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Synthesis and in vivo biological activity of large-ringed calixarenes against *Mycobacterium tuberculosis*

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ABSTRACT

A series of large-ringed calix[6,7,8]arene analogues have been synthesised and their affect against *Mycobacterium tuberculosis* in vivo established. In general, when *p*-phenylcalixarenes and *tert*-butylcalixarenes were not functionalised at the lower rim, low biological activities were observed. However on going from partially to fully lower rim pegylated calixarenes the anti-mycobacterial properties improved. The addition of cyanopropoxy groups at the lower rim gave rise to low activities, whereas the addition of acetate moieties interestingly had pro-TB effects. Two upper rim sulfonated calixarenes showed promising properties. In the course of this work, a high yielding procedure to synthesise *p*-phenylcalix[7]arene was also established.

1. Introduction

Mycobacterium tuberculosis is still a major cause of death worldwide and infects one-third of the world's population. Recent estimates are that there were 9.3 million new cases in 2007 and 1.7 million deaths from tuberculosis (TB), with 23% of the total deaths among HIV-positive TB cases.[1] In addition, the increase in multi-drug-resistant (MDR) TB cases has led to interest in new drug therapies and mechanisms of intervention.

The chemistry of calixarenes, and use in a wide range of applications, has received much attention in recent years due to their multi-functional capabilities and use as building blocks for host molecules. For example, they have been used as enzyme mimics,[2] gene and drug delivery reagents,[3] as sensors[4] and in catalysis.[5] One particularly interesting application has been as anti-mycobacterial agents and the synthesis of these compounds has been explored.[6–17] Early work in the 1950s established that macrocyclon **1** exhibited chemotherapeutic activity against experimental TB in the guinea pig[6] and mouse.[6–8] Macrocyclon, also referred to as HOC 12.5 EO, was prepared from a *tert*-octylphenol derived calix[8]arene (known as HOC **2**, Fig. 1) and ethylene oxide (EO) under basic conditions, resulting in the formation of polyethyleneglycol (PEG) chains of on average 12.5 units attached to the calixarene lower rim.[8] At the time it was believed that HOC was the *p*-*tert*-octyl-calix[4]arene, although later work established that macrocyclon was comprised of the larger *p*-*tert*-octyl-calix[8]arene possessing eight PEG chains at the lower rim of approximately 12.5 EO units.[9]

An analogue of macrocyclon, HOC-60 EO **3**, was also noted as having a pro-TB effect, enhancing the tuberculosis infection.[8] Over several years the heterogeneous mixture, macrocyclon, was used in a range of experiments: control of a *Leishmania donovani* infection in vivo was observed,[10] and it was used in chemotherapeutic trials in pulmonary tuberculosis and leprosy where no serious toxicities were noted.[11,12] Studies also indicated that **1** acted directly, modifiying host function, and may be stored in macrophages, possibly associated with lysosomes.[13] In addition, experiments established that **1** inhibited and **3** stimulated triacylglycerol lipase from macrophage extracts[14] and phospholipase A_2 activity in a model system.[15]

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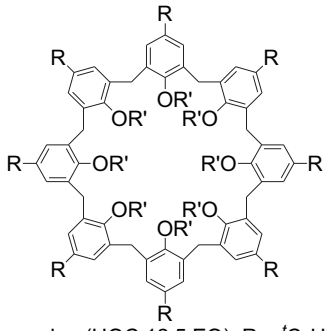


1 macrocyclon (HOC 12.5 EO); R = $^{t}C_8H_{17}$, R' = $(CH_2CH_2O)_nH$ where n is avg. of 12.5
2 HOC; R = $^{t}C_8H_{17}$, R' = H
3 HOC 60 EO; R = $^{t}C_8H_{17}$, R' = $(CH_2CH_2O)_nH$ where n is avg. of 60
4 R = $^{t}C_4H_9$, R' = $(CH_2CH_2O)_6H$

Fig. 1. Macrocyclon and structures of compounds **2**–**4**.

More recent studies involved the use of macrocyclon and *p*-*tert*-butyl-calix[6,8]arene analogues with defined *n*-ethylene glycol units, referred to as polyethyleneglycol (PEG) chains at the lower rim, and significant anti-mycobacterial activities were observed.[16,17] Notably, the PEG6-OH analogue **4** (Fig. 1) had similar anti-mycobacterial activities in vivo to macrocyclon, as did the equivalent calix[6]arene compound **5** (Fig. 2).[17] It was also established that the effect of macrocyclon was independent of the action of $CD4^+$ and $CD8^+$ T cells. Further experiments showed that macrocyclon-induced anti-mycobacterial activity was partly mediated by an L-arginine-dependent mechanism, and inducible nitric oxide synthase (iNOS) activity was required.[17]

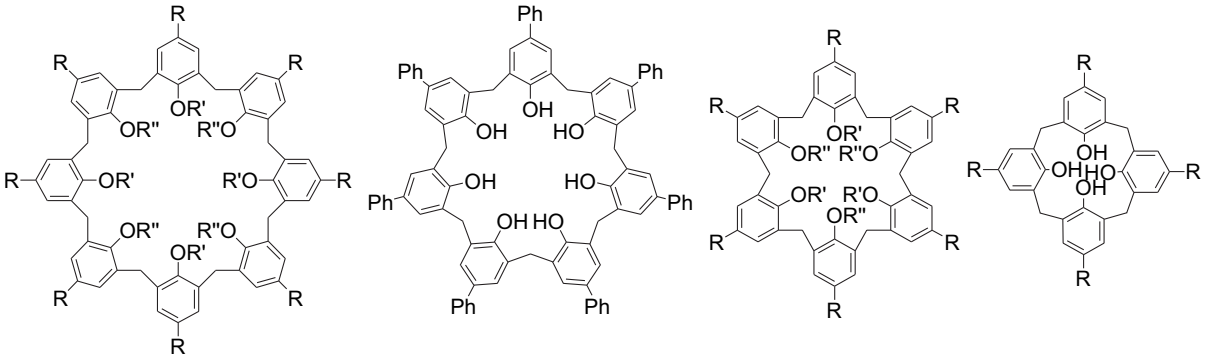
In current work, a series of calixarene analogues were prepared to establish the importance of functional groups at the upper and lower calixarene rim for anti-mycobacterial properties. Here we describe the synthesis and biological activities of selected calixarenes with a view to enhancing our understanding regarding structure and activity of these interesting compounds. Notably compounds with full and partial pegylations at the lower rim were investigated together with non-pegylated compounds and those with alternative groups at the upper rim.

2. Results and discussion

2.1. Chemistry

Several calixarenes were selected for synthesis and biological evaluation in vivo against *M. tuberculosis* infection in mice. These included *p*-*tert*-butylcalixarenes with short PEG chains such as PEG3-OMe and partial functionalisation with PEG chains, since previous studies with fully PEG6-OH pegylated compounds, *p*-*tert*-butyl-calix[6,8]arenes **5** and **4**, gave comparable activities to macrocyclon. *p*-*tert*-Butylcalix[8]arene **6** and *p*-*tert*-butylcalix[6]arene **7** were converted to the PEG3-OMe analogue **8** and alternate pegylated calixarenes **9**–**12** using potassium carbonate as a base and the corresponding brominated PEG chains as previously described.[16] The biological activity of these compounds could then be compared to the fully pegylated analogues **4**, **5** and **13**. For comparison purposes *p*-*tert*-butylcalix[4]arene **14** was also used in biological screens.

