CH_2NH); 4.53 (br s, 2H, NH); 5.15 (s, 4H, ArOCH_2); 6.90 (d, *J* = 8.56 Hz, 4H, ArH); 7.09 (d, *J* = 8.28 Hz, 4H, ArH); 7.36 (m, 2H, ArH); 7.50 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 28.42, 35.39, 42.01, 68.05, 79.38, 114.95, 128.43, 128.94, 129.75, 131.50, 135.20, 155.97, 157.30. Anal. calcd for $C_{34}H_{44}O_6N_2$, 0.5H_2O (585.75): C, 69.72; H, 7.74; N, 4.78; found: C, 69.54; H, 7.34; N, 4.74. ES-MS (pos. mode): 599.18 $[M+Na]^+$.

4.1.1.3. Bis-[α,α′-[*para*-(Boc-aminoethyl)-phenoxy]-*m*-xylene (12). From **A** (0.36 g, 1.52 10^{-3} mol), K_2CO_3 (0.21 g, 1.52 10^{-3} mol), dry MeCN (25 mL) and *m*-dibromomethylbenzene **11** (0.20 g, 0.76 10^{-3} mol) (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 98:1). Chromatography (SiO_2; CH_2Cl_2/MeOH 100:0 to 98:2). **12** (0.41 g, 0.71 10^{-3} mol, 93.0%). White powder. Mp: 132–134 °C. IR (KBr): 1683.35 (CO). UV–vis (CH_2Cl_2): 277.0 (3324). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 18H, Me_3C); 2.73 (t, *J* = 6.92 Hz, 4H, CH_2CH_2NH); 3.34 (br s, 4H, CH_2CH_2NH); 4.52 (br s, 2H, N*H*); 5.06 (s, 4H, ArOCH_2); 6.91 (d, *J* = 8.84 Hz, 4H, Ar*H*); 7.10 (d, *J* = 8.56 Hz, 4H, Ar*H*); 7.39 (br s, 3H, Ar*H*); 7.50 (s, 1 H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.4, 35.4, 42.3, 69.9, 79.4, 115.0, 126.5, 127.0, 128.9, 129.8, 131.4, 137.5, 155.9, 157.4. Anal. calcd for $C_{34}H_{44}O_6N_2$ (576.32): C, 70.81; H, 7.69; N, 4.86; found: C, 70.52; H, 7.79; N, 4.88. ES-MS (pos. mode): 577.23 $[M+H]^+$, 599.25 $[M+Na]^+$, 615.24 $[M+K]^+$.

4.1.1.4. Bis-[α,α′-[*para*-(Boc-aminoethyl)-phenoxy]]-*p*-xylene (17). From **A** (1.13 g, 4.76 10^{-3} mol), K_2CO_3 (0.63 g, 4.54 10^{-3} mol), dry MeCN (40 mL), and *p*-dibromomethylbenzene **16** (0.6 g, 2.27 10^{-3} mol) (TLC monitoring SiO_2, CH_2Cl_2/MeOH 98:2). Chromatography (SiO_2, CH_2Cl_2/MeOH 100:0 to 98:2). **17** (1.28 g, 97%). White solid. Mp: 173–174 °C. IR (KBr): 3360.55 (NH); 1681.84 (CO). UV–vis (CH_2Cl_2): 276 (4600) ^{1}H NMR (400 MHz, DMSO-d_6): 1.36 (s, 18H, Me_3C), 2.61 (t, *J* = 7.1 Hz, 4H, CH_2CH_2NH), 3.08 (m, 4H, CH_2CH_2NH), 5.07 (s, 4H, CH_2Ph), 6.84 (m, 2H, N*H*), 6.92 (d, *J* = 8.2 Hz, 4H, Ar*H*), 7.09 (d, *J* = 8.2 H, 4H, Ar*H*), 7.44 (s, 4H, Ar*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 28.83, 35.71, 42.36, 70.16, 79.59, 115.34, 128.09, 130.18, 131.77, 137.26, 156.30, 157.78. Anal. calcd for $C_{34}H_{34}O_6N_2$ (576.72): C, 70.81; H, 7.69; N, 4.86; found: C, 70.51; H, 7.66; N, 4.92. ES-MS (pos. mode): 599.2 $[M+Na^+]^+$.

4.1.1.5. Tris-[α,α′,α″-[*para*-(Boc-aminoethyl)-phenoxy]]-mesitylene (22). From **A** (0.39 g, 1.64 10^{-3} mol), K_2CO_3 (0.23 g, 1.67 10^{-3} mol), dry MeCN (30 mL) and mesitylene bromide **21** (0.20 g, 0.56 10^{-3} mol) (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 99.8:0.2). Chromatography (SiO_2; CH_2Cl_2/MeOH 99.8:0.2). **22** (0.32 g, 0.39 10^{-3} mol, 70%). Mp: 64–66 °C. IR (KBr): 1697.23. UV–vis ($CHCl_3$): 277 (6428). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 27H, Me_3C); 2.73 (t, *J* = 6.80 Hz, 6H, CH_2CH_2NH); 3.34 (br s, 6H, CH_2CH_2NH); 4.55 (br s, 3H, N*H*); 5.06 (s, 6H, ArCH_2O); 6.91 (d, *J* = 8.80 Hz, 6H, Ar*H*); 7.10 (d, *J* = 7.56 Hz, 6H, Ar*H*); 7.46 (s, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 28.43, 35.38, 42.00, 69.78, 79.26, 114.98, 125.94, 129.80, 131.47, 137.99, 155.90, 157.34. Anal. calcd for $C_{48}H_{63}O_9N_3$ (826.03): C, 69.79; H, 7.69; N, 5.09; found: C, 70.28; H, 7.74; N, 5.15. ES-MS (pos. mode): 848.47 $[M+Na]^+$, 627.31 $[M-2COOCMe_3+2H]^+$.

