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FULL PAPER

Synthesis and Evaluation of a Series of Bis(pentylpyridinium) Compounds as Antifungal Agents

Daniel Obando,[#, [a]] Yasuko Koda,[#, [a], [b]] Namfon Pantarat,[b] Sophie Lev,[b] Xiaoming Zuo,[b] Johanes Bijosono Oei,[b] Fred Widmer,[b] Julianne T. Djordjevic,[b] Tania C. Sorrell[b] and Katrina A. Jolliffe*[a]

[a] Dr Daniel Obando, Dr Yasuko Koda, Professor K. A. Jolliffe (ORCID Katrina A. Jolliffe 0000-0003-1100-4544)
School of Chemistry
The University of Sydney
2006, NSW, Australia
E-mail: kate.jolliffe@sydney.edu.au

[b] Dr Yasuko Koda, Ms Namfon Pantarat, Dr Sophie Lev, Dr Xiaoming Zuo, Mr Johanes Bijosons Oei, Dr Fred Widmer, Associate Professor Julianne T. Djordjevic, Professor Tania C. Sorrell
Centre for Infectious Diseases and Microbiology, Westmead Millennium Institute and the Centre for Clinical Research Excellence in Infections, Bioethics and Haematological Malignancies
The University of Sydney
Westmead, 2145, NSW, Australia

# These authors contributed equally

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Abstract: A series of bis(4-pentylpyridinium) compounds with a variety of spacers between the pyridinium headgroups was synthesised and the antifungal activity of these compounds has been investigated. Lengthening the alkyl spacer between the pentylpyridinium headgroups from twelve to sixteen methylene units resulted in increased antifungal activity against *C. neoformans* and *C. albicans*, but also resulted in increased hemolytic activity and cytotoxicity against mammalian cells. However, inclusion of an *ortho*-substituted benzene ring in the centre of the alkyl spacer resulted in reduced cytotoxicity and hemolytic activity, while maintaining antifungal potency. Replacement of the alkyl and aromatic-containing spacers by more hydrophilic ethylene glycol groups resulted in a loss of antifungal activity. Some of the compounds inhibited fungal PLB1 activity, but the low correlation of this inhibition with antifungal potency indicates PLB1 inhibition is unlikely to be the predominant mode of antifungal action of this class of compounds, with preliminary studies suggesting they may act via disruption of fungal mitochondrial function.

Introduction

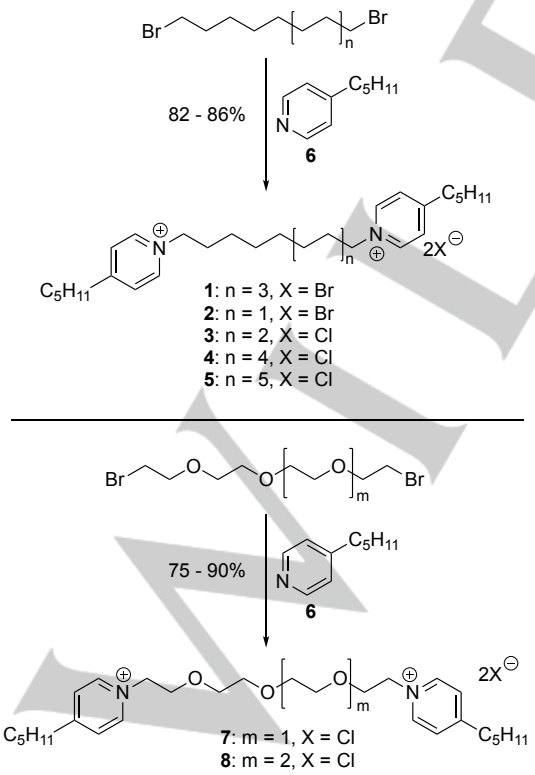
The incidence of life-threatening invasive fungal infections (mycoses) has increased significantly over the past two decades.[1] These are associated with high morbidity and mortality and a significant global economic burden. While *Candida* and *Aspergillus* infections are historically the major causes of mycoses, *Cryptococcus* is the commonest cause of fungal meningitis, affecting in particular, HIV-infected patients in developing countries; globally, cryptococcal meningitis is responsible for 15% of AIDS-related deaths.[2] The number of currently available drugs for treatment of invasive fungal infections is limited and limitations of marketed drugs include an incomplete spectrum of activity against pathogenic fungi, toxicities, poor bioavailability, poor solubility or stability, and/or high cost. In addition, resistance has emerged to commonly used treatments (*e.g.* fluconazole)[3] and there are currently no effective drugs against emerging pathogens such as some *Fusarium* species and *Lomentospora* prolificans.[4] It is widely recognised that new antifungal agents with different modes of action to those of currently available drugs are required. Given the significant burden of infection in the developing world, it is imperative that the search for such drugs should include compounds with characteristics appropriate for use in resource-poor, as well as resource-rich countries. These include ease of manufacture, potent antifungal activity, an excellent safety profile and low cost. Fungal virulence factors such as phospholipase B (PLB) are potential targets for the development of new antifungal drugs. PLB is a proven virulence determinant of the pathogenic fungi, *Candida albicans* and *Cryptococcus neoformans*,[5-9] and is also secreted by other pathogenic fungi including *Aspergillus spp.*[10] Cryptococcal PLB has been well characterised[11] and a number of biscationic compounds with antifungal activity have been found to inhibit PLB activity to some extent. For example, a series of bisquaternary ammonium salts with a spacer twelve methylene units in length between the positively charged head groups exhibit antifungal activity with MICs of 1 to 2.5 μg/mL against *C. neoformans* and *C. albicans* and for this series of compounds, antifungal activity correlated with inhibition of cryptococcal PLB1 activity.[12] In contrast, a series of bis(aminopyridinium)alkanes was found to exhibit good antifungal activity, but these compounds did not inhibit cryptoccocal PLB. However, this second class of compounds was significantly less toxic towards human erythrocytes than the bis(quaternary ammonium) class.[13] Combining the structural features of these two classes of compounds led to the investigation of a series of bis(alkylpyridinium)alkanes in which positively charged alkylpyridinium headgroups were separated by a 12 carbon alkane spacer. These compounds exhibited useful antifungal activities against a spectrum of common and more resistant emerging fungal pathogens and more favourable cytotoxicity profiles than the bis(quaternary ammonium) series.[14,15] While the antifungal activity of this class of compounds may be partially attributable to the inhibition of cryptococcal PLB, this is unlikely to be their major mode of action.

Previous studies of the antifungal activities of these biscationic compounds have focused on changes to the structure of the biscationic headgroups, rather than the linker between them. We

now report the antifungal activity of a series of bis(4-pentylpyridinium) compounds in which the two cationic headgroups are held constant while the linker between these is modified. Modification of the linker in such compounds provides a wide scope to allow optimisation of the cytotoxicity and pharmacokinetic properties of these bispyridinium compounds. We also report our preliminary investigations into the mode of action of this class of antifungal compound.

Results and Discussion

Chemistry. In previous studies, we found that 1,12-bis(pentylpyridinium)dodecane **1** had broad spectrum antifungal activity with low haemolytic activity and low cytotoxicity against mammalian cells.[14] Therefore, a series of compounds were designed based on the structure of **1**, in which the pentylpyridinium headgroups were held constant while the linker between the two headgroups was varied. Compounds **1-5**, in which the alkyl chain length was varied from eight to sixteen methylene units, were readily prepared upon reaction of the appropriate α,ω-dibromoalkane with 2.2 equivalents of 4-pentylpyridine **6** (Scheme 1). While **2** and **3** were solids and could be purified by recrystallisation as the dibromide salts, compounds **1**, **4** and **5** were purified by column chromatography, followed by ion exchange to give the dichloride salts. We have previously established that changing the counterion from bromide to chloride has no effect on the biological activity of these cationic compounds.[12] To probe the effect of the hydrophobicity of the spacer between the headgroups, compounds **7** and **8**, in which the alkyl chain was replaced by ethylene glycol, were prepared in a similar manner from 1,11-dibromo(tetraethyleneglycol)[16] and 1,14-dibromo(pentaethyleneglycol),[17] respectively.



Scheme 1. Synthesis of the bis(pentylpyridinium) compounds **1 - 5** and **7 - 8**.

In order to investigate the effect of the incorporation of rigidity into the linker between the headgroups, compounds **9-13** were prepared, in which either one or two multiple bonds were included in the spacer (Figure 1). Compounds **14-29** (Figure 2) were also prepared in which aromatic groups were incorporated into the spacer units. Two series of these compounds were prepared; one in which alkyl groups were appended directly to the aromatic spacer, together with a second series of analogues in which the inclusion of two alkynes conjugated to the aromatic ring provided spacers with a greater degree of rigidity.