In addition, the *p*-phenylcalixarene series were of interest due to their reported extended hydrophobic cavities. If the biological activity of the calixarenes is related to their ability to complex small molecules or interact with hydrophobic membranes it was envisaged that a larger hydrophobic cavity may result in interesting biological properties. Accordingly, the synthesis of large-ringed *p*-phenylcalixarenes was investigated. Gutsche has reported the synthesis of *p*-phenylcalix[8]arene **15** utilising an adapted Zinke–Cornforth procedure with aqueous formaldehyde in 14% yield.[18] Modification of the reaction conditions with increased base afforded *p*-phenylcalix[6]arene **16** in 10% yield.[18] Application of the one-step Petrolite procedure using paraformaldehyde gave **15** in 4% yield together with what was suggested to be a cyclic heptamer **15**, but no pure material could be isolated.[18] More recently, the synthesis of *p*-phenylcalix[4,5,6,8]arenes were described utilising a modified Petrolite procedure with paraformaldehyde in tetralin, but also varying the concentration of potassium hydroxide or sodium hydroxide used.[19] A higher molar ratio of base gave a mixture



6 R = $^{t}C_4H_9$, R' = R'' = H
8 R = $^{t}C_4H_9$, R' = $(CH_2CH_2O)_3Me$, R'' = R'
9 R = $^{t}C_4H_9$, R' = $(CH_2CH_2O)_6H$, R'' = H
11 R = $^{t}C_8H_{17}$, R' = $(CH_2CH_2O)_6H$, R'' = H
12 R = $^{t}C_4H_9$, R' = $(CH_2CH_2O)_{12}H$, R'' = H
13 R = $^{t}C_4H_9$, R' = $(CH_2CH_2O)_{12}H$, R'' = R'
15 R = Ph, R' = R'' = H
19 R = Ph, R' = R'' = Ac
20 R = $^{t}C_4H_9$, R' = R'' = Ac
22 R = $^{t}C_4H_9$, R' = R'' = $(CH_2)_3CN$
23 R = Ph, R' = R'' = $(CH_2)_3CN$

18

5 R = $^{t}C_4H_9$, R' = R'' = $(CH_2CH_2O)_6H$
7 R = $^{t}C_4H_9$, R' = R'' = H
10 R = $^{t}C_4H_9$, R' = $(CH_2CH_2O)_6H$, R'' = H
16 R = Ph, R' = R'' = H
21 R = $^{t}C_4H_9$, R' = R'' = $(CH_2)_3CN$
24 R = Ph, R' = R'' = $(CH_2)_3CN$

14 R = ^{t}Bu
17 R = Ph

Fig. 2. Structures of compounds **5**–**24**.

of ring sizes, including the octamer **15** (38% yield) and hexamer **16** (7% yield) (Table 1, entry 1).[19] Subsequently, a 'solventless' approach was described utilising aqueous formaldehyde: use of potassium hydroxide gave the hexamer **16** in 50% yield (Table 1, entry 2), highlighting the sensitivity of these procedures to reaction conditions.[20] With a view to generating *p*-phenylcalix[8]arene as the major isomer, with little smaller-ringed calixarenes produced to enhance ease of purification, several reaction conditions were investigated.

Table 1
Base and formaldehyde used, molar ratio of base to *p*-phenylphenol, and resulting yields of *p*-phenylcalix[*n*]arenes

Entry	Base	Molar ratio (base/phenol)	**17** (*n*=4) yield (%)	**16** (*n*=6) yield (%)	**18** (*n*=7) yield (%)	**15** (*n*=8) yield (%)
1[a,19]	KOH (15 M)	0.45	10	7	0	38
2[b,20]	KOH	0.05	10	50	0	0
3[a]	NaOH (50%)[c]	0.06	7	4	0	16
4[a]	NaOH (40%, 10 M)[c]	0.03	1	4	0	13
5[a]	KOH (40%, 10 M)[c]	0.03	0	0.2	0	27
6[a]	KOH (50%)[c]	0.07	2	8	60	0
7[a]	CsOH (50%)[c]	0.07	0	1	0	28
8[a]	CsOH (50%)[c]	0.06	0.5	1.5	0	34
9[a]	CsOH (50%)[c]	0.02	3	0.7	0	21

[a] Paraformaldehyde.
[b] Aqueous formaldehyde.
[c] Aqueous solution (wt/vol).

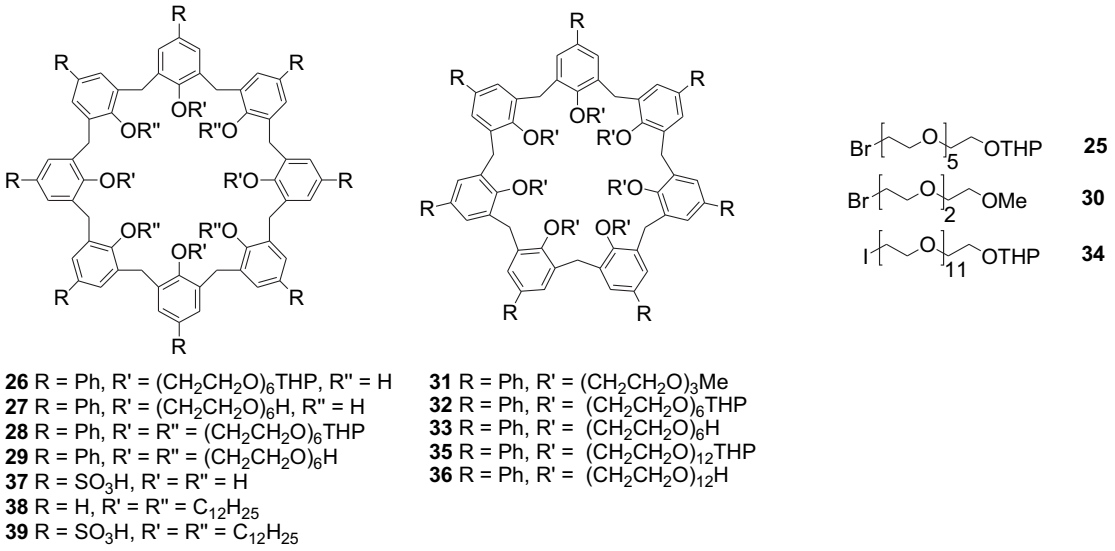
Use of the Petrolite procedure with paraformaldehyde (Table 1, entry 3) gave the *p*-phenylcalix[4]arene **17**, together with **16** and **15** in 7%, 4%, and 16% yields, respectively. Gutsche's reaction conditions in our hands gave the cyclic products in equally low yields (entry 4).[18] When the same reaction conditions were utilised, but with potassium hydroxide as the base, which may via the templating effect increase the proportion of the larger ringed calixarenes formed, the octamer **15** was formed in 27% yield with negligible **16** and no **17** (entry 5). To enhance the proportion of **15** formed, similar reaction conditions to those used in entry 3, but using potassium hydroxide were used (entry 6). Initially it was believed that the octamer **15** was formed in high yield, however MS analysis revealed that the heptamer **18** had been generated in 60% yield with hexamer **16** and tetramer **17** in a combined yield of 10%. This was unexpected: the cyclic heptamers have not been obtained in comparable yields to the major calixarenes, for example, optimised yields for the generation of *p*-*tert*-butylcalix[7]arene have been reported in the range 11–17%.[21] The formation of calix[7]arenes with *p*-methyl and *p*-ethyl substituents has also been described in low yield.[22] Gutsche and co-workers had mentioned what they believed to be *p*-phenylcalix[7]arene **18** as a trace compound formed in an inseparable mixture using the Petrolite procedure. This paper referred to a Japanese patent that claimed formation of the compound, although only sparse characterisation data was presented.[18,23] The use of CsOH as a base (Table 1, entries 7–9) gave higher yields of **15**, no heptamer **18**, and small amounts of hexamer **16** and tetramer **17**.

The spectral characterisation of heptamer **18** revealed several interesting features. Notably the δ_{OH} NMR resonance in $CDCl_3$ was observable at 10.54 ppm, similar to the value for *tert*-butylcalix[7]arene (δ_{OH} at 10.32).[21] Also the methylene bridge protons were observed as a broad singlet at δ 3.95 ppm, while the ^{13}C NMR spectrum revealed the corresponding carbons at δ 32.6 ppm. This suggested that the aryl groups were in a *syn* orientation by application of the 'de Mendoza rule', which has been reliably applied to the calix[4,5,6]arenes.[24] Solid state ^{13}C NMR measurements corroborated the solution state conformation, with the methylene signal observable at approximately δ 32.6 ppm and revealed the position of C–OH at δ 149.1 ppm.