4.1.1.6. Tetra-[α,α′,α″,α‴-[*para*-(Boc-aminoethyl)-phenoxy]]-durene (27). From **A** (0.21 g, 0.89 10^{-3} mol), K_2CO_3 (0.13 g, 0.94 10^{-3} mol), dry MeCN (20 mL) and 1,2,4,5-tetrabromomethyl benzene **26** (0.10 g, 0.22 10^{-3} mol) (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 99.8:0.2). Chromatography (SiO_2; CH_2Cl_2/MeOH 97.8:3). **27** (0.23 g, 98%). Mp: 92–94 °C. IR (KBr): 1702.8 (CO). UV–vis (CH_2Cl_2): 276.0 (10400). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 36H, Me_3C); 2.73 (t, J = 6.92 Hz, 8H, CH_2CH$_2$NH); 3.33 (br t, 8H, CH$_2$CH_2NH); 4.60 (br s, 4H, N*H*); 5.15 (s, 8H, ArOCH_2); 6.89 (d, J = 8.56 Hz, 8H, Ar*H*); 7.09 (d, J = 8.56 Hz, 8H, Ar*H*); 7.67 (s, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 28.8, 35.8, 42.4, 68.1, 79.6, 115.3, 130.0, 130.2, 132.0, 135.7, 156.30; 157.6. Anal. calcd for $C_{62}H_{82}O_{12}N_4$ (1075.33): C, 69.25; H, 7.69; N, 5.21; found: C, 69.00; H, 7.61; N, 5.18. ES-MS (pos. mode): 1097.58 [M+Na]$^+$, 1075.60 [M+H]$^+$.

4.1.1.7. Hexakis-[*para*-(Boc-aminoethyl)-phenoxymethyl]-benzene (32). From **A** (0.22 g, 0.93 10^{-3} mol), K_2CO_3 (0.13 g, 0.94 10^{-3} mol), dry MeCN (20 mL) and hexabromomethylbenzene **31** (0.10 g, 0.16 10^{-3} mol) (TLC monitoring; SiO_2, CH_2Cl_2/MeOH 99:1). After cooling, the solvent was evaporated to dryness, and the solid residue was dissolved in CH_2Cl_2. The solution was washed with H_2O (2 × 20 mL), the aqueous phase was rinsed with CH_2Cl_2 (3 × 15 mL) and the combined organic phases were dried over Na_2SO_4, filtered then concentrated prior chromatography (SiO_2; CH_2Cl_2/MeOH 99.5:0.5). **32** (0.22 g, 97%). Mp: 154–156 °C. IR (KBr): 1699.77 (CO). UV–vis ($CHCl_3$): 276 (11950). ^{1}H NMR (400 MHz, $CDCl_3$): 1.43 (s, 54H, Me_3C); 2.73 (t, J = 6.80 Hz, 12H, CH_2CH_2NH); 3.33 (br s, 12H, CH_2CH_2NH); 4.60 (br s, 6H, NH); 5.15 (s, 12H, ArCH$_2$O); 6.89 (d, J = 8.30 Hz, 12H, ArH); 7.09 (d, J = 8.30 Hz, 12H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): 28.5, 35.5, 42.2, 63.7, 79.4, 114.9, 129.9, 131.9, 138.0, 156.0, 157.2. Anal. calcd for $C_{90}H_{120}O_{18}N_6$ (1573.95): C, 68.68; H, 7.68; N, 5.34; found: C, 68.37; H, 7.59; N, 5.33. ES-MS (pos. mode): 1596.85 [M+Na]$^+$, 809.42 [M+2Na]$^{2+/2}$.

4.1.2. Compounds 3, 8, 13, 18, 23, 28, 33—general procedure

A solution of (Boc-amino) derivative in a mixture of CH_2Cl_2 and TFA was stirred at rt under Ar during X h. The solvent was evaporated under vacuum and the residual acid was eliminated by multiple dissolution/co-evaporation cycles in CH_2Cl_2 until formation of a solid material. The latter was treated by multiple trituration–filtration sequences with dry Et_2O, until pH of the filtrate remained neutral, to give the desired trifluoroacetate salt.

4.1.2.1. α-[*para*-(Aminoethyl)-phenoxy]toluene, trifluoroacetate (3). From **2** (0.10 g, 0.30 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (15 mL), 5 h. **3** (0.10 g, 96%). White powder. Mp: 148–150 °C. IR (KBr): 3037.75 (NH_3^+); 1676.29, 1634.02 (CO). UV–vis (D_2O): 273 (1641.9). ^{1}H NMR (400 MHz, D_2O): 2.89 (t, J = 7.04 Hz, 2H, ArCH_2CH$_2$NH); 3.19 (t, J = 7.18 Hz, 2H, ArCH$_2$CH_2NH); 5.13 (s, 2H, ArOCH_2); 7.02 (d, J = 8.56 Hz, 2H, Ar*H*); 7.22 (d, J = 8.28 Hz, 2H, Ar*H*); 7.37–7.47 (m, 5H, Ar*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 32.57, 48.13, 69.51, 115.30, 127.97, 128.16, 128.80, 130.10, 137.51, 147.51, 157.16. Anal. calcd for $C_{15}H_{17}NO$, CF_3COOH, 0.25H_2O (345.82): C, 59.04; H, 5.39; N, 4.05; found: C, 59.37, H, 5.29, N, 4.09. ES-MS (pos. mode): 228.05 [M−CF_3COOH+H]$^+$, 210.72 [M−CF_3COOH−NH_2]$^+$.

4.1.2.2. Bis-[α,α′-[*para*-(aminoethyl)-phenoxy]-*o*-xylene, bis-trifluoroacetate (8). From **7** (0.26 g, 0.44 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (20 mL), 5 h. **8** (0.20 g, 71%). White solid. Mp: 127–128 °C. IR (KBr): 1678.40 (CO). UV–vis (H_2O): 273.0 (2970.1). ^{1}H NMR (400 MHz, D_2O): 2.81 (t, J = 7.18 Hz, 4H, CH_2CH$_2$NH); 3.11 (t, J = 7.18 Hz, 4H, CH$_2$CH_2NH); 5.15 (s, 4H, ArOCH_2); 6.89 (d, J = 8.32 Hz, 4H, Ar*H*); 7.12 (d, J = 8.32 Hz, 4H, Ar*H*); 7.35 (m, 2H, Ar*H*); 7,45 (m, 2H, Ar*H*). ^{13}C NMR (100 MHz, D_2O): 31.84, 40.61, 68.21, 115.44, 129.04, 129.52, 129.98, 130.05, 134.97, 156.84. Anal. calcd for $C_{24}H_{28}O_2N_2$, 2CF_3COOH, 2H_2O (640.22): C, 52.50; H, 5.35; N, 4.37; found: C, 52.49; H, 5.58; N, 4.13. ES-MS (pos. mode): 377.17 [M−2CF_3COOH+H]$^+$.