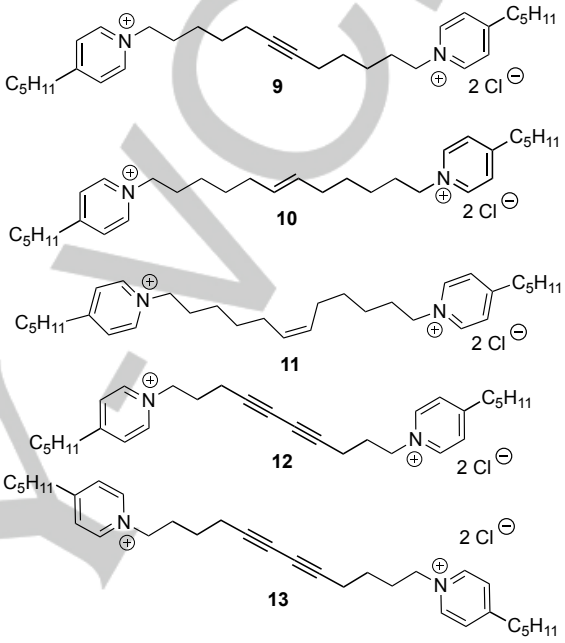


Figure 1. Structures of compounds **9-13**.

The linkers required for synthesis of **9-11** containing either one alkene of defined stereochemistry [(*E*)- or (*Z*)-] or an alkyne between the central carbon atoms of the spacer unit were prepared from a common precursor. Hept-3-yn-1-ol was treated with sodium hydride and diaminopropane in an alkyne 'zipper' reaction[18] to give the terminal alkyne **30** (Scheme 2). Following protection of the alcohol with a tetrahydropyranyl (THP) ether, the alkyne **31**[19] was treated with *n*-butyllithium and the resulting alkynoate was reacted with the known bromide **32**[20] to provide the symmetrically substituted alkyne **33**. Removal of the THP groups upon treatment with *p*-toluenesulfonic acid gave the diol **34**. Stereoselective reduction of the alkyne to provide the (*E*)-alkene **35** was achieved upon treatment of **34** with lithium metal in ammonia.[21] Unfortunately, hydrogenation of **34** in the presence of the Lindlar catalyst was not stereoselective, giving a 1.6:1 mixture of the (*Z*)- and (*E*)-alkenes (**36**). This mixture was used in the subsequent formation of the bis(pentylpyridinium) salt.

To convert alcohols **34-36** to the bis(pentylpyridinium) compounds **9-11**, the alcohols were treated with *p*-toluenesulfonylchloride to provide the intermediate ditosylates **37-39**, which were then reacted with 4-pentylpyridine in a similar manner to that described for the dibromides above. Ion exchange provided **9-11** as their dichloride salts. Compounds **12** and **13**, which incorporate diyne linkers and therefore have increased

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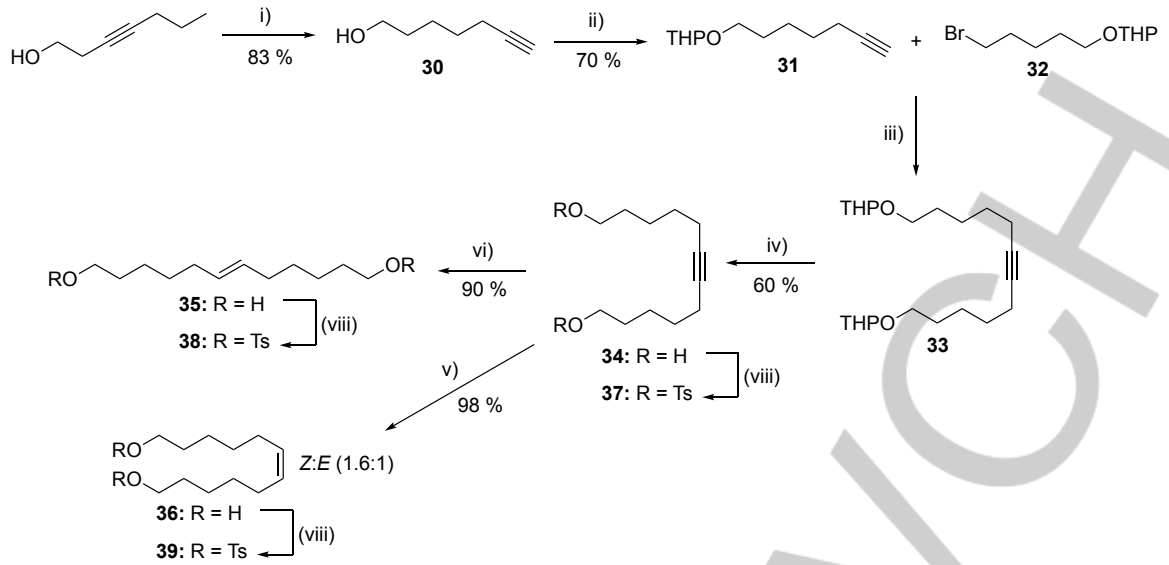


Scheme 2. Synthesis of linkers **37-39**.

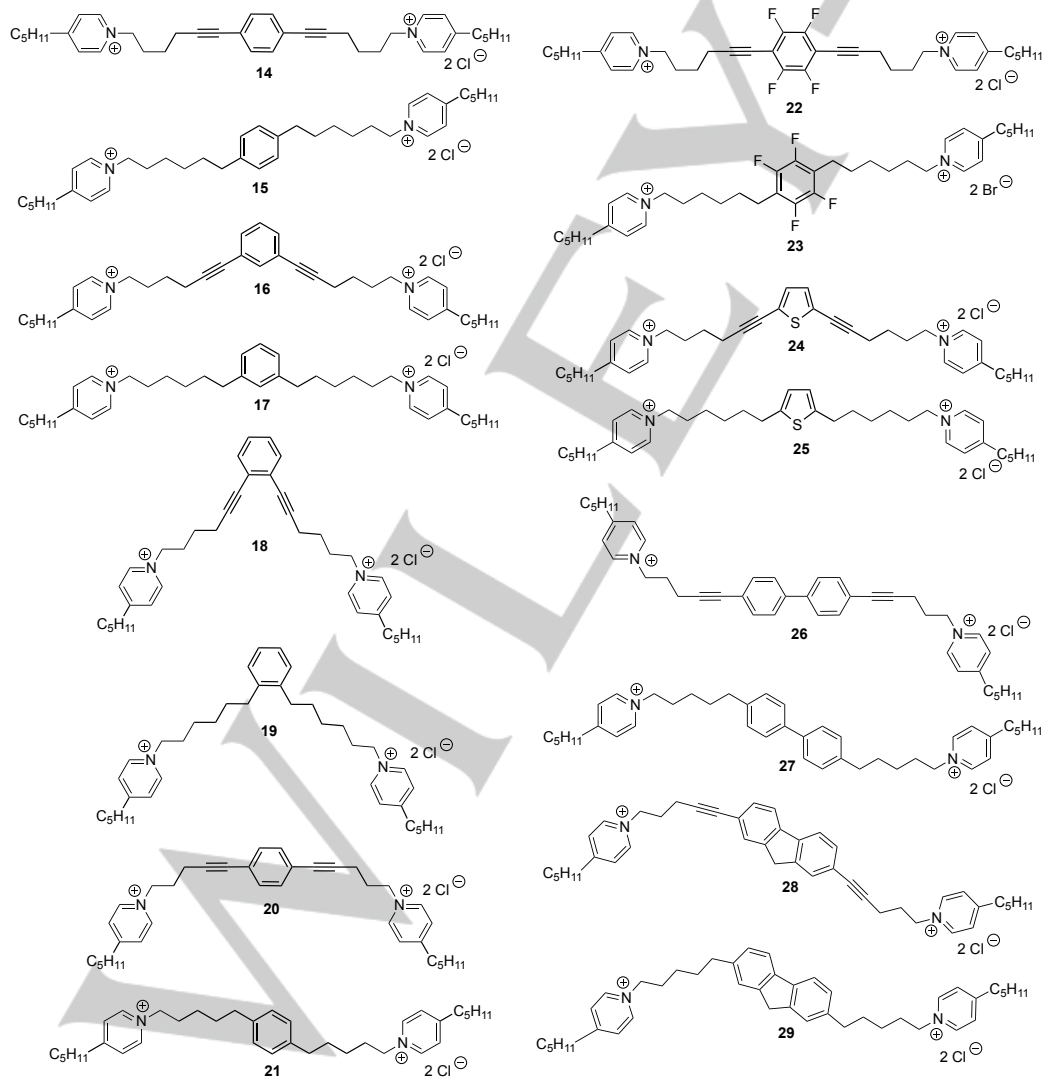
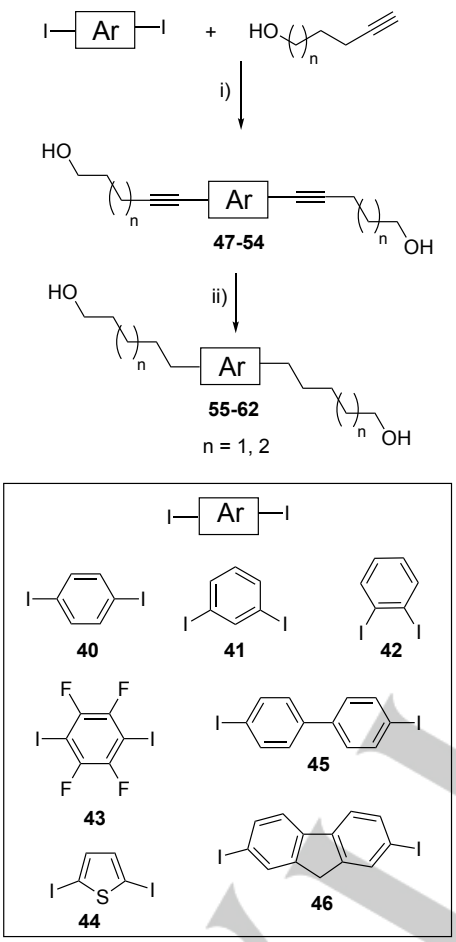