With the focus on large-ringed calixarenes, selected derivatisations of the *p*-phenyl octamer **15** and heptamer **18** were explored. Initially, the fully substituted acetate of **15** was prepared under standard conditions, using sodium hydride and acetyl chloride to give **19** in 46% isolated yield. The corresponding *p*-*tert*-butylcalix[8]arene acetate **20** was also synthesised as previously described, for comparison purposes.[25] The *p*-*tert*-butyl-octakis(cyanopropoxy)calix[6,8]arenes **21** and **22** had previously been prepared for testing as representative non-pegylated analogues,[16] and therefore the corresponding cyanopropoxy derivatives of octamer **15** and heptamer **16** were also synthesised using equivalent procedures to generate **23** and **24**, respectively (Fig. 2). Compounds **23** and **24** were formed in 9% and 18% yield and for reactions with octamer **15** to **23**, higher equivalents of base and electrophile were required compared to the previous reaction conditions reported to generate **21** and **22**. This highlighted that functionalisation at the lower rim required more forcing reaction conditions, perhaps due to steric or conformational effects.

To compare biological activities to the pegylated *tert*-butylcalixarenes, several pegylated *p*-phenylcalixarenes were also synthesised using the same method as that used to generate the corresponding *tert*-butylcalixarenes **4**, **5**, **8**–**12**. The approach was to achieve tetra-alkylation using potassium carbonate as base with the electrophile, and a second procedure with sodium hydride as base to the fully alkylated products. Accordingly, octamer **15** was reacted with 17-tetrahydropyranyloxy-3,6,9,12,15-pentaoxaheptadecyl (**25**) using potassium carbonate as base to give a pegylated analogue. By analogy to the same procedure used with *tert*-butylcalix[8]arene and from MS data, where the molecular ion and loss of THP groups were observed, this was assigned to be the tetra-substituted PEG6-OTHP derivative **26**.[16] It was also tentatively assigned as the 1,3,5,7-symmetrically substituted product, although line broadening and poor relaxation of the aromatic ^{13}C NMR signals meant that this could not be confirmed. Full characterisation was carried out on the THP protected analogues due to the ability of the deprotected calixarenes to complex several metal ions and fragment under MS conditions where it became more difficult to observe the molecular ions. This had been observed previously with other pegylated calixarenes.[16] Compound **26** was then deprotected as described previously for the pegylated *tert*-butylcalix[8]arene **9** and NMR spectroscopy confirmed removal of the protecting group to give **27**.[16] The protected intermediate **26** was further reacted with bromide **25** using sodium hydride as base to effect the full pegylation, giving **28** in 20% yield, which was confirmed by MALDI-TOF MS.[16] In addition, a shift in the UV maxima from 290 nm in to 255 nm was observed, consistent with alkylation of the calixarenes.[26] Deprotection of **28** produced the fully substituted PEG6-OH analogue **29** (Fig. 3). Again, the reaction yields when functionalizing the *p*-phenylcalix[8]arene were lower than for the comparable *tert*-butylcalix[8]arene.

Heptamer **18** was then functionalised with PEG chains. Reaction with 1-(2-bromoethoxy)-2(2-methoxyethoxy)ethane **30** (8 equiv) and potassium carbonate as base (16 equiv) readily gave the fully alkylated product **31** in 31% isolated yield, in contrast to the



26 R = Ph, R' = $(CH_2CH_2O)_6$THP, R'' = H
27 R = Ph, R' = $(CH_2CH_2O)_6$H, R'' = H
28 R = Ph, R' = R'' = $(CH_2CH_2O)_6$THP
29 R = Ph, R' = R'' = $(CH_2CH_2O)_6$H
37 R = SO_3H, R' = R'' = H
38 R = H, R' = R'' = $C_{12}H_{25}$
39 R = SO_3H, R' = R'' = $C_{12}H_{25}$

31 R = Ph, R' = $(CH_2CH_2O)_3$Me
32 R = Ph, R' = $(CH_2CH_2O)_6$THP
33 R = Ph, R' = $(CH_2CH_2O)_6$H
35 R = Ph, R' = $(CH_2CH_2O)_{12}$THP
36 R = Ph, R' = $(CH_2CH_2O)_{12}$H

Fig. 3. Structures of compounds **25**–**39**.

partially alkylated products observed for the hexamer **5** and octamers **2** and **6**[16] under similar conditions, and fewer equivalents of base and electrophile were also required. Full alkylation was confirmed by the shift in the UV absorption band from 310 nm in **18** to 260 nm in **31**.[26] In recently reported work, Hii and co-workers have described pegylations at the lower rim of calixarenes where cesium carbonate was used to effect full alkylation, though higher equivalents of reagents were used and it was noted that potassium carbonate gave products in much lower yields.[27] The ease with which the etherification of **18** was observed was surprising. Functionalisation with a longer ethylene glycol chain **25** confirmed the ease of addition to the heptamer, generating **32** in 49% isolated yield. Characterisation was confirmed by NMR and MALDI-TOF MS, where the molecular ion was observed complexed to sodium (*m*/*z* 3746). Finally, deprotection of **32** yielded heptamer **33**, bearing hexamethylene glycol chains, in 96% yield. Using an analogous procedure **18** was also reacted with THP protected PEG12-iodide **34** to give the fully alkylated *p*-calix[7]arene **35**. Again, compound characterisation was more readily achieved on **35** with the THP protected PEG chain present, and deprotection as previously gave **36**. The reason why the heptamer affords fully pegylated compounds so readily is unclear. However *tert*-butylcalix[7]arene has been reported to be more conformationally flexible than particularly the *tert*-butylcalix[4,8]arenes where favourable intramolecular hydrogen bonding exists.[28] It is possible that by analogy the phenyl heptamer ring is significantly more conformationally

Table 2
Calix[*n*]arenes tested for biological activity in vivo

Compound number	Ring size	R	R′	R′	Chemotherapeutic activity[a]
1	8	$^tC_8H_{17}$	$(CH_2CH_2O)_nH$ (*n* avg 12.5)	R′	+++
4[16]	8	$^tC_4H_9$	$(CH_2CH_2O)_6H$	R′	+++[17]
5[16]	6	$^tC_4H_9$	$(CH_2CH_2O)_6H$	R′	+++[17]
6[16]	8	$^tC_4H_9$	H	H	+
7[16]	6	$^tC_4H_9$	H	H	None[17]
8[16]	8	$^tC_4H_9$	$(CH_2CH_2O)_3Me$	R′	+++
9[16]	8	$^tC_4H_9$	$(CH_2CH_2O)_6H$	H	+
10[16]	6	$^tC_4H_9$	$(CH_2CH_2O)_6H$	H	+
11[16]	8	$^tC_8H_{17}$	$(CH_2CH_2O)_6H$	H	+
12[16]	8	$^tC_4H_9$	$(CH_2CH_2O)_{12}H$	H	+
13[16]	8	$^tC_4H_9$	$(CH_2CH_2O)_{12}H$	R′	+++[17]
14	4	$^tC_4H_9$	H	H	None
15	8	Ph	H	H	+
18	7	Ph	H	H	±
19	8	Ph	$COCH_3$	$COCH_3$	Pro-TB effect[b]
20	8	$^tC_4H_9$	$COCH_3$	$COCH_3$	Pro-TB effect
21[16]	6	$^tC_4H_9$	$(CH_3)_2CN$	$(CH_3)_2CN$	±
22[16]	8	$^tC_4H_9$	$(CH_3)_2CN$	$(CH_3)_2CN$	±
27	8	Ph	$(CH_2CH_2O)_6H$	H	±
29	8	Ph	$(CH_2CH_2O)_6H$	R′	±
31	7	Ph	$(CH_2CH_2O)_3Me$	—	±
33	7	Ph	$(CH_2CH_2O)_6H$	—	+
35	7	Ph	$(CH_2CH_2O)_{12}H$	—	+++
37	8	SO_3H	H	R′	++
39	8	SO_3H	$OC_{12}H_{25}$	R′	++

All activities measured from *M. tuberculosis* growth in mice spleens and lungs, as determined by CFU bacterial counts of infection. Groups of 3–5 mice were treated with macrocyclon, control and the new calixarene compounds. All calixarenes were tested in athymic *nu*/*nu* mice with the exception of compounds **14** and **20**, which were tested in BALB/c mice. Compounds **1**, **6** and **7** were also tested in BALB/c mice with similar results. The activity is calculated from the median change in colony counts of $\log_{10}$ CFUs/tissue, from the spleen and lung, compared to macrocyclon (100% reduction) and control (0% reduction).