4.1.2.3. Bis-[α,α′-[*para*-(aminoethyl)-phenoxy]-*m*-xylene, bis trifluoroacetate (13). From **12** (0.39 g, 0.68 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (30 mL), 5 h. **13** (0.38 g, 92%). White solid. Mp: deliquescent. IR (neat):1670.35 (CO). UV–vis (H_2O): 273.0 (2650). ^{1}H NMR (400 MHz, D_2O): 2.81 (t, J = 7.30 Hz, 4H, CH_2CH$_2$NH); 3.11 (t, J = 7.30 Hz, 4H, CH$_2$CH_2NH); 5.03 (s, 4H, ArOCH_2); 6.91 (d, J = 8.56 Hz, 4H, Ar*H*); 7.13 (d, J = 8.56 Hz, 4H, Ar*H*); 7.34 (s, 3H, Ar*H*); 7.41 (s, 1H, Ar*H*). ^{13}C NMR (100 MHz, D_2O): 31.89, 40.56, 69.47, 115.22, 126.81, 127.33, 128.80, 129.25, 129.88, 136.90, 156.91, 116.39 (q, J = 292 Hz, CF_3CO), 162.73 (q, J = 35 Hz, CF_3CO). Anal. calcd for $C_{24}H_{28}O_2N_2$, 2CF_3COOH (604.20): C, 55.63; H, 5.00; N, 4.63; found: C, 55.32; H, 5.01; N, 4.70. ES-MS (pos. mode): 377.20 [M−2CF_3COOH+H]$^+$.

4.1.2.4. Bis-[α,α′-[*para*-(aminoethyl)-phenoxy]-*p*-xylene, bis trifluoroacetate (18). From **17** (1.28 g, 2.22 10^{-3} mol), in a 85:15 mixture of CH_2Cl_2 and TFA (70 mL), 12 h. **18** (1.13 g, 85%). White solid. Mp: 192–193 °C. IR (KBr): 1676.86 (CO). UV–vis (H_2O): 272 (3203.0). ^{1}H NMR (400 MHz, DMSO-d_6): 2.78 (t, J = 8.1 Hz, 4H, CH_2CH$_2$NH), 3.00 (m, 4H, CH$_2$CH_2NH), 5.09 (s, 4H, CH_2Ph), 6.97 (d, J = 8.6 Hz, 4H, Ar*H*), 7.17 (d, J = 8.6 H, 4H, Ar*H*), 7.45 (s, 4H, Ar*H*), 7.82 (br s, 2H, N*H*). ^{1}H NMR (400 MHz, D_2O): 2.97 (t, J = 7.1 Hz, 4H, CH_2CH$_2$NH), 3.28 (t, J = 8.3 Hz, 4H, CH$_2$CH_2NH), 5.20 (s, 4H, CH_2Ph), 7.09 (d, J = 8.0 Hz, 4H, Ar*H*), 7.30 (d, J = 8.6 H, 4H, Ar*H*), 7.54 (s, 4H, Ar*H*). ^{13}C NMR (100 MHz, D_2O): 32.27, 41.06 (*C*H$_2$CH$_2$NH), 70.47 (OCH$_2$), 116.12, 128.79, 130.04, 130.56, 137.06, 157.33 (*C* Ar). Anal. calcd for $C_{28}H_{30}F_6N_2O_6$, 0.25H_2O (609.04): C, 55.22; H, 5.05; N, 4.60; found: C, 55.23; H, 5.07; N, 4.71. ES-MS (pos. mode): 377.20 [M−2(CF_3COOH)+H]$^+$; 360.24 [M−2(CF_3COOH)−NH_2+H]$^+$.

4.1.2.5. Tris-[α,α′,α″-[*para*-(aminoethyl)-phenoxy]]-mesitylene, tris trifluoroacetate (23). From **22** (0.15 g, 0.18 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (15 mL), 5 h. After trituration–filtration sequences with dry Et_2O, the white solid was dissolved in H_2O and the resulting solution was lyophilized to give **23** (0.15 g, 98%). Mp: deliquescent. IR (KBr): 1677.34 (CO). UV–vis (H_2O): 273.0 (4076). ^{1}H NMR (400 MHz, D_2O): 2.84 (t, J = 7.44 Hz, 6H, CH_2CH$_2$NH); 3.13 (t, J = 7. 42 Hz, 6H, CH$_2$CH_2NH); 4.81 (s, 6H, ArOCH_2); 6.84 (t, J = 8.56 Hz, 6H, Ar*H*); 7.13 (t, J = 7.56 Hz, 6H, Ar*H*); 7.25 (s, 3H, Ar*H*). ^{13}C NMR (100 MHz, D_2O): 31.90, 40.57, 69.27, 115.33, 126.45, 129.40, 129.95, 137.49, 156.82, 116.36 (q, J = 292 Hz, CF_3CO), 162.80 (CF_3CO). Anal. calcd for $C_{33}H_{39}N_3O_3$, 3CF_3COOH, H_2O (885.29): C, 52.88; H, 5.01; N, 4.74; found: C, 52.96; H, 4.83; N, 4.80. ES-MS (pos. Mode): 526.32 [M−3CF_3COOH+H]$^+$.

4.1.2.6. Tetra-[α,α′,α″,α‴-[*para*-(aminoethyl)-phenoxy]]-durene, tetra trifluoroacetate (28). From **27** (0.22 g, 0.20 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (20 mL), 24 h. **28** (0.19 g, 80%). White solid. Mp: deliquescent. IR (KBr): 1681.42 (CO). UV–vis (H_2O): 273.0 (6050). ^{1}H NMR (400 MHz, DMSO-d_6): 2.79 (br t, 8H, CH_2CH$_2$NH); 2.99 (br t, 8H, CH$_2$CH_2NH); 5.20 (s, 8H, ArOCH_2); 6.98 (d, J = 8.81 Hz, 8H, Ar*H*); 7.17 (d, J = 8.80 Hz, 8H, Ar*H*); 7.68 (s, 2H, ArH), 7.98 (br s, 12H, NH_3^+). ^{13}C NMR (100 MHz, DMSO-d_6): 32.3, 40.2, 66.8, 115.0, 128.9, 129.7, 129.8, 135.1, 157.1, 157.1, 158.4 (q, J = 30.8 Hz).