Figure 2. Structures of compounds **14-29**

rigidity and a more linear shape than **9-11** were prepared in a similar manner from the known precursors 1,10-dihydroxy-dec-4,6-diyne,[22] and 1,12-dihydroxy-dodec-5,7-diyne,[23] respectively. The synthesis of the required linkers for compounds **14-29** (Figure 2) was achieved from appropriately substituted diiodo-aromatic precursors. A range of aromatic cores **40-46** was used to provide linkers with different geometries and substitution patterns. Treatment of **40-46** with either 5-hexyn-1-ol or 4-pentyn-1-ol in double Sonogashira couplings[24,25] allowed the preparation of linkers **47-54** in which the rigid aromatic region of the linker was extended by conjugation to alkynes (Scheme 3). Subsequent hydrogenation of the triple bonds provided efficient access to more flexible analogues **55-62**. In all cases, the resulting diols were converted into either the dibromides or ditosylates and further reacted with 2.2 equivalents of 4-pentylpyridine as described above to yield the bis(pentylpyridinium) salts **14-29**.



Scheme 3. Synthesis of linkers **47-62**.

Antifungal Activity. Compounds **1-5** and **7-29** were assayed by a standardised serial dilution microtitre sensitivity test for their antifungal activities against reference strains of *Cryptococcus neoformans, Candida albicans* and *Aspergillus fumigatus* (Table 1).[26,27] All compounds were fully soluble in the assay media at twice the highest concentrations tested. While none of the compounds, with the exception of **19**, exhibited significant antifungal activity against the mold, *A. fumigatus*, strong activity against both yeasts, *C. albicans* and *C. neoformans*, was observed for many of these compounds. For the series **1-5**, in which the length between the headgroups was increased (from eight to sixteen methylene units), while the spacer was maintained as an alkyl chain, antifungal activity increased against both *C. albicans* and *C. neoformans* with increased spacer length. This trend was clearest for *C. neoformans* with the MIC decreasing significantly (44 μM for **2** to 0.7 μM for **5**). However, increasing the hydrophilicity of the spacer by replacing the alkyl chains with polyethylene glycol derivatives (compounds **7** and **8**) resulted in a loss of antifungal activity. Rigidifying the spacer between the headgroups through the incorporation of bis-alkynes, while maintaining the same overall chain length of 10-12 carbon atoms (**12** vs **3** (10 carbon spacer) and **13** vs **1** (12 carbon spacer), respectively) also resulted in a significant loss of activity although the same trend for higher activity for the compound containing the 12 carbon spacer over that with the 10 carbon spacer was observed. Comparison of the antifungal activity for compounds **1**, **9**, **10** and **11**, in which the spacer length was maintained at 12 carbons, but the bond between the central two carbon atoms was varied between alkane, alkene (both *E* and *Z* geometries) and alkyne, suggests that a small amount of rigidity at this position is tolerated with activity of both alkyne-containing **9** and *Z*-alkene containing **11** being within two dilution steps of that of **1**. However, **10** with the *E*-alkene spacer has the lowest antifungal activity of this group of compounds indicating that, despite the flexibility of these chains, the geometry of the alkene is important and suggesting that placing a kink in the spacer (as for *Z*-alkene containing **11**) might lead to increased antifungal activity. We therefore synthesised the series of compounds **14-29** to further explore these tentative structure-activity relationships.

We first prepared a series of compounds (**20**, **21**, **14** and **15**) with spacer lengths approximately equivalent to those of the alkyl chain spacers of **4** and **5** (fourteen and sixteen methylene units, respectively), but with differing rigidity, through the incorporation of a *para*-substituted benzene ring in the centre of the spacer, flanked by alkynes in the case of **20** and **14**. In comparison to **4**, both **20** and **21** exhibit slightly decreased antifungal activity against both *C. albicans* and *C. neoformans*, while the slightly longer chain compounds **14** and **15** have similar activity to **5** against *C. neoformans*, but slightly reduced activity against *C. albicans*. In this compound series, the additional rigidity imparted by the flanking alkynes did not substantially alter the observed antifungal activity. As was observed for the alkyl chain series, increasing the length of the spacer (**14** and **15** vs **20** and **21**) resulted in an increase in antifungal activity.

Altering the substitution pattern of the central benzene ring of the spacer from *para*- (**14**, **15**) to *meta*- (**16**, **17**) and *ortho*- (**18**, **19**) while maintaining the overall spacer length and rigidity did not have a significant effect on activity against *C. neoformans* and *C. albicans*. However, a notable increase in activity against *A. fumigatus* was observed for the *ortho*-substituted compounds, with **19** the only compound tested to show strong activity against this mold. Replacing the *para*-substituted benzene spacer of **14** and **15** with either a *para*-substituted tetrafluorobenzene (**22**, **23**) or 2,5-thiophene spacer (**24**, **25**) did not result in significant changes in antifungal activity, suggesting that the polarity of this aromatic group is not important for activity.

Compounds **26-29** contain larger and more rigid aromatic groups (biphenyl and fluorene) in the centre of the spacers with shorter alkyl substituents, such that the overall length of these spacers is

comparable to that of **5**, **14** and **15**. These compounds have slightly reduced antifungal activity, particularly in the case of **26** and **28** in which the flanking alkynes provide an extended rigid core, suggesting that while linear rigidity in the spacers is tolerated to some extent, some flexibility is required.

Table 1. Influence of linker structure on *in vitro* antifungal potency, reported as MIC (μM).

Compound	X⁻	*C. neoformans* (ATCC 90112)	*C. albicans* (ATCC 10231)	*A. fumigatus* (ATCC 204305)
2	Br	44	2.7	nd
3	Br	11	2.7	nd
1[a]	Cl	1.4	2.7	88
4	Cl	1.4	0.7	nd
5	Cl	0.7	0.7	nd
7	Cl	88	>175	nd
8	Cl	88	>175	nd
9	Cl	2.7	5.5-11	175
10	Cl	22	44	>350
11	Cl	5.5	11	>350
12	Cl	100	100	>100
13	Cl	25	50	>100
14	Cl	1.5	6.2	100
15	Cl	0.8	6.2	50
16	Cl	1.5	6.2	50
17	Cl	0.8	6.2	50
18	Cl	1.3	5.5-11	44
19	Cl	1.3	2.7	11
20	Cl	6.2	12.5	100
21	Cl	6.2	12.5	50
22	Cl	1.3	1.3	44
23	Br	1.3	1.3	88
24	Cl	0.7-1.3	1.3-2.7	88
25	Cl	0.7-1.3	1.3	88
26	Cl	22	22	>350
27	Cl	11	11	>350
28	Cl	22	22	>350
29	Cl	5.5	2.7	88

[a] The activity of this compound has been reported previously.[14]

Haemolytic assays. Since several of the compounds exhibited strong antifungal activity against both *C. albicans* and *C. neoformans*, we next evaluated a selected subset of compounds for their haemolytic activity (Table 2), since this compound class bears some structural resemblance to bolaform surfactants. Compounds **2- 4**, **9**, **10**, **18-21** and **29** did not lyse erythrocytes at any of the concentrations tested (up to 350 μM). However, some haemolysis was observed for compounds where the alkyl chain length between the head groups was extended to 16 carbons (compound **5**) and for compounds **15-17**, **22**, **24**, **25** and **27** bearing aromatic groups in the linker. Haemolytic activity varied with the substitution pattern on the benzene spacer with the *para*- and *meta*-substituted derivatives **15** and **1**7 exhibiting higher haemolytic activity than the *ortho*- (**19**) substituted compound and a similar trend observed for the *meta*- and *ortho*-substituted derivatives, **16** and **18**, with extended alkyne cores. Shortening the length of the spacer resulted in lower haemolytic activity (HC_{50} = 109 μM for **15**, vs >350 μM for **21**). A comparison of compounds **14**, **22** and **24** in which the *para*-substituted benzene core of **14** was replaced by tetrafluorophenyl and thiophene cores, respectively shows that the modified aromatics provide a slight improvement in haemolytic activity. Overall, the incorporation of aromatic rings into the spacers results in slightly increased haemolytic activity over that observed for compounds with alkyl spacers of comparable chain length, with the exception of the *ortho*-substituted derivatives.