[a] None, no detectable anti-TB activity; ±0–20% activity of macrocyclon compared to control; + 20–50% activity of macrocyclon compared to control; ++ 50–80% activity of macrocyclon compared to control; +++ 80–100% activity of macrocyclon.

[b] Experiments terminated due to severity of infection.

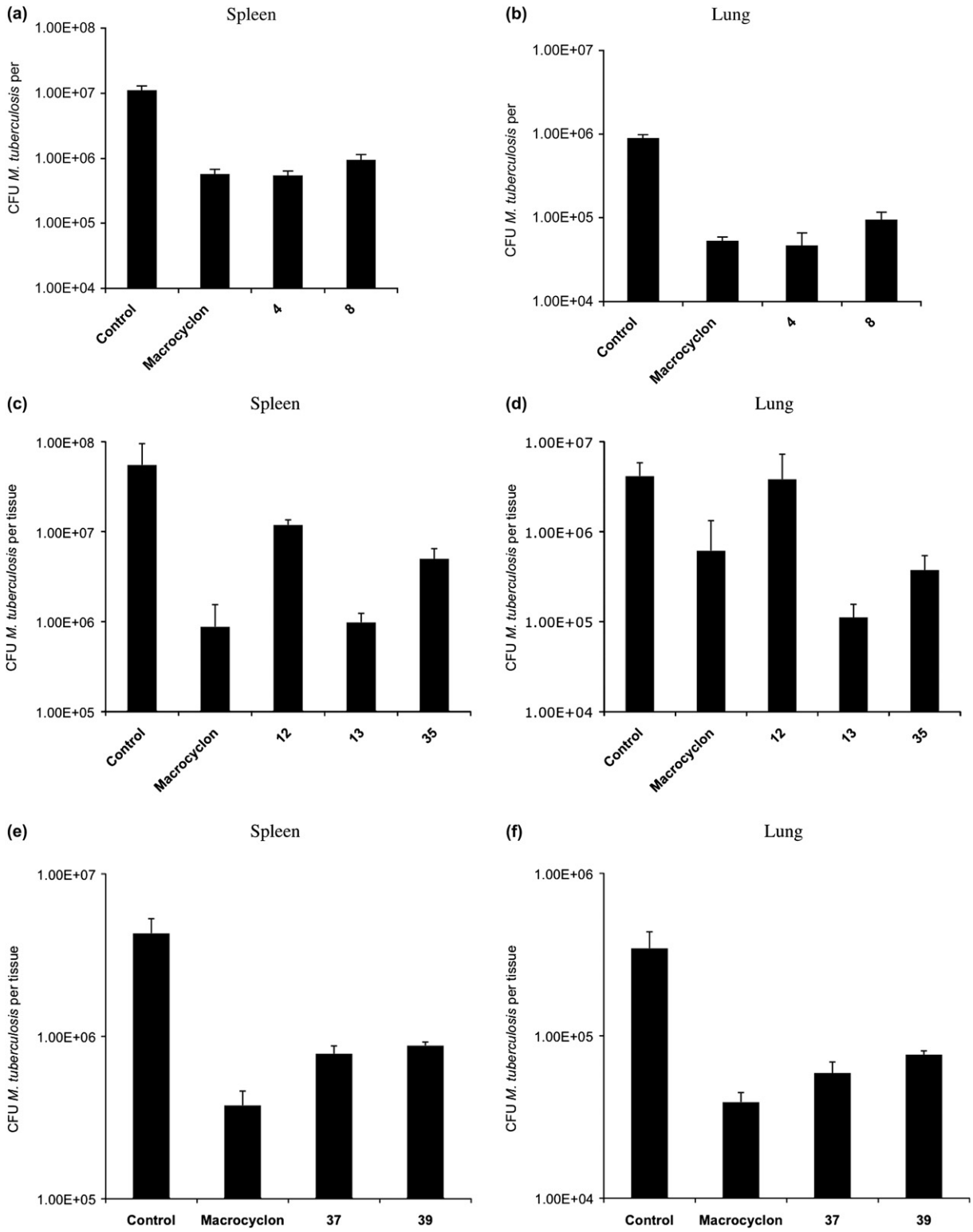


Fig. 4. Representative anti-mycobacterial data for compounds **4**, **8**, **12**, **13**, **35**, **37**, **39** (Table 2) compared to saline controls and macrocyclon. All activities were determined by measuring *M. tuberculosis* growth in mice spleens and lungs, as determined by CFU bacterial counts of infection as previously described.[17] Groups of 3–5 mice were treated with macrocyclon, control and the new calixarene compounds. All calixarenes shown in Fig. 4 were tested in athymic *nu*/*nu* mice. The activity was calculated from the median change in colony counts of $\log_{10}$ CFUs/tissue, from the spleen and lung.

flexible than the octamer, so that the free hydroxyls are not as sterically hindered by adjacent PEG chains as they are in the octamer.

Several calixarene derivatives were then available for screening bearing *tert*-butyl and phenyl groups at the upper rim, which could also be compared to the previously synthesised calixarenes with pegylation at the lower rim. Also butyronitriles and acetates at the lower rim, and a couple of other calixarenes were selected for synthesis, based upon the fact that sulfonatocalixarenes have well precedented water solubilities. Firstly, calix[8]arene-*p*-octasulfonic acid **37** (Fig. 3), which has good water solubility, as do the pegylated calixarenes, was prepared as previously described from calix[8]

arene in 90% yield.[29] In addition an analogue of **37**, alkylated with dodecyl chains at the lower rim was prepared, to give a calixarene with a hydrophobic and hydrophilic region comparable to the pegylated calixarene **4**. Accordingly, calix[8]arene was alkylated at the lower rim using an excess of sodium hydride and 16 equiv of bromododecane. The product **38** was formed in 68% yield and was characterised by NMR spectroscopy using deuterated pyridine as solvent. This was then readily sulfonated in refluxing sulfuric acid, using an identical procedure to that used to prepare **37** to afford **39** in 76% yield. With these compounds in hand (Figs. 2 and 3 and Table 2) the biological activities in vivo were determined.

2.2. Biological results

Previous work had established that macrocyclon **1** acted by modifying host function, and for this reason experiments were carried out in vivo against *M. tuberculosis* infection using either BALB/c mice or in most cases athymic *nu*/*nu* mice as previously described.[17] Mice were treated with the calixarenes 2 d prior to intravenous infection, followed by a second identical dose of calixarene 2–3 d after infection. Compounds were screened against macrocyclon **1** and controls (treatment with saline solution) and from this the relative activity of the calixarenes determined (Table 2). Compound toxicities of selected calixarenes were in accordance with those previously reported for macrocyclon, indeed at the concentrations used no toxicities were noted.[8,26]

Previous work had established that the unsubstituted *tert*-butylcalix[6,8]arenes, compounds **7** and **6**, respectively, had either a low anti-mycobacterial effect (**7**), or in the case of the hexamer no activity.[17] Further studies established that the tetramer **14** was inactive, that the *p*-phenylcalixarene **15** had some moderate activity and that heptamer **18** exhibited low levels of activity. This suggested that with no functionalisation at the lower rim, the calix[8]arenes had some low anti-mycobacterial activity, but that the smaller ring sizes, *p*-phenylcalix[7]arene and *tert*-butylcalix[4,6]arenes, had less or no activity, respectively. In addition, different bacterial colony counts were measured in the spleen compared to the lung, suggesting the low solubility of these compounds in aqueous media (typically 0.25 mg/mL in water) resulted in poor biodistribution. It was for these reasons that functionalisation of the calixarenes was focused on the larger calix[6,7,8]arene ring sizes.

Previous work had established that the *tert*-butylcalix[6,8]arenes, compounds **5** and **4**, with full pegylation with PEG6-OH at the lower rim had comparable activities to that of macrocyclon **1**, so the effect of the pegylation was investigated in more detail.[17] First compound **8**, with PEG3-OMe groups at all eight phenolic positions was compared to analogue **4** and macrocyclon **1**. Levels of anti-mycobaterial activity were approximately 80% that of **1** and **4** (Fig. 4a and b), suggesting that shorter *n*-ethylene oxide chains could be tolerated. Partially pegylated compounds **9**–**12** were also tested and compared to macrocyclon **1** and the fully pegylated compounds such as **13**. Interestingly, the compounds all had less anti-mycobacterial activity than the fully pegylated calixarenes (Fig. 4c and d), regardless of ring size and length of PEG chain (6 or 12). In addition, the group at the upper rim, whether it was *tert*-octyl or *tert*-butyl made little difference, and PEG-OH chains greater than six repeat units did not improve the activity further as had been observed previously with the fully alkylated analogues **5** and **13**.[17] Other lower rim fully substituted calixarenes were then investigated.