Anal. calcd for $C_{42}H_{50}O_4N_4$,4 CF_3COOH, H_2O (1151.0): C, 52.18; H, 5.08; N, 4.87; found: C, 51.94; H, 4.84; N, 4.87. ES-MS (pos. mode): 789.3 $[M-3CF_3COOH+H]^+$; 675.39 $[M-4CF_3COOH+H]^+$.

4.1.2.7. Hexakis-[*para*-(aminoethyl)-phenoxymethyl]benzene (33).

From **32** (0,20 g, 0.13 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (20 mL), 3 h. **33** (0.19 g, 88%). White solid. Mp: deliquescent. IR (KBr): 1677.1 ($-NH_3^+$). UV–vis (H_2O): 272 (8985). ^1H NMR (400 MHz, D_2O): 2.87 (t, J = 7.30 Hz, 12H, ArCH_2CH$_2$NH); 3.17 (t, J = 7.42 Hz, 12H, ArCH$_2$CH_2NH); 5.35 (s, 12H, ArCH_2O); 6.91 (d, J = 8.56 Hz, 12H, ArH); 7.14 (d, J = 8.80 Hz, 12H, ArH). ^{13}C NMR (100 MHz, D_2O): 31.9, 40.6, 64.3, 115.2, 129.6, 129.7, 137.8, 156.9. Anal. calcd for $C_{60}H_{72}N_6O_6$, 6 CF_3COOH, 2H_2O (1692.5): C, 51.07; H, 4.67; N, 4.96; found: C, 51.12; H, 4.67; N, 4.96. ES-MS (pos. mode): 487.27 $[M-6CF_3COOH+2H]^{2+/2}$; 325.19 $[M-6CF_3COOH+3H]^{3+/3}$; 308.17 $[M-6CF_3COOH-3NH_2+3H]^{3+/3}$.

4.1.3. Compounds 4, 14, 19, 24, 29, 34—general procedure

The ammonium salts were suspended in dry CH_2Cl_2, and solubilized with the minimum of MeOH; Et_3N was added, then *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*″-triflylguanidine **B**. The mixture was stirred at rt under Ar during X h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH, X:Y%). After evaporation of the solvent, the residue was dissolved in CH_2Cl_2, and the solution was washed with H_2O; the aqueous phase was washed with CH_2Cl_2, and the combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness. The resulting solid was chromatographed (SiO_2, CH_2Cl_2/MeOH, gradient) to give the desired –(di-Boc-guanidinoet hyl)– derivatives.

4.1.3.1. α-[*para*-(di-Boc-guanidinoethyl)-phenoxy]toluene (4).

From **3** (0.10 g, 0.29 10^{-3} mol), dry CH_2Cl_2 (10 mL), Et_3N (112 μL, 0.87 10^{-3} mol), **B** (0.114 g, 0.29 10^{-3} mol), 9 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH, 99.9:0.1). Residue of evaporation dissolved in CH_2Cl_2 (30 mL), washed with H_2O (30 mL); washing of aqueous phase with CH_2Cl_2 (2 × 20 mL). Chromatography (SiO_2, CH_2Cl_2/MeOH, 100:0 to 99.5:0.5). **4** (0.12 g, 85%). White solid. Mp: 99–100 °C. IR (KBr): 1730.4 (CO). UV–vis ($CDCl_3$): 276 (1577.5). ^1H NMR (400 MHz, $CDCl_3$): 1.48 (s, 9H, *Me*$_3$C); 1.51 (s, 9H, *Me*$_3$C); 2.81 (t, J = 7.18 Hz, 2H, ArCH_2CH$_2$NH); 3.64 (m, 2H, CH$_2$CH_2NH); 5.04 (s, 2H, ArOCH_2); 6.91 (d, J = 8.56 Hz, 2H, ArH); 7.13 (d, J = 8.56 Hz, 2H, ArH); 7.37–7.43 (m, 5H, ArH); 8.37 (br t, 1 H, NH); 11.49 (br s, 1 H, NHguan). ^{13}C NMR (100 MHz, $CDCl_3$): 28.46, 28.74, 34.80, 42.85, 70.42, 79.61, 83.38, 115.36, 127.87, 128.32, 128.97, 130.19, 131.29, 137.52, 153.56, 156.55, 157.93, 164.03. Anal. calcd for $C_{26}H_{35}O_5N_3$, 0.1 C(NH)$(NH_2)_2$, 0.3 CH_2Cl_2 (500.96): C, 63.20; H, 7.23; N, 9.26; found: C, 63.29; H, 7.26; N, 9.23. ES-MS (pos. mode): 319.11 $[M-COOCMe_3-Me_3CO+Na+H]^+$.

4.1.3.2. Bis-[α,α′-[*para*-(di-Boc-guanidinoethyl)-phenoxy]-*m*-xylene (14).

From **13** (0.17 g, 0.28 10^{-3} mol), dry CH_2Cl_2 (15 mL), MeOH (0.3 mL), Et_3N (250 μL, 1.66 10^{-3} mol), **B** (0.22 g, 0.56 10^{-3} mol), 24 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH, 99.9:0.1). Residue of evaporation dissolved in CH_2Cl_2 (50 mL), washed with H_2O (40 mL); washing of aqueous phase with CH_2Cl_2 (2 × 20 mL). Chromatography (SiO_2, CH_2Cl_2). **14** (0.23 g, 98.8%). White solid. Mp: 98–100 °C. IR (KBr): 1721.70, 1638.94 (CO). UV–vis (CH_2Cl_2) 276 (2746.31). ^1H NMR (400 MHz, $CDCl_3$): 1.48 (s, 18H, *Me*$_3$C); 1.50 (s, 18H, *Me*$_3$C); 2.81 (t, J = 7.30 Hz, 4H, ArCH_2CH$_2$NH$_2$); 3.62 (m, 4H, ArCH$_2$CH_2NH$_2$); 5.05 (s, 4H, ArOCH_2); 6.91 (d, J = 8.52 Hz, 4H, ArH); 7.13 (d, J = 8.56 Hz, 4H, ArH); 7.39 (br s, 3H, ArH); 7.50 (s, 1 H, *Ar*H); 8.36 (br s, 2H, NH); 11.47 (s, 2H, NH_{guan}). ^{13}C NMR (100 MHz, $CDCl_3$): 28.44, 28.70, 34.77, 42.87, 70.29, 79.64, 83.41, 115.33, 126.87, 127.43, 129.24, 130.20, 131.32, 137.89, 153.54, 156.57, 157.87, 163.92. Anal. calcd for $C_{46}H_{64}O_{10}N_6$, 0.75 CH_2Cl_2 (924.17): C, 60.75; H, 7.14; N, 9.10; found: C, 60.49; H, 6.95; N, 9.61. ES-MS (pos. mode): 883.43 $[M+Na]^+$, 861.53 $[M+H]^+$, 761.42 $[M-COOCMe_3+2H]^+$, 431.00 $[M+2H]^{2+/2}$.