Table 2 Influence of linker structure on haemolytic activity and mammalian cytotoxicity.

Compound	X⁻	HC_{50} (μM)[b] Erythrocytes	CC_{50} (μM)[c] A549	CC_{50} (μM)[c] MDCK
2	Br	>350	nd	nd
3	Br	>350	nd	nd
1[a]	Cl	>350	110±7	153±8
4	Cl	>350	52±12	41±10
5	Cl	250	9.6±0.9	15±5
9	Cl	>350	>350	>350
10	Cl	>350	nd	nd
12	Cl	nd	>350	>350
13	Cl	nd	>350	>350
15	Cl	109	45±7	68±6
16	Cl	95	nd	nd
17	Cl	175	36±8	60
18	Cl	>350	70±8	120±9
19	Cl	>350	>350	>350
20	Cl	>350	116±13	140±10
21	Cl	>350	74±12	165
22	Cl	182	43±12	60±8
24	Cl	233	80±10	105±8
25	Cl	194	62±6	113±9
27	Cl	90	nd	nd
29	Cl	>350	nd	nd

[a] The activity of this compound has been reported previously.[14] [b] Assays were performed in triplicate and in all cases standard errors were <5%. [c] Data are presented as the means ± SD of 3 independent experiments.

Cytotoxicity. We subsequently evaluated the broader cytotoxicity of this subset of compounds against two mammalian cell lines [human lung epithelial cells (A549) and Madin-Darby Canine Kidney cells (MDCK)] using an MTS assay (Table 2).[28] In general, mammalian cell cytotoxicity of the compounds correlated with their haemolytic activity, suggesting that the cytotoxicity may be a result of cell lysis. Increasing the length of the spacer between the two pyridinium head groups resulted in a significant increase in cytotoxicity (CC_{50} against A548 cells = 115 μM for **1** compared with 10 μM for **5**) and this trend was maintained when a *para*-substituted phenyl group was incorporated in the spacer. Incorporation of a *para*- or *meta*- substituted phenyl spacer (**15** and **17**, respectively) or a 2,5-substituted thiophene (**25**) within the linker led to a slight decrease in cytotoxicity in comparison to a compound containing an alkyl chain of approximately the same length (**5**), whereas the inclusion of an *ortho*-substituted phenyl ring resulted in a significant decrease in cytotoxicity (**19**; CC_{50} >350 mM for both A549 and MDCK cell lines). The majority of strongly antifungal compounds exhibited cytotoxicity at levels well above their MIC values against fungi, with therapeutic indices (calculated as CC_{50} (MDCK) / MIC_{50} against *C. neoformans*) in the range 14 - >107.

Phospholipase inhibition. Given that bis-cationic compounds, such as **1**, had previously been found to inhibit cryptococcal PLB1, we evaluated the inhibitory activity of selected compounds against both cryptoccocal PLB1 and a representative mammalian phospholipase, porcine pancreatic PLA_2 ($ppPLA_2$) (Table 3) to evaluate whether this might be their mode of antifungal action. Crude secretions, in which the phospholipase activity is attributable to PLB1,[9] were collected from concentrated cryptococcal cell suspensions following an overnight incubation. Inhibition assays against its three activities (PLB: Phospholipase B; LPL: lysophospholipase and LPTA: lysophospholipase acyltransferase) were then carried out at pH 4, under previously optimised conditions.[29] Inhibition of $ppPLA_2$ was determined using the method of De Haas *et. al.*[30] Compounds were initially tested at concentrations of 250, 25, 2.5 and 0.25 μM against both $ppPLA_2$ and cryptococcal PLB1, and compounds which showed inhibition were re-assayed over an appropriate concentration range to determine their IC_{50}s.

None of the compounds exhibited significant inhibition of the LPL or LPTA activities of PLB1 at any of the concentrations tested, however some compounds showed significant inhibition of the PLB activity of this enzyme. Increasing the length of the alkyl spacer from 12 to 14 methylene units resulted in an approximately 2.5–fold increase in inhibition of PLB activity. In general, the incorporation of an aromatic group into the spacer resulted in increased inhibition of PLB, in comparison to that exhibited by **1**. However, in contrast to the low inhibition of $ppPLA_2$ activity observed for **1** and related compounds with a 12 carbon spacer,[14] significant inhibition of $ppPLA_2$ was also observed for the majority of compounds containing an aromatic group in the spacer. The exception to this was compound **24**, containing a thiophene group in the spacer, which exhibited significant inhibition of PLB (IC_{50} = 6 μM), but did not inhibit $ppPLA_2$ at any of the concentrations tested (IC_{50} >250 μM).

Table 3. Inhibition of fungal PLB and $ppPLA_2$ by bis(alkylpyridinium)alkanes.

Compound	PLB IC_{50} (μM)	$ppPLA_2$ IC_{50} (μM)
1[a]	104	>250
4	39	130
9	13	25
14	27	24
15	7	35
18	18	128
19	12	27
22	17	25
24	6	>250
29	6	16

[a] The activity of this compound has been reported previously.[14]

While most of the compounds evaluated had increased inhibitory activity against fungal PLB1 compared to **1**, there was no strong correlation between their antifungal activity and PLB1 inhibitory activity, suggesting that this may not be the primary mode of antifungal action. This was further supported by evaluation of the antifungal activity of **1** against four strains of *C. neoformans* with differing levels of PLB1 secretion, strain H99 and the three mutant strains created from it: *Δplb1, Δplb1::REC15* and *Δsec14-1*.[9,31] These strains had either normal (H99 and *Δplb1::REC15*), significantly reduced (*Δsec14-1*) or absent (*Δplb1*) PLB1 secretion. No significant differences were observed in the susceptibility of these mutants to **1** (MIC_{50} = 1.4 μM in all cases, Figure S1), confirming that the antifungal activity of these compounds is unlikely to be a result of inhibition of PLB1.

Disruption of mitochondrial function. The bispyridinum compounds **1-5** and **7-29** are lipophilic cations, similar in structure to compounds known to disrupt mitochondrial function by altering mitochondrial membrane potential, such as the mitochondrial poison, dequalinium.[32] It has recently been suggested that disruption of mitochondrial function in fungi might provide a novel target for antifungal drug discovery,[33] and there are several reports of antifungal activity that is correlated with disruption of mitochondrial function.[34] In efforts to determine the mode of antifungal action of the bispyridinium compounds, we performed preliminary investigations of their effect on mitochondrial membrane potential. We selected two compounds for this purpose; **1** which shows strong antifungal activity against both *C. albicans* and *C. neoformans* and **8**, which has reduced lipophilicity and did not exhibit antifungal activity.

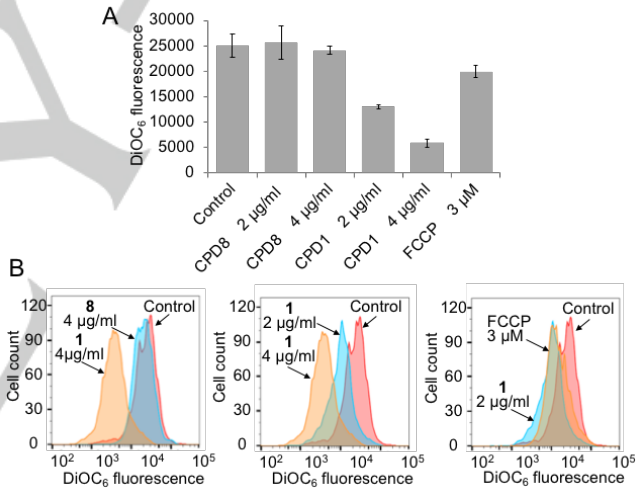


Figure 3. Assessment of mitochondrial membrane potential by flow cytometry using $DiOC_6$. Fungal cells were incubated with 2-4 μg/ml **1** or 2-4 μg/ml **8** or 3 μM FCCP for 3.5 hours at 30°C and stained with $DiOC_6$ (0.3 μM). After 20 min of staining at ambient temperature, the incorporation of the dye was quantified by flow cytometry. (**A**) Average $DiOC_6$ fluorescence. Error bars indicate standard deviation. The assay was performed in triplicate. (**B**) Histograms illustrating accumulation of $DiOC_6$ across fungal cell populations following treatments as indicated.