The fully acetylated calix[8]arenes **19** and **20** gave interesting data in that pro-TB activity was detected in both cases. It is unclear why this is the case, particularly because the phenolic acetates are likely to be hydrolysed in vivo and both non-functionalised calixarenes, **15** and **6**, demonstrated anti-TB properties. However, these compounds could be readily accessed molecular probes to use in future experiments for identifying the mode of pro-TB activity, also reported for HOC-60 when HOC is conjugated to approximately PEG-60 chains at the lower rim.

The two (cyanopropoxy)calix[6,8]arenes **21** and **22** gave rise to detectable but low anti-mycobacterial properties, and so the other (cyanopropoxy)calixarenes **23** and **24** were not tested. Nevertheless, it did highlight that substituted but non-pegylated calixarenes did retain some measure of activity. Next, the upper rim *p*-phenylcalix[7,8]arenes were examined. The partially PEG6-OH substituted calix[8]arene **27** showed only slight anti-TB properties, consistent with the data for compounds **9**–**12** bearing *tert*-butyl or *tert*-octyl groups at the upper rim. Interestingly however, the fully PEG6-OH substituted calix[8]arene **29**, similarly only had slight levels of activity. Moving to the pegylated *p*-phenylcalix[7]arenes, with PEG3-OMe (**31**), PEG6-OH (**33**), or PEG12-OH (**35**) substitutions, increase of the PEG chain length increased the anti-mycobacterial activity markedly, and this was a much clearer trend than for the comparable *tert*-butyl series. In general, the large-ringed *tert*-butylcalixarenes had higher activities than for the phenylcalixarenes, with the same PEG chain lengths. For example, *p*-phenylcalix[7]arene required PEG12-OH chains at the lower rim to achieve similar levels of activity to that of macrocyclon and *tert*-butylcalix[8]arene with PEG6-OH (Fig. 4a–d). This could reflect requirements for a shorter aryl cavity or hydrophobic region, or a certain balance between hydrophobicity and hydrophilicity that is needed for better biodistribution and transportation through cell membranes.

Finally, the two highly water soluble sulfonated calixarenes **37** and **39** were tested, and whether non-functionalised, or alkylated at the lower rim, the activity was comparable, and in both cases approximately 70% of that of macrocyclon **1** (Fig. 4e and f). These results highlighted that for good anti-mycobacterial activity larger ringed calixarenes are required with, ideally, good water solubilities. The advantage of compounds such as **4** and **37** exhibiting good anti-TB activity is that they are synthetically readily available for further modification and also the preparation of labelled molecular probes.

2.3. Conclusion

In summary, a range of calixarenes have been prepared for preliminary screening as anti-TB compounds. Several novel pegylated large-ringed calixarenes were prepared, including derivatisation with a phenyl group at the upper rim. In the course of this work we also identified a new procedure to *p*-phenylcalix[7]arene in high yield that was particularly amenable to derivatisation with activated PEG chains in comparatively high yield and one step only.

The compounds prepared and previous calixarenes synthesised in our group were used in preliminary in vivo experiments against *M. tuberculosis* in mice. These indicated that when not pegylated at the lower rim, *p*-*tert*-butylcalix[8]arene and *p*-phenylcalix[8]arene were more active than the *p*-*tert*-butylcalix[4,6]arenes and *p*-phenylcalix[7]arene, and that the highly water soluble sulfonate **37** showed activities approaching those of macrocyclon. When derivatising with PEG chains at the lower rim, this readily enhanced the anti-mycobacterial properties of the *tert*-butylcalix[6,8]arenes, but for the *p*-phenylcalix[7]arenes longer PEG chains were required to achieve this effect. For the *tert*-butylcalix[6,8]arenes, full pegylations gave rise to higher anti-mycobacterial properties, than the corresponding partial pegylations. Lower rim substitution with acetate groups gave rise to pro-TB activities, and lower rim functionalisation with non-PEG chains such as cyanopropoxy gave compounds with low activities. Further experiments with selected compounds will now be performed to probe the mode of action of this interesting family of anti-mycobacterial compounds.

3. Experimental

3.1. General chemical experimental

Unless otherwise noted, solvents and reagents were reagent grade from commercial suppliers and used without further purification. THF was dried by distillation from a sodium/benzophenone suspension under a dry N_2 atmosphere. CH_2Cl_2 was dried by distillation from CaH_2 under a dry N_2 atmosphere. All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} plates with detection by UV, or permanganate, ninhydrin (for ureas) and phosphomolybdic acid stains. Flash column chromatography was carried out using silica gel (particle size 40–63 μm). Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ at the field indicated. Compounds, **1**, **4**–**14**, **20**, **25**, **28** and **30**, were prepared as previously described.[16,25,29] Selected spectra and the biological screening data are in Supplementary data.

3.2. 5,11,17,23,29,35,41,47-Octaphenyl-49,50,51,52,53,54,55,56-octahydroxycalix[8]arene (*p*-phenylcalix[8]arene)[18] 15

A mixture of *p*-phenylphenol (8.50 g, 0.050 mol), paraformaldehyde (3.00 g, 0.10 mol) and caesium hydroxide (0.85 mL, 2.83 mmol; 50% aqueous solution) in xylene (50 mL) was heated at 130 °C for 4 h under nitrogen, with the azeotropic removal of water. The resulting precipitate was collected by filtration in vacuo and washed with water (20 mL), ethyl acetate (20 mL) and acetone (20 mL). The mixture was purified using flash silica chromatography (hexane/ethyl acetate, 3:1) to yield **15** as a white powder (3.10 g, 34%). R_f 0.25 (ethyl acetate/hexane, 2:1); λ_{max}/nm (KOH/MeOH) 230, 290; ν_{max}/cm^{-1} (KBr) 3200, 2920, 2960–2850; δ_H (300 MHz; DMSO-d_6) 4.01 (16H, br s, $ArCH_2Ar$), 7.20–7.71 (56H, m, ArH); δ_C (100 MHz; acetone-d_6) 33.5 (CH_2), 126.8, 127.2, 127.9, 129.4, 130.4, 132.8 (C-5), 142.3 (C-6), 154.0 (C–OH); *m*/*z* (ES^+) 1479 (MNa^+, 5%).

3.3. 5,11,17,23,29,35-Hexaphenyl-37,38,39,40,41,42-hexahydroxycalix[6]arene (*p*-phenylcalix[6]arene)[18] 16 and 5,11,17,23,29,35,41-heptaphenyl-43,44,45,46,47,48,49-heptahydroxycalix[7]arene (*p*-phenylcalix[7]arene) 18

A mixture of *p*-phenylphenol (8.50 g, 0.050 mol), paraformaldehyde (3.00 g, 0.100 mol) and potassium hydroxide (0.40 mL, 3.60 mmol; 50% aqueous solution) in xylene (50 mL) was heated at 130 °C for 4 h under nitrogen, with the azeotropic removal of water. The resulting precipitate was collected by filtration in vacuo and washed with water (20 mL), ethyl acetate (20 mL) and acetone (20 mL). Purification by flash silica chromatography (hexane/ethyl acetate, 3:1) yielded **16** (0.730 g, 8%) and **18** as a white powder (5.46 g, 60%). Data for **16**: R_f 0.85 (ethyl acetate/hexane, 2:1); ν_{max}/cm^{-1} (KBr) 3068, 3040; δ_H (300 MHz; DMSO-d_6) 4.10 (12H, s, $ArCH_2Ar$), 7.21–7.64 (42H, m, ArH); δ_C (100 MHz; acetone-d_6) 33.4 (CH_2), 126.7, 127.1, 127.8, 129.2, 130.3, 132.7 (C-5), 142.2 (C-6), 154.0 (C–OH); *m*/*z* (ES^+) 1093 (MH^+, 1%). Data for **18**. Mp >300 °C (ethyl acetate/hexane); λ_{max}/nm (KOH/MeOH) 275; ν_{max}/cm^{-1} (KBr) 3190, 2980; δ_H (500 MHz; $CDCl_3$) 4.10 (14H, br s, $ArCH_2Ar$), 7.28 (7H, t, *J* 7.5 Hz, Ph-*para H*), 7.38 (14H, t, *J* 7.5 Hz, Ph-*meta H*), 7.44 (14H, s, Ar-*meta H*), 7.49 (14H, d, *J* 7.5 Hz, Ph-*ortho H*), 10.54 (7H, br s, OH); δ_C (125 MHz; acetone-d_6) 32.6 (CH_2), 126.6, 126.8 (CH), 127.7, 128.9 (CH), 129.3, 133.5, 141.6; δ_C (75 MHz; CPMAS) 32.7 (CH_2), 128.2 (signals superimposed), 134.1 (C-5), 140.1 (C-6), 149.1 (C–OH); *m*/*z* (FAB^+) 1298 (MNa^+, 1%), 1276 (M^+, 1), 648 (10); *m*/*z* ($HRFAB^+$) found (MH^+) 1275.5235; $C_{91}H_{71}O_7$ requires 1275.5200.