4.1.3.3. Bis-[α,α′-[*para*-(di-Boc-guanidinoethyl)-phenoxy]-*p*-xylene (19).

From **18** (1.00 g, 1.65 10^{-3} mol), dry CH_2Cl_2 (50 mL), MeOH (5 mL), Et_3N (1.38 mL, 9.92 10^{-3} mol), **B** (1.36 g, 3.47 10^{-3} mol), 7 h (TLC monitoring SiO_2, CH_2Cl_2/MeOH 99.9:0.1). Residue of evaporation dissolved in CH_2Cl_2 (50 mL), washed with H_2O (30 mL). Chromatography (SiO_2, CH_2Cl_2). **19** (1.23 g, 87%). White solid. Mp: 104–105 °C. IR (pure): 1714.72, 1610.56 (CO). UV–vis (CH_2Cl_2): 275 (2244). ^1H NMR (400 MHz, $CDCl_3$): 1.48 (s, 18H, *Me*$_3$C), 1.50 (s, 18H, *Me*$_3$C), 2.82 (t, J = 7.3 Hz, 4H, CH_2CH$_2$NH), 3.64 (q, J = 6.8 Hz, 4H, CH$_2$CH_2NH), 5.05 (s, 4H, CH_2Ph), 6.90 (d, J = 8.6 Hz, 4H, ArH), 7.13 (d, J = 8.6 H, 4H, ArH), 7.44 (s, 4H, ArH), 8.37 (s large, 2H, NH), 11.46 (s, 2H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 28.43, 28.71, 34.77, 42.85, 70.10, 79.64, 83.40, 115.33, 128.05, 130.19, 131.28, 137.22, 153.53, 156.54, 157.85, 163.95. Anal. calcd for $C_{46}H_{64}N_6O_{10}$ (861.03): C, 64.17; H, 7.49; N, 9.76; found: C, 64.17; H, 7.54; N, 9.79. ES-MS (pos. mode): 899.44 $[M+K]^+$, 883.46 $[M+Na]^+$, 861.48 $[M+H]^+$, 761.42 $[M-COOCMe_3+2H]^+$, 431.21 $[M+2H]^{2+/2}$.

4.1.3.4. Tris-[α,α′,α″-[*para*-(di-Boc-guanidinoethyl)-phenoxy]]-mesitylene (24).

From **23** (0.14 g, 0.16 10^{-3} mol), dry CH_2Cl_2 (5 mL), MeOH (3 mL), Et_3N (0.27 mL, 1.93 10^{-3} mol), **B** (0.19 g, 0.49 10^{-3} mol), 24 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH, 99.9:0.1). Residue of evaporation dissolved in CH_2Cl_2 (25 mL), washed with H_2O (10 mL); washing of aqueous phase with CH_2Cl_2 (3 × 10 mL). Chromatography (SiO_2, CH_2Cl_2). **24** (0.18 g, 89.9%). White solid. Mp: 80–82 °C. IR (KBr): 1721.31, 1638.71 (CO). UV–vis (CH_2Cl_2): 276 (7220). ^1H NMR (400 MHz, $CDCl_3$): 1.47 (s, 27H, *Me*$_3$C); 1.50 (s, 27H, *Me*$_3$C); 2.81 (t, J = 7.30 Hz, 6H, CH_2CH$_2$NH); 3.63 (t, J = 6.53 Hz, 6H, CH$_2$CH_2NH); 5.06 (s, 6H, ArOCH_2); 6.91 (d, J = 8.56 Hz, 6H, ArH); 7.13 (d, J = 7.80 Hz, 6H, ArH); 7.47 (s, 3H, ArH); 8.36 (t, J = 4.78 Hz, 3H, CH$_2$NH); 11.46 (s, 3H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): 28.46, 28.72, 34.83, 42.85, 70.18, 79.64, 83.41, 115.32, 126.40, 130.22, 131.42, 138.34, 138.40, 153.57, 156.52, 157.83, 164.02. Anal. calcd for $C_{66}H_{93}O_{15}N_9$, 0.5CH_2Cl_2 (1335.63): C, 61.68; H, 7.32; N, 9.73; found: C, 61.67; H, 7.24; N, 9.89. ES-MS (pos. mode): 1252.67 $[M+H]^+$; 1274.66 $[M+Na]^+$; 1290.61 $[M+K]^+$.