The effect on these two compounds on mitochondrial function was evaluated in *C. neoformans* by staining with 3,3'-dihexyloxacarbocyanine iodide ($DiOC_6$), a cell-permeable, fluorescent, lipophilic dye which accumulates in highly polarized membranes. At low concentration, $DiOC_6$ accumulation was shown to reflect mitochondrial membrane potential.[35] Fungal cells were treated with the compounds **1** and **8**, and a potent oxidative phosphorylation uncoupler, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) as a control for compromised mitochondrial function.[36] $DiOC_6$ accumulation was assessed using flow cytometry. Figure 3 shows that untreated

cells and cells exposed to **8** accumulate similar amount of $DiOC_6$. However, treatment with compound **1** and FCCP caused reduction in $DiOC_6$ accumulation. Furthermore, compound **1** affects $DiOC_6$ accumulation in a dose-dependent manner. These findings are consistent with a highly specific, detrimental effect of the compound **1** on fungal mitochondrial potential, similar to that of FCCP.

To further assess the impact of **1** and **8** on fungal mitochondrial function, the morphology of treated cells and their $DiOC_6$ staining pattern were assessed by microscopy. Cell nuclei were visualized by staining with DAPI. In control cells and cells treated with **8** (4 μg/ml) for 7 hours, brightly stained punctate mitochondrial structures were observed by $DiOC_6$ staining, indicative of functional mitochondria with strong membrane potential (Figure 4).[37] In contrast, cells treated with **1** (4 μg/ml) for 7 hours were significantly enlarged and the punctate mitochondrial structures were no longer visible, indicative of mitochondrial damage and disruption of mitochondrial membrane potential. Consistent with the flow cytometry data, the intensity of $DiOC_6$ staining in cells treated with **1** was dramatically reduced.

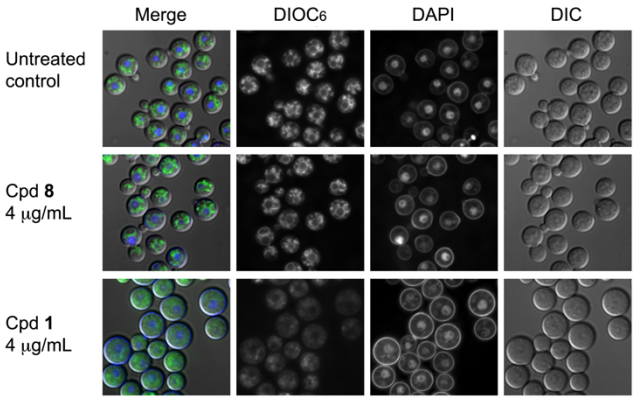


Figure 4. Fluorescence microscope images of *C. neoformans* cells stained with $DiOC_6$ (to visualise mitochondrial membrane potential) and DAPI (to visualise fungal nuclei). Cells were incubated with no drug (untreated), compound **1** (4 μg/mL) or compound **8** (4 μg/mL) for 7 h at 30 ºC prior to imaging. Due to the weak staining of **1**-treated cells with $DiOC_6$, image acquisition time and brightness of the resulting images were adjusted to allow comparison of the $DiOC_6$ staining pattern with the control cells. DIC - differential interference contrast.

Conclusions

The biscationic 1,12-bis(4'-pentylpyridinium)dodecane has previously been reported to exhibit antifungal activity against a range of yeasts (but not molds).[14,15] Using this compound as a starting point, a series of bis(4-pentylpyridinium)s, in which the spacer between the two headgroups was varied, were designed and synthesised to probe the antifungal structure-activity relationship of this class of compounds. The haemolytic activity and mammalian cell cytotoxicity of a selected group of compounds were also evaluated, as was their ability to inhibit fungal PLB and mammalian PLA_2. It was found that lengthening the alkyl spacer between the two headgroups from twelve to sixteen methlyene units resulted in increased antifungal potency against *C. neoformans* and *C. albicans*, but this was accompanied by significant increases in haemolytic activity and cytotoxicity towards mammalian cells, leading to decreased therapeutic indices. Replacement of the alkyl spacer by more hydrophilic ethylene glycol derivatives resulted in a complete loss of antifungal activity. The incorporation of aromatic groups into the alkyl spacer resulted in changes to antifungal activity, haemolytic activity and mammalian cytotoxicity, with larger, more rigid groups resulting in a reduction in antifungal activity, while the incorporation of an *ortho*-substituted benzene group into the spacer gave improved activity against *A. fumigatus*, whilst activity against *C. neoformans* and *C. albicans* was maintained. Both haemolytic activity and mammalian cytotoxicity were reduced in comparison to compounds with alkyl spacers of equivalent length, providing compounds with increased therapeutic indices.

While some of the compounds inhibited the PLB activity of fungal PLB1, there was a poor correlation between antifungal potency and enzyme inhibition, indicating that inhibition of PLB1 is unlikely to be the major mode of antifungal action of these compounds. This was supported by the lack of variation in MIC_{50} when the antifungal activity of compound **1** was evaluated against four strains of *C. neoformans* with either normal (H99 and *Δplb1::REC15*), significantly reduced (*Δsec14-1*) or absent (*Δplb1*) PLB1 secretion levels. However, preliminary studies suggest that these compounds may act via disruption of mitochondrial function, since treatment of *C. neoformans* with compound **1**, which exhibits strong antifungal activity, resulted in mitochondrial damage and loss of mitochondrial membrane potential, as evidenced by staining cells treated with **1** with $DiOC_6$. In contrast, compound **8**, which does not display antifungal activity, did not appear to affect mitochondrial function in *C. neoformans*. This opens up a new avenue for investigation of the antifungal activity of these and related biscationic compounds.

Experimental Section

Chemistry. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance DPX 200 or a Bruker Avance DPX 300 spectrometer. The solvent ^1H and ^{13}C signals, δ_H 7.26 for residual $CHCl_3$ and δ_C 77.0 for $CDCl_3$, δ_H 3.31 and δ_C 49.0 for CD_3OD, δ_H 2.50 and δ_C 39.5 for d_6-DMSO were used as internal references. Low resolution mass spectra were recorded on a Finnigan LCQ ion trap mass spectrometer (ESI and APCI). High resolution mass spectra were recorded on a Bruker 7T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (ESI and APCI). Analytical thin layer chromatography (TLC) was performed using precoated silica gel plates (Merck Kieselgel 60 F254) and visualized using a basic $KMnO_4$ staining reagent. Preparative column chromatography was carried out using Merck Kieselgel 60 silica gel (SiO_2, 0.04 – 0.065 mm) or neutral alumina (activity II – III) with the indicated solvent systems (v/v). Ion exchange chromatography was performed on Lewatit MP-64 resin (chloride form), which was swollen in H_2O, washed with 3 bed volumes of 0.1 M HCl (aq) and then washed with H_2O until the washings were neutral. Analytical RP-HPLC was performed using an XTerraRP C18 column (5 μm, 4.6 mm ID, 250 mm) with a flow rate of 0.2 mL/min. Preparative RP-HPLC was performed using an XTerraRP C18 column (10 μm, 22 mm ID, 300 mm) with a flow rate of 7.0 mL/min.] 1,11-Dibromotetraethyleneglycol,[16] 1,14-dibromopenta(ethylene glycol),[17] 1,10-dihydroxy-dec-4,6-diyne,[22] 1,12-dihydroxy-dodec-5,7-diyne,[23] 1,4-bis(6'-hydroxy-1'-hexynyl)benzene,[25] 1,4-bis(5'-hydroxy-1'-pentynyl)benzene,[25] 1,4-bis(5'-hydroxypentyl)benzene,[25] 1,4-bis(6'-hydroxyhexyl)benzene,[25] 1,4-bis(6'-bromohexyl)benzene,[38] compounds **1**,[14] **6**,[39] **31**,[19] and **32**,[20] were prepared according to literature methods. Detailed synthetic methods for precursors are provided in the supporting information.

General methods

General method for bromination Carbon tetrabromide (1.1 eq per hydroxyl group) was added to a solution of alcohol (1 eq.) in dry CH_2Cl_2 (1 mL / 15 mg substrate) and the resulting mixture was stirred at RT for 10 min. Triphenylphosphine (1.1 eq per hydroxyl) in dry CH_2Cl_2 (1 mL / 50 mg triphenylphosphine) was added to the solution at 0 °C and the mixture was allowed to warm to RT and stirred until complete by TLC (5-9 h) under a nitrogen atmosphere. The solvent was removed under reduced pressure then hexane or hexane/EtOAc (1:1 v/v) was added to the residue. The resulting solution was washed with $NaHCO_3$ (2 x 30 mL) and brine (2 x 30 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was triturated with hexane then filtrates were collected, and the solvent was removed under reduced pressure. The filtrate was collected and the solvent was removed under reduced pressure. The residue was purified by flash chromatography to give the desired dibromide product.