3.4. 5,11,17,23-Tetraphenyl-25,26,27,28-tetrahydroxycalix[4]arene (*p*-phenylcalix[4]arene)[29] 17

A mixture of *p*-phenylphenol (8.50 g, 0.050 mol), paraformaldehyde (3.00 g, 0.100 mol) and sodium hydroxide (0.20 mL, 0.003 mol, 50% aqueous solution) in xylene (50 mL) was heated at 130 °C, under nitrogen for 4 h with the azeotropic removal of water. The resulting precipitate was collected by filtration and washed with water (20 mL), ethyl acetate (20 mL) and acetone (20 mL). The product was purified by flash chromatography (hexane/ethyl acetate, 3:1) to give **17** as a white powder (0.640 g, 7%). R_f 0.93 (ethyl acetate/hexane, 2:1); ν_{max}/cm^{-1} (KBr) 3084, 3045, 748; δ_H (300 MHz; DMSO-d_6) 4.91 (8H, s, $ArCH_2Ar$), 7.38–7.72 (28H, m, ArH); δ_C (100 MHz; acetone-d_6) 32.6 (CH_2), 126.2, 126.6, 127.2, 128.7, 129.7, 132.2 (C-5), 141.7 (C-6), 149.6 (C–OH); *m*/*z* ($HRFAB^+$) found (M^+) 728.2899; $C_{52}H_{40}O_4$ requires 728.2927.

3.5. 5,11,17,23,29,35,41,47-Octaphenyl-49,50,51,52,53,54,55,56-octakis(acetyl)calix[8]arene[18] 19

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (720 mg, 0.018 mol) in THF/DMF (4:1, 40 mL), *p*-phenylcalix[8]arene (874 mg, 0.600 mmol) was added in portions. Following stirring for 15 min acetyl chloride (750 mg, 9.55 mmol) was added dropwise. The resulting solution was heated at 70 °C for 24 h. The reaction mixture was quenched with iced water (20 mL), the crude product extracted into chloroform (3×50 mL) and washed with saturated lithium chloride solution (2×20 mL). Drying ($MgSO_4$), filtration and evaporation in vacuo afforded a crude oil, which upon flash chromatography (hexane/ethyl acetate, 3:1) afforded **19** as colourless crystals (490 mg, 46%). Mp 235–239 °C (ethyl acetate/hexane) (lit. 230–265 °C dec; chloroform/ethanol);[30] ν_{max}/cm^{-1} (KBr) 2954, 2854, 1759, 1597, 1555; δ_H (300 MHz; $CDCl_3$) 1.85 (24H, s, Me), 3.78 (16H, s, $ArCH_2Ar$), 7.23–7.81 (56H, m, ArH); δ_C (100 MHz; $CDCl_3$) 21.2 (CH_3), 31.0 ($ArCH_2Ar$), 125.5, 126.2 (signals superimposed), 127.9, 133.4, 141.0 (signals superimposed), 152.9 (C–O), 168.0 (C=O); *m*/*z* (FAB^+) 1793 (M^+, 1%).

3.6. 5,11,17,23,29,35,41,47-Octaphenyl-49,50,51,52,53,54,55,56-octakis-(cyanopropoxy)calix[8]arene 23

The reaction was carried out under anhydrous conditions. To a solution of **15** (100 mg, 0.07 mmol) in acetonitrile (50 mL), potassium carbonate (606 mg, 4.39 mmol) was added and the reaction mixture stirred at 40 °C for 15 min. 4-Bromobutyronitrile (0.44 mL, 4.39 mmol) was added and the reaction mixture stirred at 80 °C for 14 d. Water (10 mL) was added, the solution dried ($MgSO_4$) and evaporated in vacuo to afford the crude product as a yellow oil. Distillation under reduced pressure then flash silica chromatography (ethyl acetate/hexane, 2:1) afforded **23** as a yellow oil (12 mg, 9%). ν_{max}/cm^{-1} (film) 2950, 2890, 2246, 1076; δ_H (300 MHz; $CDCl_3$) 1.84–2.02 (16H, m, 8×OCH_2CH_2), 2.38–2.51 (16H, m, 8×CH_2CN), 3.43–3.95 (32H, m, 8×OCH_2, 8×$ArCH_2Ar$), 7.18–7.72 (56H, m, ArH); δ_C (100 MHz; $CDCl_3$) 14.8 (OCH_2CH_2), 25.8 (CH_2CN), 31.0 ($ArCH_2Ar$), 71.3 (CH_2O), 117.8 (CN), 126.8, 128.9, 129.4, 134.7, 136.4, 141.9, 157.1 ($COCH_2$); *m*/*z* (FAB^+) 1993 (M^+, 15%).

3.7. 5,11,17,23,29,35,41-Heptaphenyl-43,44,45,46,47,48,49-heptakis-(cyanopropoxy)-calix[7]arene 24

The reaction was carried out under anhydrous conditions. To a suspension of **18** (100 mg, 0.08 mmol) in acetonitrile (50 mL) potassium carbonate (345 mg, 2.50 mmol) was added and the reaction mixture stirred for 15 min at 40 °C. 4-Bromobutyronitrile (0.25 mL, 2.50 mmol) was added and the reaction mixture stirred vigorously for 14 d at 80 °C. Water (10 mL) was added, the solution dried

($MgSO_4$) and evaporated in vacuo to afford the crude product as a yellow oil. Distillation under reduced pressure then flash silica chromatography (ethyl acetate/hexane, 2:1) afforded **24** as a yellow oil (24 mg, 18%). ν_{max}/cm^{-1} (film) 2910, 2329, 1076; δ_H (300 MHz; $CDCl_3$) 1.65–1.90 (14H, m, 7×OCH_2CH_2), 2.45 (14H, t, *J* 7.1, 7×CH_2CN), 3.74–4.00 (28H, t, *J* 5.9, 7×OCH_2, 7×$ArCH_2Ar$), 7.08–7.63 (49H, m, ArH); δ_C (75 MHz; $CDCl_3$) 16.4 (OCH_2CH_2), 28.6 (CH_2CN), 31.3 ($ArCH_2Ar$), 71.2 (CH_2O), 119.7 (CN), 127.1, 128.3, 129.2, 134.3, 137.4, 140.7, 154.6 ($COCH_2$); *m*/*z* (FAB^+) 1745 (MH^+, 12%), 1676 ($M^+-(CH_2)_3CN$, 10), 1608 ($M^+-2[(CH_2)_3CN]$, 8), 1540 ($M^+-3[(CH_2)_3CN]$, 11).