4.1.3.5. Tetra-[α,α′,α″,α‴-[*para*-(di-Boc-guanidinoethyl)-phenoxy]]-durene (29).

From **28** (0.18 g, 0.16 10^{-3} mol), dry CH_2Cl_2 (10 mL), MeOH (10 mL), Et_3N (0.28 mL, 2.00 10^{-3} mol), **B** (0.26 g, 0.66 10^{-3} mol), 24 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH, 99.9:0.1). Residue of evaporation dissolved in CH_2Cl_2 (20 mL), washed with H_2O (2 × 20 mL); washing of aqueous phase with CH_2Cl_2 (3 × 20 mL). Chromatography (SiO_2, CH_2Cl_2/MeOH, 100:0 to 99:1). **29** (0.16 g, 61%). White powder. Mp: 134–136 °C. IR (KBr): 1638.74, 1721.54 (CO). UV–vis (CH_2Cl_2): 276 (9980). ^1H NMR (400 MHz, $CDCl_3$): 1.46 (s, 36H, *Me*$_3$C); 1.49 (s, 36H, *Me*$_3$C); 2.80 (t, J = 7.16 Hz, 8H, CH_2CH$_2$NH); 3.61 (m, 8H, CH$_2$CH_2NH); 5.13 (s, 8H, ArOCH_2); 6.88 (d, J = 8.08 Hz, 8H, ArH); 7.11 (d, J = 7.84 Hz, 8H, ArH); 7.67 (s, 2H, ArH); 8.37 (br s, 4H, NH); 11.30 (br s, 4H, NH_{guan}). ^{13}C NMR (100 MHz, $CDCl_3$): 28.4, 28.6, 34.7, 42.7, 67.9, 79.6, 83.3, 115.2, 129.9, 130.2, 131.5, 135.5, 153.5, 156.4, 157.6, 163.9. Anal. calcd for $C_{86}H_{122}O_{20}N_{12}$ (1643.96): C, 62.83; H, 7.48; N, 10.22; found: C, 62.79; H, 7.50; N, 10.02. ES-MS (pos. mode): 1643.88 $[M+H]^+$; 1681.83 $[M+K]^+$; 1666.85 $[M+Na]^+$; 822.9 $[M+2H]^{2+/2}$.

4.1.3.6. Hexakis-[*para*-(di-Boc-guanidinoethyl)-phenoxymethyl]benzene (34).

From **33** (0.18 g, 0.106 10^{-3} mol), dry CH_2Cl_2 (10 mL), MeOH (10 mL), Et_3N

(0.28 mL, 2.00 10^{-3} mol), **B** (0.26 g, 0.66 10^{-3} mol), 15 h (TLC monitoring, SiO_2, CH_2Cl_2/MeOH, 99.9:0.1). Residue of evaporation dissolved in CH_2Cl_2 (20 mL), washed with H_2O (2 × 25 mL); washing of aqueous phase with CH_2Cl_2 (3 × 10 mL). Chromatography (SiO_2, CH_2Cl_2/MeOH, 99:1). **34** (0.22 g, 84%). White powder. Mp: 152–154 °C IR (KBr): 1721.91, 1638.39, 1615.16 (CO). UV (CH_2Cl_2): 275.0 (10486). ^{1}H NMR (400 MHz, $CDCl_3$): 1.45 (s, 54H, Me_3C); 1.49 (s, 54H, Me_3C); 2.79 (t, J = 6.80 Hz, 12H, ArCH_2CH$_2$NH); 3.60 (br t, 12H, ArCH$_2$CH_2NH); 5.15 (s, 12H, ArCH_2O); 6.81 (d, J = 8.08 Hz, 12H, ArH), 7.06 (d, J = 8.04 Hz, 12H, ArH); 8.39 (br s, 6H, NH); 11.46 (br s, 6H, NH_{guan}). ^{13}C NMR (100 MHz, $CDCl_3$): 28.04; 28.31; 34.46; 42.49; 63.64; 79.30; 83.05; 114.81; 129.84; 131.31; 137.88; 153.15; 156.06;157.21; 163.48. Anal. calcd for $C_{126}H_{180}O_{30}N_{18}$, 0.3$CH_2Cl_2$ (2452.36): C, 61.86; H, 7.42; N, 10.28; found: C, 61.83; H, 7.07; N, 10.50. ES-MS (pos. mode): 2426.32 [M+H]$^+$.

4.1.3.7. Bis-[α,α′-[*para*-(di-Boc-guanidinoethyl)-phenoxy]-*o*-xylene (9). The salt **8** (0.30 g, 4.70 10^{-4} mol) was solubilised in anhydrous DMF (30 mL), then Et_3N (1.11 mL, 7.95 10^{-3} mol) and the 1,3-bis-(*tert*-butoxycarbonyl)-2-methyl-2-thiopseudourea **C** (0.73 g, 2.50 10^{-3} mol) were added. The mixture was stirred under Ar at 4 °C, then $HgCl_2$ (0.68 g, 2.50 10^{-3} mol. **Warning: $HgCl_2$ must be handled with care**) was added in one portion. After stirring at rt under Ar overnight, the solvent was evaporated under high vacuum at 30 °C. The resulting solid was dissolved in CH_2Cl_2 and the insoluble material was filtered off over Celite. The filtrate was concentrated then chromatographed (Al_2O_3, CH_2Cl_2/MeOH 99.8/0.2) to give **9** (0.39 g, 94%). White powder. Mp: 85–86 °C. IR (KBr): 1721.70, 1638.94 (CO). UV–vis (CH_2Cl_2): 276.0 (5382.4). ^{1}H NMR (400 MHz, $CDCl_3$): 1.47 (s, 18H, Me_3C); 1.50 (s, 18H, Me_3C); 2.81 (t, J = 7.30 Hz, 4H, CH_2CH$_2$NH$_2$); 3.64 (q, J = 6.90 Hz, 4H, CH$_2$CH_2NH); 5.14 (s, 4H, ArOCH_2); 6.90 (d, J = 8.52 Hz, 4H, ArH); 7.12 (d, J = 8.56 Hz, 4H, ArH); 7.35 (m, 2H, ArH); 7.51 (m, 2H, *Ar*H); 8.39 (br s, 2H, NH); 11.33 (br s, 2H, NH_{guan}). ^{13}C NMR (100 MHz, $CDCl_3$): 28.04, 28.31, 34.40, 42.55, 67.99, 79.44, 83.13, 114.96, 128.37, 128.86, 129.83, 131.00, 135.17, 153.14, 156.06, 157.38, 163.99. Anal. calcd for $C_{46}H_{64}O_{10}N_6$, 1.5H_2O (887.49): C, 62.25; H, 7.61; N, 9.47; found: C, 61.96; H, 7.35; N, 9.80. ES-MS (pos. mode): 861.6 [M+H]$^+$, 761.4 [M−COOCMe$_3$+H]$^+$, 431.4 [M+2H]$^{2+/2}$.

4.1.4. Compounds 5, 10, 15, 20, 25, 30, 35—general procedure

A solution of (di-Boc-guanidino) derivative in a mixture of CH_2Cl_2 and TFA was stirred at rt under Ar during X h. The solvent was evaporated under vacuum and the residual acid was eliminated by multiple dissolution/co-evaporation cycles in CH_2Cl_2 until formation of a solid material. The latter was treated by multiple tri turation–ultrasonication–filtration sequences with dry Et_2O, until pH of the filtrate remained neutral. The solid material was dissolved in H_2O and lyophilized to give the desired trifluoroacetate salt.