General method for the preparation of bispyridinium salts The dibromide or ditosylate (1 eq) was dissolved in 4-methyl-2-pentanone (1 mL per 50 mg dibromide) and **6** (2.2 eq. per 1 eq. dibromide) was added. The mixture was stirred at reflux for 15 - 18 h under a nitrogen atmosphere then the solvent was removed under reduced pressure and the residue was triturated with Et_2O or EtOAc (8 × 10 mL), then purified by chromatography on neutral Al_2O_3 (activity II-III), (98:2 $CHCl_3$/MeOH → 90:10 $CHCl_3$/MeOH). The required fractions were combined and the solvent removed under reduced pressure. The residue was passed down a column of Lewatit MP-64 anion resin (Cl^-), eluting with EtOH. The required fractions were combined and the solvent removed under reduced pressure.

1,8-Bis(4'-pentylpyridinium)octane dibromide (2) 1,8-Dibromooctane (0.50 g, 1.84 mmol) was dissolved in 4-methyl-2-pentanone (2.0 mL) and **6** (0.68 g, 4.56 mmol) was added. The mixture was stirred at reflux for 18 h under a nitrogen atmosphere, and the solvent was removed under reduced pressure. The crude product was dissolved in methanol (25 mL) and decolourising charcoal was added, then the mixture was boiled for 5 min. The charcoal was removed by filtration and the solvent removed under reduced pressure. The residue was recrystallised from MeOH/Et_2O to yield **2** as a colourless solid (0.87 g, 83 %); m.p. 190 – 191 ºC; ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.00 (4H, d, *J* = 6.5 Hz), 8.03 (4H, d, *J* = 6.5 Hz), 4.54 (4H, t, *J* = 7.6 Hz), 2.87 (4H, t, *J* = 7.5 Hz,), 1.89 (4H, m), 1.66 (4H, m), 1.29 (16H, m), 0.86 (6H, m); ^{13}C NMR (75 MHz, d_6-DMSO): δ 163.4, 144.9, 128.5, 60.7, 35.4, 31.5, 31.4, 29.6, 29.0, 26.2, 22.6, 14.6; *m/z* ESI (positive ion) 205 $[M\text{-}2Br^-]^{2+}$ (100 %), 489 $[M\text{-}^{81}Br^-]^+$ (15), 491 $[M\text{-}^{79}Br^-]^+$ (15). Found $[M\text{-}^{81}Br^-]^+$ 489.2850, $[C_{28}H_{46}N_2Br]^+$ requires 489.2839. Anal. ($C_{28}H_{46}Br_2N_2$) calcd, C 58.95; H 8.1; N 4.9. Found C 58.9; H 8.0; N 4.9.

1,10-Bis(4'-pentylpyridinium)decane dibromide (3) 1,10-Dibromodecane (0.50 g, 1.66 mmol) was dissolved in 4-methyl-2-pentanone (2.0 mL) and **6** (0.69 g, 4.20 mmol) was added. The mixture was stirred at reflux for 18 h under a nitrogen atmosphere, and the solvent was removed under reduced pressure. The crude product was dissolved in methanol (25 mL) and decolourising charcoal was added, then the mixture was boiled for 5 min. The charcoal was removed by filtration and the solvent removed under reduced pressure. The residue was recrystallised from MeOH/Et_2O to yield **3** as a colourless solid (0.82 g, 82 %); m.p. 173 – 174 ºC; ^{1}H NMR (300 MHz, d_6-DMSO): δ 9.01 (4H, d, *J* = 6.5 Hz), 8.03 (4H, d, *J* = 6.5 Hz), 4.55 (4H, t, *J* = 7.5 Hz), 2.87 (4H, t, *J* = 7.5 Hz), 1.89 (4H, m), 1.65 (4H, m), 1.29 (20H, m), 0.86 (6H, m); ^{13}C NMR (75 MHz, d_6-DMSO): δ 163.4, 144.8, 128.5, 60.7, 35.4, 31.5, 31.4, 29.6, 29.5, 29.2, 26.3, 22.6, 14.6; *m/z* ESI (positive ion) 219 $[M\text{-}2Br^-]^{2+}$ (100 %), 437 $[M\text{-}2Br^-\text{-}H^+]^+$ (40), 517 $[M\text{-}^{81}Br^-]^+$ (40), 519 $[M\text{-}^{79}Br^-]^+$ (40). Found $[M\text{-}^{81}Br^-]^+$ 517.3159, $[C_{30}H_{50}N_2Br]^+$ requires 517.3152. Anal. ($C_{30}H_{50}Br_2N_2$) calcd, C 60.2; H 8.4; N 4.7. Found C 60.3; H 8.5; N 4.6.

1,14-Bis(4'-pentylpyridinium)tetradecane dichloride (4) 1,14-Dibromotetradecane (0.50 g, 1.40 mmol) was dissolved in 4-methyl-2-pentanone (2.0 mL) and **6** (0.52 g, 3.50 mmol) was added. The mixture was stirred at reflux for 18 h under a nitrogen atmosphere, then the solvent was removed under reduced pressure. The residue was then diluted with H_2O (~ 15 mL) and washed with Et_2O (3 x 20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL), then the combined CH_2Cl_2 phases were concentrated under reduced pressure. The residue was purified by chromatography on neutral Al_2O_3 (activity II-III), (98:2 $CHCl_3$/MeOH → 90:10 $CHCl_3$/MeOH). The required fractions were combined and the solvent removed under reduced pressure. The residue was passed down a column of Lewatit MP-64 anion resin (Cl^-), eluting with EtOH. The resulting fractions were combined and the solvent removed under reduced pressure to give **4** as a pale brown waxy oil (0.67 g, 85 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.94 (4H, d, *J* = 6.5 Hz), 8.02 (4H, d, *J* = 6.5 Hz), 4.65 (4H, t, *J* = 7.5 Hz), 3.02 (4H, t, *J* = 7.5 Hz), 2.07 (4H, m), 1.82 (4H, m), 1.42 (28H, m), 0.98 (6H, m); ^{13}C NMR (75 MHz, CD_3OD): δ 164.2, 144.1, 128.5, 61.2, 35.6, 31.6, 31.5, 29.8, 29.7, 29.6, 29.2, 26.2, 22.5, 13.6, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 247 $[M\text{-}2Cl^-]^{2+}$ (100 %), 494 $[M\text{-}2Cl^-\text{-}H^+]^+$ (80), 529 $[M\text{-}^{37}Cl^-]^+$ (60), 531 $[M\text{-}^{35}Cl^-]^+$ (25). Found $[M\text{-}2Cl^-]^{2+}$ 247.2293, $[C_{34}H_{58}N_2]^{2+}$ requires 247.2294. Anal. ($C_{34}H_{58}Cl_2N_2{\cdot}2.5H_2O$) calcd, C 66.8; H 10.3; N 4.6. Found C 66.8; H 9.8; N 4.6.

1,16-Bis(4'-pentylpyridinium)hexadecane dichloride (5) 1,16-Dibromohexadecane (0.20 g, 0.49 mmol) was dissolved in 4-methyl-2-pentanone (2.0 mL) and **6** (0.18 g, 1.24 mmol) was added. The mixture was stirred at reflux for 18 h under a nitrogen atmosphere, and the solvent was removed under reduced pressure. The residue was then diluted with H_2O (~ 15 mL) and washed with Et_2O (3 x 20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL), then the combined CH_2Cl_2 phases were concentrated under reduced pressure. The residue was purified by chromatography on neutral Al_2O_3 (activity II-III), (98:2 $CHCl_3$/MeOH → 90:10 $CHCl_3$/MeOH). The required fractions were combined and the solvent removed under reduced pressure. The residue was passed down a column of Lewatit MP-64 anion resin (Cl^-), eluting with EtOH. The resulting fractions were combined and the solvent removed under reduced pressure to give **5** as a pale brown waxy oil (0.25 g, 86 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.99 (4H, d, *J* = 6.5 Hz), 8.02 (4H, d, *J* = 6.5 Hz), 4.66 (4H, t, *J* = 7.5 Hz), 2.98 (4H, t, *J* = 7.5 Hz), 2.03 (4H, m), 1.79 (4H, m), 1.41 (32H, m), 0.93 (6H, m); ^{13}C NMR (75 MHz, CD_3OD): δ 164.0, 144.3, 128.5, 61.2, 35.6, 31.6, 31.5, 29.9, 29.8, 29.6, 29.5, 29.2, 26.3, 22.5, 13.5, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 261 $[M\text{-}2Cl^-]^{2+}$ (100 %), 521 $[M\text{-}2Cl^-\text{-}H^+]^+$ (90), 557 $[M\text{-}^{37}Cl^-]^+$ (45), 559 $[M\text{-}^{35}Cl^-]^+$ (20). Found $[M\text{-}2Cl^-]^{2+}$ 261.2454, $[C_{36}H_{62}N_2]^{2+}$ requires 261.2451. Anal. ($C_{36}H_{62}Cl_2N_2{\cdot}2.5H_2O$) calcd, C 67.6; H 10.6; N 4.4. Found C 67.3; H 10.4; N 4.3.