3.8. 5,11,17,23,29,35,41,47-Octaphenyl-49,51,53,55-tetrakis-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy)-50,52,54,56-tetrahydroxycalix[8]arene 27

The reaction was carried out under anhydrous conditions. To a suspension of **13** (500 mg, 0.343 mmol) and potassium carbonate (758 mg, 5.49 mmol) in acetonitrile (60 mL) at rt, mono-THP-mono-bromo-hexaethyleneglycol **25** (1.18 g, 2.75 mmol) in acetonitrile (10 mL) was added and the reaction mixture heated at reflux for 4 d. Water was added (30 mL), the crude product extracted into dichloromethane (50 ml), dried (phase separator) and evaporated in vacuo to afford the crude product as a yellow oil. Purification using flash silica chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded **26** as a viscous yellow oil (196 mg, 20%). ν_{max}/cm^{-1} (film) 2868, 1456, 1122; λ_{max}/nm (KOH/MeOH) 230, 255; δ_H (300 MHz; CD_2Cl_2) 1.67–1.98 (24H, m, 4×THP $(CH_2)_3$), 3.55–4.00 (approx. 120H, m, 4×THP–OCH_2, 8×$ArCH_2Ar$, 48×PEG–OCH_2), 4.76 (4H, br s, 4×THP–O*CH*), 7.45–7.77 (56H, m, ArH); δ_C (100 MHz; $CDCl_3$) 18.9 (CH_2), 24.9 (CH_2), 29.8 (CH_2), 30.0 (CH_2), 61.0 (CH_2), 66.1 (CH_2), 70.0–70.8 (signals superimposed), 98.3 ($CHOCH_2$), 126.2 (CH), 126.7 (CH), 127.1 (CH), 127.5 (CH), 128.1, 133.3, 140.2, 140.4, 157.9 ($COCH_2$); *m*/*z* (MALDI-TOF) 2872 (MNa^+, 40%), 2849 (M^+, 30), 2786 ($MNa^+-C_5H_{10}O$, 45), 2680 ($M^+-2[C_5H_{10}O]$, 35). *p*-Phenycalix[8]arene-tetra-hexaethyleneglycolTHP **26** (50.0 mg, 0.02 mmol) was added in portions to a solution of hydrochloric acid (5 mL; 18 M) and dichloromethane (5 mL) at rt. The resulting solution was stirred at rt for 2 h. Removal of solvents afforded a brown oil, which was suspended in a 10% aqueous solution of sodium hydroxide (10 mL) and stirred vigorously for 30 min. The product was extracted into dichloromethane (5 mL), dried ($MgSO_4$) and evaporated to afford **27** as a yellow viscous oil (43 mg, 85%). ν_{max}/cm^{-1} (film) 3240, 2868, 1456, 1122; δ_H (300 MHz; CD_2Cl_2) 3.55–4.00 (approx. 110H, m, 8×$ArCH_2Ar$, 48×PEG–OCH_2), 7.45–7.77 (56H, m, ArH); δ_C (100 MHz; $CDCl_3$) 62.2 (CH_2), 70.0–73.2 (signals superimposed), 125.4, 126.2, 127.7, 127.9, 128.0, 134.2, 140.2, 156.5 ($COCH_2$); *m*/*z* (ES-TOF) found 2514.2686 (MH^+); $C_{152}H_{177}O_{32}$ requires 2514.2223.

3.9. 5,11,17,23,29,35,41,47-Octaphenyl-49,50,51,52,53,54,55,56-octakis-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy)calix[8]arene 29

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (60%; 44 mg, 1.10 mmol) in THF (40 mL), **27** (196 mg, 0.069 mmol) in THF (10 mL) and mono-bromo-mono-THP-hexaethyleneglycol **26** (236 mg, 0.55 mmol) in THF (10 mL) was added and the reaction mixture heated at reflux temperature for 4 d. Water was added (10 mL), the product extracted into dichloromethane (40 mL), dried (phase separator), and evaporated to afford the crude product as a yellow oil. Purification using flash silica chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded **28** as a viscous yellow oil (58 mg, 20%). ν_{max}/cm^{-1} (film) 3240, 2868, 1456, 1122; λ_{max}/nm (KOH/MeOH) 230, 262; δ_H (300 MHz; $CDCl_3$) 1.44–1.66 (48H, m, 8×THP(CH_2)$_3$), 3.41–3.83 (approx. 220H, m, 8×THP–OCH_2, 8×$ArCH_2Ar$, 96×PEG–OCH_2), 4.58 (8H, t, *J* 2.6, 4×THP–O*CH*), 7.24–7.78 (56H, m, ArH); δ_C (100 MHz; $CDCl_3$) 19.2 (CH_2), 25.1 (CH_2), 30.1 (CH_2), 61.9 (CH_2), 66.9 (CH_2), 70.2–70.4 (signals superimposed), 98.6 ($CHOCH_2$), 126.8 (CH), 127.6, 128.5 (CH), 134.2, 136.3, 140.7, 154.3 ($COCH_2$); *m*/*z* (MALDI-TOF) 1001.6 ($[MNa-3[THP]]^{4+}$/4); *m*/*z* (ES^+) found 1791.83 ($MNa-8THP-H)^{2+}$/2; $[C_{210}H_{294}NaO_{56}]$/2 requires 1791.88. To a solution of hydrochloric acid (5 mL; 18 M) and dichloromethane (5 mL), **28** (50 mg, 0.01 mmol) was added and the resulting solution stirred at rt for 2 h. Evaporation in vacuo afforded a brown oil, which was suspended in a 10% aqueous solution of sodium hydroxide (10 mL) and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 mL), dried ($MgSO_4$) and evaporated to afford **29** as a viscous yellow oil (36 mg, 86%). ν_{max}/cm^{-1} (film) 3140, 2908, 1454, 1118; δ_H (300 MHz; $CDCl_3$) 3.52–4.10 (approx. 210H, m, 8×$ArCH_2Ar$, 96×PEG–OCH_2), 7.45–7.77 (56H, m, ArH).

3.10. 5,11,17,23,29,35,41-Heptaphenyl-43,44,45,46,47,48,49-heptakis-(9-methoxy-3,6-dioxadecyloxy)-calix[7]arene 31

The reaction was carried out under anhydrous conditions. To a solution of *p*-phenylcalix[7]arene (500 mg, 0.39 mmol) in acetonitrile (100 mL) at rt, potassium carbonate (879 mg, 6.33 mmol) was added and the reaction mixture stirred at 40 °C for 1 h. 1-(2-Bromoethoxy)-2(2-methoxyethoxy)ethane **30** (709 mg, 3.12 mmol) in acetonitrile (10 mL) was added dropwise and the reaction mixture was stirred at 80 °C for 4 d. Water (10 mL) was added and the crude product extracted into dichloromethane (30 mL) then dried ($MgSO_4$) and evaporated in vacuo to afford the crude product as a brown oil. Flash silica chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded the product, which was stirred in brine (20 mL) overnight. Extraction into dichloromethane (10 mL), drying (hydrophobic frit) and evaporation in vacuo afforded the *title compound* as an orange oil (281 mg, 31%). ν_{max}/cm^{-1} (film) 2870, 2322, 1438, 1119; λ_{max}/nm (KOH/MeOH) 230, 260; δ_H (300 MHz; $CDCl_3$) 3.38 (21H, s, Me), 3.48–3.93 (approx. 100H, m, 42×PEG–OCH_2, 7×$ArCH_2Ar$), 7.25–7.60 (49H, ArH); δ_C (100 MHz; $CDCl_3$) 30.1 (CH_2), 58.8 (OCH_3), 70.3–71.7 (signals superimposed), 126.6 (CH), 128.4 (signals superimposed), 133.6, 136.6, 140.3, 154.5 ($COCH_2$); *m*/*z* (ES^+) 2320 (MNa^+, 30%), 1172 ($[MNa_2]^{2+}$/2, 60).