4.1.4.1. α-[*para*-(Guanidinoethyl)-phenoxy]toluene, trifluoroacetate (5). From **4** (0.150 g, 0.32 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (15 mL), 6 h. **5** (0.12 g, 94%). White powder. Mp: 160–162 °C. IR (KBr): 1664.5 (CO). UV–vis (H_2O): 274.0 (1026.00). ^{1}H NMR (400 MHz, D_2O): 2.81 (t, J = 6.68 Hz, 2H, CH_2CH$_2$NH); 3.40 (t, J = 6.68 Hz, 2H, CH$_2$CH_2NH); 5.15 (s, 2H, ArOCH_2); 7.02 (d, J = 8.56 Hz, 2H, ArH); 7.22 (d, J = 8.28 Hz, 2H, ArH); 7.38–7.46 (m, 5H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): 33.91, 42.62, 69.50, 115.05, 127.98, 128.13, 128.78, 130.16, 130.78, 137.55, 157.15, 157.34. Anal. calcd for $C_{16}H_{19}ON_3$, CF_3COOH, 0.5H_2O (392,37): C, 55.10; H, 5.39; N, 10.71; found: C, 55.03; H, 5.43; N, 10.97. ES-MS (pos. mode): 270.28 [M−CF_3COOH+H]$^+$.

4.1.4.2. Bis-[α,α′-[*para*-(guanidinoethyl)-phenoxy]-*o*-xylène, bis trifluoroacetate (10). From **9** (0.50 g, 0.58 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (30 mL), 24 h. **10** (0.30 g, 75.6%). White solid. Mp: deliquescent. IR (pure): 1671.9 (CO). UV–vis (H_2O): 272 (3394). ^{1}H NMR (400 MHz, D_2O): 2.80 (t, J = 6.42 Hz, 4H, CH_2CH$_2$NH); 3.39 (t, J = 6.66 Hz, 4H, CH$_2$CH_2NH); 5.24 (s, 4H, ArOCH_2); 6.96 (d, J = 8.32 Hz, 4H, ArH); 7.18 (d, J = 8.56 Hz, 4H, ArH); 7.45 (m, 2H, ArH); 7.54 (m, 2H, ArH). ^{13}C NMR (100 MHz, D_2O): 33.55, 42.74, 68.12, 115.25, 128.86, 129.58, 130.18, 131.32, 135.21, 156.96, 157.01. Anal. calcd for $C_{26}H_{32}O_2N_6$, 2 CF_3COOH, H_2O (706.63): C, 50.99; H, 5.14; N, 11.89; found: C, 51.09; H, 4.92; N, 11.54. ES-MS (pos. mode): 461.26 [M−2CF_3COOH+H]$^+$, 230.82 [M−2CF_3COOH+2H]$^{2+/2}$.

4.1.4.3. Bis-[α,α′-[*para*-(guanidinoethyl)-phenoxy]]-*m*-xylene, bis trifluoroacetate (15). From **14** (0.15 g, 0.17 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (20 mL), 5 h. **15** (0.12 g, 0.17 10^{-3} mol, 98%). White solid. Mp: deliquescent. IR (neat): 1672.28, 1654.92, 1624.06, 1616.35 (CO). UV–vis (H_2O): 273.0 (3302.0). ^{1}H NMR (400 MHz, D_2O): 2.69 (t, J = 6.56 Hz, 4H, CH_2CH$_2$NH); 3.27 (t, J = 6.66 Hz, 4H, CH$_2$CH_2NH); 4.94 (s, 4H, ArOCH_2); 6.82 (d, J = 8.28 Hz, 4H, ArH); 7.06 (d, J = 8.32 Hz, 4H, ArH); 7.29 (br s, 3H, ArH); 7.32 (br s, 1H, ArH). ^{13}C NMR (100 MHz, D_2O): 33.55; 42.74; 68.12; 115.25; 128.86; 129.58; 130.18; 131.32; 135.21; 156.96; 157.01; 165.21. Anal. calcd for $C_{26}H_{32}O_2N_6$, 2CF_3COOH, 1/2H_2O (697.63): C, 51.65; H, 5.06; N, 12.05; found: C, 51.60; H, 4.86, N12.06. ES-MS (pos. mode): 461.25 [M−2CF_3COOH+H]$^+$; 231.05 [M−2CF_3COOH+2H]$^{2+/2}$.

4.1.4.4. Bis-[α,α′-[*para*-(guanidinoethyl)-phenoxy]]-*p*-xylene, bis trifluoroacetate (20). From **19** (0.40 g , 0.46 10^{-3} mol), in a 80:20 mixture of CH_2Cl_2 and TFA (25 mL), 24 h. **20** (0.25 g, 79%). White powder. Mp: deliquescent. IR (pure): 1612.49, 1629.85 (CO). UV–vis (H_2O): 273 (3032). ^{1}H NMR (400 MHz, D_2O): 2.89 (t, J = 6.8 Hz, 4H, CH_2CH$_2$NH), 3.47 (t, J = 6.8 Hz, 4H, CH$_2$CH_2NH), 5.22 (s, 4H, CH_2Ph), 7.06 (d, J = 8.8 Hz, 4H, ArH), 7.29 (d, J = 8.8 H, 4H, ArH), 7.57 (s, 4H, ArH). ^{1}H NMR (400 MHz, DMSO-d_6): 2.70 (t, J = 7.1 Hz, 4H, CH_2CH$_2$NH), 3.30 (q, J = 6.5 Hz, 4H, CH$_2$CH_2NH), 5.07 (s, 4H, CH_2Ph), 6.94 (d, J = 8.3 Hz, 4H, ArH), 7.17 (d, J = 8.3H, 4H, ArH), 7.22 (br s, 8H, NH), 7.44 (s, 4H, ArH), 7.66 (t, J = 4.9 Hz, 2H, CH$_2$CH$_2$NH). ^{13}C NMR (100 MHz, DMSO-d_6): 36.35, 45.04 (*C*H$_2$CH$_2$NH), 71.70 (OCH$_2$), 117.51, 130.50, 132.58, 133.24, 139.56, 159.61, 159.75 (*C* Ar and $C_{guanidine}$). Anal. calcd for $C_{26}H_{32}O_2N_6$, 2CF_3COOH (688.62): C, 52.33; H, 4.98; N, 12.20; found: C, 52.37; H, 5.05; N, 12.16. ES-MS (pos. mode): 461.27 [M−2CF_3COOH+H]$^+$.