1,11-Bis(4'-pentylpyridinium)tetra(ethyleneglycol) dichloride (7) Treatment of the 1,11-dibromotetraethyleneglycol (0.21 g, 0.65 mmol) and **6** (0.20 g, 1.38 mmol) according to the general method for bispyridinium formation gave **7** as a pale yellow waxy oil (0.31 g, 90 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.96 (4H, d, *J* = 6.5 Hz), 8.04 (4H, d, *J* = 6.5 Hz), 4.87 (4H, t, *J* = 7.0 Hz), 4.04 (4H, t, *J* = 7.4 Hz), 3.63 (4H, m), 3.54 (4H, m), 2.99 (4H, t, *J* = 6.4 Hz), 1.70 (4H, m), 1.40 (8H, m), 0.96 (6H, t, *J* = 6.6 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.4, 144.9, 127.9, 70.5, 69.4, 65.9, 60.8, 35.9, 31.5, 29.8, 22.5, 14.8; *m/z* ESI (positive ion) 229 $[M\text{-}2Cl^-]^{2+}$ (100 %), 457 $[M\text{-}2Cl^-\text{-}H^+]^+$ (55), 493 $[M\text{-}^{35}Cl^-]^+$ (25). Found $[M\text{-}2Cl^-]^{2+}$ 229.1750, $[C_{28}H_{46}N_2O_3]^{2+}$ requires 229.1749. Anal. ($C_{28}H_{46}Cl_2N_2O_3{\cdot}2H_2O$) calcd, C 59.5; H 8.9; N 4.95. Found C 59.45; H 9.0; N 5.0.

1,14-Bis(4'-pentylpyridinium)penta(ethyleneglycol) dichloride (8) Treatment of the 1,14-dibromopenta(ethyleneglycol) (0.23 g, 0.65 mmol) and **6** (0.19 g, 1.32 mmol) according to the general method for bispyridinium formation gave **8** as a pale yellow waxy oil (0.27 g, 75 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.82 (4H, d, *J* = 6.5 Hz), 7.94 (4H, d, *J* = 6.5 Hz), 4.75 (4H, m), 3.95 (4H, m), 3.59- 3.49 (12H, m), 2.93 (4H, m), 1.73 (4H, m), 1.35 (8H, m), 0.89 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 167.1, 147.4, 130.5, 73.2, 73.0, 72.0, 63.5, 38.3, 34.1, 32.2, 25.0, 15.9, 1 signal obscured or overlapping. MS: *m/z* ESI (positive ion) 251 $[M\text{-}2Cl^-]^{2+}$ (100 %), 502 $[M\text{-}2Cl^-\text{-}H^+]^+$ (30). Found $[M\text{-}2Cl^-]^{2+}$ 251.1879,

$[C_{30}H_{50}N_2O_4]^{2+}$ requires 251.1880. Anal. ($C_{30}H_{50}Cl_2N_2O_4 \cdot 2H_2O$) calcd, C 57.4; H 9.0; N 4.5. Found C 57.4; H 9.0; N 4.4.

1,12-Dihydroxydodec-6-yne (34) To a stirred solution of heptynol-THP[19] (0.31 g, 1.55 mmol) in THF (2.5 mL) at 0 °C, under an argon atmosphere, was added *n*-BuLi (1.40 mL, 2.10 mmol, 2.5 M in hexanes). After 15 min, HMPA (2.5 mL) was added to the bright yellow solution, then after a further 15 min, a solution of 1-bromo-6-(pentan-5-oxy)tetrahydropyran[20] (0.47 g, 1.86 mmol) in THF (1.5 mL) was added. The mixture was allowed to stir at RT for 8 h then MeOH (0.5 mL) was added to quench excess base, and the solution was diluted with EtOAc/Hex (20 mL, 1:1) and washed with sat. NH_4Cl (2 x 20 mL) and brine (20 mL). The combined aqueous layers were extracted with EtOAc/Hex (60 mL, 1:1), the combined organic layers were dried ($MgSO_4$) and the solvent was removed under reduced pressure. To the residue were added MeOH (6.0 mL) and *p*-TsOH (0.10 g, 0.57 mmol), and the mixture was stirred at 40 °C for 4 h. The solvent was removed under reduced pressure to give a brown residue that was purified by flash chromatography (Hex/EtOAc 1:1 → 1:3) and the required fractions were concentrated to give the diol **34** as a pale yellow liquid (0.18 g, 59 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.64 (4H, t, *J*= 7.5 Hz), 2.16 (4H, m), 1.62 – 1.50 (12H, m); ^{13}C NMR (50 MHz, $CDCl_3$): δ 80.3, 63.0, 32.4, 28.9, 25.1, 18.8; *m/z* APCI (positive ion) 199 $[M+H]^+$.

(*Z*)-1,12-Dihydroxydodec-6-ene (35) To a solution of **34** (37 mg, 0.19 mmol) in MeOH (2.0 mL) was added Lindlar catalyst (Pd-C/$CaCO_3$, 0.13 g). The suspension was stirred under an atmosphere of H_2 for 30 min and filtered through a Celite® pad. Evaporation of the solvent gave a mixture of the *Z* alkene **35** and the *E* alkene **36** (**35**:**36** ratio = 1.6:1, 38 mg, 100 %) as a colourless oil. Data for *Z* isomer (**35**): ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.38 (2H, m), 3.62 (4H, t, *J*= 7.5 Hz), 2.01 (4H, m), 1.65 – 1.54 (4H, m), 1.37 (8H, m). ^{13}C NMR (75 MHz, $CDCl_3$): δ 130.4, 63.3, 33.0, 29.8, 27.5, 25.9; *m/z* APCI (positive ion) 201 $[M+H]^+$

(*E*)-1,12-Dihydroxy-dodec-6-ene (36) To a solution of Li (20 mg, 2.65 mmol) in liquid NH_3 (12 mL) was added a solution of **34** (35 mg, 0.18 mmol) in THF (3.0 mL) at -78 °C. The solution was stirred for 12 h at -78 °C, then the reaction was quenched by the addition of MeOH (1.0 mL). The mixture was diluted with EtOAc (20 mL), washed with H_2O (20 mL) and brine (20 mL), then the organic phase was then dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 1:1 → 1:2) and the required fractions were concentrated to give the diol **36** (32 mg, 90 %) as a colourless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.38 (2H, t, *J* = 7.4 Hz), 3.63 (4H, t, *J* = 7.5 Hz), 1.99 (4H, m), 1.56 (4H, m), 1.36 (8H, m), (OH not observed). ^{13}C NMR (75 MHz, $CDCl_3$): δ 130.9, 63.5, 33.2, 33.0, 29.8, 25.7; *m/z* APCI (positive ion) 201 $[M+H]^+$.

1,12-Bis(4'-pentylpyridinium)dodec-6-yne dichloride (9) A solution of diol **34** (15 mg, 0.07 mmol) in CH_2Cl_2 (2.5 mL) was cooled to 0 °C then NEt_3 (0.04 mL, 0.30 mmol), DMAP (4 mg, cat.) and *p*-TsCl (43 mg, 0.23 mmol) were added. The resulting solution was stirred under a nitrogen atmosphere at 4 °C for 5 h, then poured onto a mixture of ice (8 g) and HCl (1 M, 20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL), then dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) and the required fractions were concentrated to give tosylate **37** as a pale yellow liquid (23 mg, 60 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (4H, m), 7.34 (4H, m), 4.02 (4H, t, *J* = 6.5 Hz), 2.45 (6H, s), 2.09 (4H, m), 1.65 (4H, m), 1.41 (8H, m). Treatment of the ditosylate **37** (18 mg, 0.03 mmol) and **6** (12 mg, 0.08 mmol) according to the general method for bispyridinium formation gave **9** as a brown oil (16 mg, 88 %). ^{1}H NMR (200 MHz, CD_3OD): δ 8.85 (4H, d, *J* = 6.4 Hz), 7.96 (4H, d, *J* = 6.4 Hz), 4.59 (4H, t, *J* = 7.4 Hz), 2.95 (4H, t, *J* = 7.4 Hz), 2.14 (4H, m), 2.03 (4H, m), 1.74 (4H, m), 1.51 – 1.35 (16H, m), 0.93 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (50 MHz, CD_3OD): δ 164.5, 144.2, 128.4, 79.9, 61.2, 35.7, 31.6, 31.1, 29.7, 28.7, 25.5, 22.6, 18.3, 13.4; *m/z* ESI (positive ion) 231 $[M\text{-}2Cl^-]^{2+}$ (100 %), 463 $[M\text{-}2Cl^-\text{-}H^+]^+$ (55), 497 $[M\text{-}^{37}Cl^-]^+$ (100), 499 $[M\text{-}^{35}Cl^-]^+$ (35). Found $[M\text{-}2Cl^-]^{2+}$ 231.1981, $[C_{32}H_{54}N_2]^{2+}$ requires 231.1981. Anal. ($C_{32}H_{50}Cl_2N_2 \cdot 3H_2O$) calcd, C 65.4; H 9.6; N 4.8. Found C 65.8; H 9.5; N 4.8.