3.11. 5,11,17,23,29,35,41-Heptaphenyl-43,44,45,46,47,48,49-heptakis-(17-hydroxy-3,6,9,12,15-pentaoxaheptadecyloxy) calix[7]arene 33

The reaction was carried out under anhydrous conditions. To a suspension of *p*-phenylcalix[7]arene (127 mg, 0.10 mmol) in acetonitrile (40 mL) at rt, potassium carbonate (224 mg, 1.62 mmol) was added and the reaction mixture stirred at 40 °C for 15 min. Compound **25** (342 mg, 0.80 mmol) in acetonitrile (10 mL) was added dropwise and the resulting solution stirred at 80 °C for 4 d. Water was added (10 mL) and the product extracted into chloroform (2×30 mL), dried ($MgSO_4$) and evaporated in vacuo to afford crude **32** as a yellow oil. Purification via flash silica chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) gave **32** as a viscous yellow oil (181 mg, 49%). ν_{max}/cm^{-1} (film) 2869, 1456, 1123; λ_{max}/nm (KOH/MeOH) 232, 258; δ_H (400 MHz; acetone-d_6) 1.64–1.70 (42H, m, 7×THP(CH_2)$_3$), 3.62–3.93 (approx. 200H, m, 96×PEG–OCH_2, 7×$ArCH_2Ar$), 4.78 (7H, t, *J* 3.3, 7×THP–O*CH*), 7.47–7.89 (49H, m, ArH); δ_C (100 MHz; $CDCl_3$) 19.4 (CH_2), 25.4 (CH_2), 30.1 (CH_2), 30.6 (CH_2), 62.1 (OCH_2), 66.7 (OCH_2), 70.0–72.0 (signals superimposed), 98.9 ($CHOCH_2$), 126.3, 126.7, 127.6, 128.5, 134.5, 137.6, 141.0, 155.2 ($COCH_2$); *m*/*z* (TOF) found 3734.9 (MNa^+); $C_{210}H_{294}NaO_{56}$ requires 3735.0.

To a solution of hydrochloric acid (18 M; 5 mL) and dichloromethane (5 mL), **32** (60 mg, 0.02 mmol) was added and the resulting solution stirred at rt for 2 h. Evaporation in vacuo afforded a brown oil, which was suspended in an aqueous solution of sodium hydroxide (10%; 10 mL) and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 mL), dried ($MgSO_4$) and evaporated in vacuo to afford **33** as a viscous yellow oil (48 mg, 96%). ν_{max}/cm^{-1} (film) 3120, 2848, 1447, 1117; λ_{max}/nm (KOH/MeOH) 230, 260; δ_H (300 MHz; $CDCl_3$) 3.43–3.99 (182H, m, 96×PEG–OC*H*$_2$, 7×ArC*H*$_2$Ar), 6.97–7.89 (49H, m, ArH); δ_C (100 MHz; $CDCl_3$) 30.1 (CH_2), 61.5 (OCH_2), 69.5–72.4 (signals superimposed), 126.5, 126.7, 127.9, 128.5, 133.6, 137.0, 140.5, 155.1 (*C*OCH$_2$); *m*/*z* (ES^+) 3126 (MNa^+, 30%).

3.12. 5,11,17,23,29,35,41-Heptaphenyl-43,44,45,46,47,48,49-heptakis-(35-hydroxy-3,6,9,12,15,18,21,24,27,30,33-undecaoxapentatriacontyloxy) calix[7]arene 36

The reaction was carried out under anhydrous conditions. To a suspension of *p*-phenylcalix[7]arene (200 mg, 0.16 mmol) and potassium carbonate (347 mg, 2.51 mmol) in acetonitrile (50 mL) at rt, **32** (929 mg, 1.26 mmol) in acetonitrile (10 mL) was added dropwise. The resulting solution was stirred at 80 °C for 7 d. Water (20 mL) was added and the crude product extracted into dichloromethane (40 mL). Drying (phase separator) and evaporation in vacuo afforded the crude product as a brown oil. Flash chromatography (ethyl acetate/hexane, 2:1, then ethyl acetate/methanol, 10:1) afforded the THP intermediate **35** as a viscous oil (190 mg, 22%). ν_{max}/cm^{-1} (film) 2873, 1442, 1182; δ_H (400 MHz; $CDCl_3$) 1.40–1.60 (42H, m, 7×THP(C*H*$_2$)$_3$), 3.14–4.69 (approx. 400H, m, 192×PEG–OC*H*$_2$, 7×ArC*H*$_2$Ar), 5.01 (7×THP–OC*H*), 7.15–7.91 (49H, m, ArH); δ_C (100 MHz; $CDCl_3$) 19.5 (CH_2), 25.4 (CH_2), 30.7 (CH_2), 32.2 (CH_2), 62.2 (OCH_2), 67.7 (OCH_2), 69.6–71.9 (signals superimposed), 98.7 (CH) 124.8, 126.5, 126.6, 128.3, 134.0, 136.4, 140.4, 154.8(*C*OCH$_2$); *m*/*z* (MALDI-TOF) 1133.6 ($[MNa_5]^{5+}$/5, 30%). To a solution of hydrochloric acid (18 M; 5 mL) and dichloromethane (5 mL), **35** (50 mg, 0.01 mmol) was added and the solution stirred at rt for 2 h. Evaporation in vacuo afforded a brown oil, which was suspended in an aqueous solution of sodium hydroxide (10%; 10 mL), and the solution was stirred vigorously for 30 min. The product was extracted into dichloromethane (10 mL), dried ($MgSO_4$) and evaporated in vacuo afforded **36** as a brown viscous oil (23 mg, 50%). ν_{max}/cm^{-1} (film) 2885, 1437, 1165; δ_H (400 MHz; $CDCl_3$) 3.21–5.22 (74H, m, 192×PEG–OC*H*$_2$, 7×ArC*H*$_2$Ar), 7.10–7.94 (49H, m, AH).

3.13. 49,50,51,52,53,54,55,56-Octadodecanecalix[8]arene 38

The reaction was carried out under anhydrous conditions. To a suspension of sodium hydride (60%; 425 mg, 10.6 mmol) in THF (40 mL), calix[8]arene (300 mg, 0.353 mmol) and bromododecane (1.36 mL, 5.68 mmol) were added. The reaction mixture was stirred at 70 °C for 3 d. Water was added (40 mL), and the precipitate collected by filtration to afford **38** as a white powder (530 mg, 68%). Mp 248–265 °C (THF/water); ν_{max}/cm^{-1} (KBr) 2849, 1462, 1376; δ_H (300 MHz; pyridine-d_6) 0.85 (24H, t, *J* 5.9, 8×CH_3), 1.19–1.31 (144H, m, 72×CH_2), 1.69–1.78 (16H, m, 8×CH_2), 3.39 (16H, t, *J* 6.8, 8×CH_2), 4.17 (16H, s, ArCH_2Ar), 7.19 (24H, apparent s, ArH); δ_C (100 MHz; pyridine-d_6) 14.2 (CH_3), 20.7 (CH_2), 29.2–30.9 (signals superimposed), 33.3 (OCH_2CH_2), 70.7 (OCH_2), 118.9, 128.7, 129.6, 155.0 (*C*OCH$_2$); *m*/*z* (FAB^-) 2192 (M–H, 1%), 848 (M–8[$(CH_2)_{11}$Me], 12).

3.14. 5,11,17,23,29,35,41,47-Octasulfonyl-49,50,51,52,53,54,55,56-octadodecanecalix[8]arene 39

Compound **38** (450 mg, 0.205 mmol) was suspended in concentrated sulfuric acid (10 mL; 18 M), and heated at 80 °C for 4 h. Water (30 mL) was added and the solution was neutralised by the addition of solid potassium carbonate. Following filtration in vacuo, sodium carbonate was added to the filtrate, and evaporation in vacuo afforded a pale yellow solid, which was dissolved in water (30 mL), and the remaining precipitate removed by filtration in vacuo. Following evaporation the crude solid was recrystallised from water and methanol to afford **39** as a white solid (430 mg, 76%). Mp >300 °C (THF/water); ν_{max}/cm^{-1} (KBr) 3400, 2858, 1454, 1376, 1051; δ_C (100 MHz; D_2O) 14.2 (CH_3), 20.8 (CH_2), 29.2–31.0 (signals superimposed), 33.3 (OCH_2CH_2), 72.7 (OCH_2), 126.5, 128.4, 139.0, 156.2 (*C*OCH$_2$); *m*/*z* (ES) 1439 ($[M+2Na]^{2+}$/2, 18%).

3.15. In vivo experiments

Experiments were performed as previously described.[17] Further details are provided in Supplementary data. Mice were infected with 2×10^5 mycobacterial cells (intravenously) and the level of infection determined by assessment of infection in the lungs and spleens.[17] In experiments with the calixarenes, macrocyclon and controls, CFUs were measured 24–35 d after infection. The doses of calixarenes in 200 μL of endotoxin-free saline were injected into each mouse intraperitoneally, in two doses, one 48–72 h before infection and one 48–72 h after infection.[17] Calixarenes were administered in doses of 2–3 mg/200 μL.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.11.034.

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