4.1.4.5. Tris-[α,α′,α″-[*para*-(guanidinoethyl)-phenoxy]]-mesitylene, tris trifluoroacetate (25). From **24** (0.16 g, 0.12 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (15 mL), 6 h. (TLC monitoring; SiO_2, CH_2Cl_2/CH_3OH 2%). **25** (0.11 g, 90%). Mp: deliquescent. IR (KBr): 1670.4 (CO). UV–vis (H_2O): 274 (4050). ^{1}H NMR (400 MHz, D_2O): 2.76 (t, J = 6.04 Hz, 6H, CH_2CH$_2$NH); 3.34 (t, J = 6.04 Hz, 6H, CH$_2$CH_2NH); 4.89 (s, 6H, ArOCH_2); 6.85 (t, J = 7.32 Hz, 6H, ArH); 7.12 (t, J = 7.56 Hz, 6H, ArH); 7.29 (s, 3H, ArH). ^{13}C NMR (100 MHz, D_20): 33.16, 42.40, 69.02, 114.95, 126.05, 129.78, 130.87, 137.39, 156.57, 156.64, 116.4 (q, J = 292 Hz, *C*F$_3$CO), 162.46 (CF$_3$*C*O). Anal. calcd for $C_{36}H_{45}N_9O_3$, 3CF_3COOH, 0.5H_2O (1002.88): C, 50.30; H, 4.92; N, 12.57; found: C, 50.16; H, 4.97; N, 12.46. ES-MS (pos. mode): 880 [M−CF_3COOH+H]$^+$; 766 [M−2CF_3COOH+H]$^+$.

4.1.4.6. Tetra-[α,α′,α″,α‴-[*para*-(guanidinoethyl)-phenoxy]]-durene, tetra trifluoroacetate (30). From **29** (0.14 g, 0.08 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (15 mL), 24 h. (TLC monitoring; SiO_2, CH_2Cl_2/CH_3OH 99:1). **30** (0.08 g,

71%). White solid. Mp: deliquescent. IR (KBr): 1671.14 (CO). UV–vis (H_2O): 272 (6126). ^{1}H NMR (400 MHz, D_2O): 2.74 (t, J = 6.78 Hz, 8H, CH_2CH_2NH); 3.31 (t, J = 6.92 Hz, 8H, CH_2CH_2NH); 5.01 (s, 8H, $ArOCH_2$); 6.82 (d, J = 8.32 Hz, 8H, Ar*H*); 7.08 (d, J = 8.28 Hz, 8H, Ar*H*); 7.49 (s, 2H, Ar*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 33.6, 42.3, 66.8, 114.7, 128.8, 129.9, 130.7, 135.1, 156.8, 156.9, 158.9 (q, J = 31.3 Hz). Anal. calcd for $C_{46}H_{58}O_4N_{12}$, 4 CF_3COOH, 1.5H_2O (1326.15): C, 48.91; H, 4.94; N, 12.67; found: C, 49.00; H, 5.27; N, 12.63. ES-MS (pos. mode): 1185.47 $[M-CF_3COOH+H]^+$, 1071.48 $[M-2CF_3COOH+H]^+$, 957.48 $[M-3CF_3COOH+H]^+$, 843.49 $[M-4CF_3COOH+H]^+$.

4.1.4.7. Hexakis-[*para*-(guanidinoethyl)-phenoxymethyl]benzene, hexa trifluoroacetate (35). From **34** (0.14 g, 0.06 10^{-3} mol) in a 75:25 mixture of CH_2Cl_2 and TFA (20 mL), 24 h. (TLC monitoring; SiO_2, CH_2Cl_2/CH_3OH 98:2). **35** (0.09 g, 79%). White solid. Mp: deliquescent. IR (KBr): 1670.26 (CO). UV–vis (H_2O): 273 (1350.0). ^{1}H NMR (400 MHz, D_2O): 2.77 (t, J = 6.80 Hz, 12H, $ArCH_2CH_2NH$); 3.35 (t, J = 6.80 Hz, 12H, $ArCH_2CH_2NH$); 5.33 (s, 12H, $ArOCH_2$); 6.87 (d, J = 8.56 Hz, 12H, Ar*H*); 7.11 (d, J = 8.56 Hz, 12H, Ar*H*). ^{13}C NMR (100 MHz, D_2O): 33.37; 42.54; 64.56; 115.25; 130.05; 131.66; 138.05; 156.72; 158.80. (no visible CF_3COOH). Anal. calcd for $C_{66}H_{84}N_{18}O_6$, 6 CF_3COOH, 2.5H_2O (1954.66): C, 47.93; H, 4.90; N, 12.90; found C 47.91; H, 5.05; N, 12.86. ES-MS (pos. mode): 613.3 $[M-6CF_3COOH+2H]^{2+/2}$; 409.2 $[M-6CF_3COOH+3H]^{3+/3}$; 307.2 $[M-6CF_3COOH+4H]^{4+/4}$; 245.9 $[M-6CF_3COOH+5H]^{5+/5}$.

4.2. Biology

4.2.1. Inhibition of mycobacterial growth

The susceptibility of the two *M. tuberculosis* strains H_{37}Rv and MYC5165 to all synthesized compounds was evaluated by determining the minimum inhibitory concentration (MIC) and the 50% inhibitory concentration (IC_{50}). We used a colorimetric microassay based on the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-di phenyltetrazolium bromide, Sigma) to formazan by metabolically active cells.[20,21,24,25] Briefly, serial twofold dilutions of each drug were prepared in 7H9 broth (Middlebrook 7H9 broth base (Difco)) using 96-well microtiter plates and 100 mL of bacterial suspension in 7H9 broth were added to each well. After 6 days of incubation, MTT was added (50 mL, 1 mg/mL). After one day of incubation, solubilisation buffer was added to each well. The optical densities were measured at 570 nm. The MIC was determined as the lowest concentration of drug that inhibited bacterial growth (absorbance from untreated bacilli was taken as a control for growth). The IC_{50} were determined by using the Graph Pad Prism 5.0 software. The reported MICs are an average of at least three individual measurements.

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