(*E*)-1,12-Bis(4'-pentylpyridinium)dodec-6-ene dichloride (10) A solution of diol **36** (16 mg, 0.08 mmol) in CH_2Cl_2 (2.5 mL) was cooled to 0 °C then NEt_3 (0.04 mL, 0.32 mmol), DMAP (4 mg, cat.) and *p*-TsCl (46 mg, 0.24 mmol) were added. The resulting solution was stirred under a nitrogen atmosphere at 4 °C for 4 h, then poured onto a mixture of ice (8 g) and HCl (1 M, 20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL), then dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) and the required fractions were concentrated to give the tosylate **39** (36 mg, 89 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (4H, m), 7.35 (4H, m), 5.30 (2H, m), 4.01 (4H, t, *J* = 6.5 Hz), 2.44 (6H, s), 1.90 (4H, m), 1.60 (4H, m), 1.29 (8H, m). Treatment of the ditosylate **39** (36 mg, 0.07 mmol) and **6** (23 mg, 0.15 mmol) according to the general method for bispyridinium formation gave **10** as a brown oil (30 mg, 80 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.85 (4H, d, *J* = 6.4 Hz), 7.96 (4H, d, *J* = 6.4 Hz), 5.41 (2H, m), 4.58 (4H, t, *J* = 7.5 Hz), 2.96 (4H, t, *J* = 7.6 Hz), 2.04 (8H, m), 1.75 (4H, m), 1.40 (16H, m), 0.93 (6H, t, *J* = 6.8 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.3, 144.1, 130.4, 128.2, 61.2, 35.5, 32.3, 31.4, 31.2, 29.5, 29.0, 25.6, 22.4, 13.2; *m/z* ESI (positive ion) 232 $[M\text{-}2Cl^-]^{2+}$ (100 %), 463 $[M\text{-}2Cl^-\text{-}H^+]^+$ (40), 499 $[M\text{-}^{37}Cl^-]^+$ (100), 501 $[M\text{-}^{35}Cl^-]^+$ (37). Found $[M\text{-}2Cl^-\text{-}H^+]^+$ 463.4050, $[C_{32}H_{52}N_2]^+$ requires 463.4047. Anal. ($C_{32}H_{52}Cl_2N_2 \cdot 4H_2O$) calcd, C 63.2; H 9.8; N 4.5. Found C 63.0; H 9.3; N 4.3.

(*Z*)-1,12-Bis(4'-pentylpyridinium)dodec-6-ene dichloride (11) A solution of diol **35** (38 mg, 0.19 mmol) in CH_2Cl_2 (4 mL) was cooled to 0 °C then NEt_3 (0.10 mL, 0.76 mmol), DMAP (4 mg, cat.) and *p*-TsCl (109 mg, 0.57 mmol) were added. The resulting solution was stirred under a nitrogen atmosphere at 4 °C for 7 h, then poured onto a mixture of ice (8 g) and HCl (1 M, 20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL), then dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) and the combined fractions were concentrated to give the tosylate **38** (87 mg, 90 %). Data for *Z* isomer: ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (4H, m), 7.33 (4H, m), 5.30 (2H, m), 4.01 (4H, t, *J* = 6.5 Hz), 2.44 (6H, s), 1.89 (4H, m), 1.63 (4H, m), 1.28 (8H, m). Treatment of the ditosylate **38** (0.09 g, 0.19 mmol) and **6** (0.06 g, 0.40 mmol) according to the general method for bispyridinium formation gave **11** as a brown wax as a 1.6:1 mixture of *Z* and *E* isomers as determined by ^{1}H NMR (78 mg, 77 %). Data for *Z* isomer: ^{1}H NMR (300 MHz, CD_3OD): δ 8.86 (4H, d, *J* = 6.4 Hz), 7.96 (4H, d, *J* = 6.4 Hz), 5.35 (2H, t), 4.59 (4H, t, *J* = 7.5 Hz), 2.96 (4H, t, *J* = 7.6 Hz), 2.04 (8H, m), 1.77 (4H, m), 1.40 (16H, m), 0.93 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.3, 144.1, 130.4, 129.7, 61.2, 35.5, 32.3, 31.3, 29.5, 29.2, 26.9, 25.8, 22.4, 13.2; *m/z* ESI (positive ion) 232 $[M\text{-}2Cl^-]^{2+}$ (100 %), 463 $[M\text{-}2Cl^-\text{-}H^+]^+$ (30), 499 $[M\text{-}^{37}Cl^-]^+$ (80), 501 $[M\text{-}^{35}Cl^-]^+$ (70). Found $[M\text{-}2Cl^-\text{-}H^+]^+$ 463.4050, $[C_{32}H_{52}N_2]^+$ requires 463.4047. Anal. ($C_{32}H_{52}Cl_2N_2 \cdot 2.5H_2O$) calcd, C 66.1; H 9.7; N 4.8. Found C 66.0; H 9.5; N 4.8.

1,10-Bis(4'-pentylpyridinium)dec-4,6-diyne dichloride (12) Treatment of dec-4,6-diyne-1,10-diol (0.10 g, 0.58 mmol) according to the general method for bromination gave **63** as a pale colourless solid (0.16 g, 94 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.51 (4H, t, *J* = 6.5 Hz), 2.46 (4H, t, *J* = 6.6 Hz), 2.05 (4H, m). Treatment of the dibromide **63** (0.16 g, 0.55 mmol) and 4-pentylpyridine **6** (0.17 g, 1.20 mmol) according to the general method for bispyridinium formation gave **12** as a dark yellow oil (0.11 g, 48 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.90 (4H, d, *J* = 6.8 Hz), 8.00 (4H, d, *J* = 6.3 Hz), 4.72 (4H, t, *J* = 7.8 Hz), 2.98 (4H, t, *J* = 6.5 Hz), 2.48 (4H, t, *J* = 6.5 Hz), 2.25 (4H, m), 1.76 (4H, m), 1.40 (8H, m), 0.93 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 163.1, 142.9, 126.1, 74.0, 64.8, 58.7, 34.2, 30.0, 28.0, 20.9, 14.4, 11.6, 1 signal obscured or overlapping; *m/z* ESI

(positive ion) 215 $[M\text{-}2Cl^-]^{2+}$ (40 %), 430 $[M\text{-}2Cl^-\text{-}H^+]^+$ (100), 465 $[M\text{-}^{37}Cl^-]^+$ (85), 467 $[M\text{-}^{35}Cl^-]^+$ (15). Found $[M\text{-}2Cl^-]^{2+}$ 215.1670, $[C_{30}H_{42}N_2]^{2+}$ requires 215.1668. Anal. ($C_{30}H_{42}Cl_2N_2{\cdot}3.5H_2O$) calcd, C 63.8; H 8.7; N 5.0. Found C 63.6; H 8.4; N 5.0.

1,12-Bis(4'-pentylpyridinium)dodec-5,7-diyne dichloride (13) Treatment of dodec-5,7-diyn-1,12-diol (0.10 g, 0.51 mmol) according to the general method for bromination gave **64** as a pale yellow liquid (0.11 g, 70 %) after flash chromatography (Hex/EtOAc 4:1). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.43 (4H, t, *J* = 6.5 Hz,), 2.32 (4H, t, *J* = 6.7 Hz), 1.99 (4H, m), 1.66 (4H, m); *m/z* APCI (positive ion) 195 $[M+H]^+$. Treatment of the dibromide **64** (61 mg, 0.19 mmol) and **6** (60 mg, 0.40 mmol) according to the general method for bispyridinium formation gave **13** as a brown wax (70 mg, 75 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (4H, d, *J* = 6.0 Hz), 7.73 (4H, d, *J* = 6.0 Hz), 4.89 (4H, t, *J* = 7.6 Hz), 2.71 (4H, t, *J* = 7.3 Hz), 2.23 (4H, m), 2.07 (4H, m), 1.53 (8H, m), 1.18 (8H, m), 0.73 (6H, t, *J* = 6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.1, 145.2, 128.3, 66.5, 60.2, 36.1, 31.4, 31.2, 29.5, 25.0, 22.5, 19.0, 14.1, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 229 $[M\text{-}2Cl^-]^{2+}$ (65 %), 458 $[M\text{-}2Cl^-\text{-}H^+]^+$ (100), 493 $[M\text{-}^{37}Cl^-]^+$ (90), 495 $[M\text{-}^{35}Cl^-]^+$ (25). Found $[M\text{-}2Cl^-]^{2+}$ 229.1826, $[C_{32}H_{46}N_2]^{2+}$ requires 229.1825. Anal. ($C_{32}H_{46}Cl_2N_2{\cdot}5H_2O$) calcd, C 62.0; H 9.1; N 4.5. Found C 61.8; H 8.8; N 4.7.

1,4-Bis[6'-(4''-pentylpyridinium)-hex-1'-ynyl]benzene dichloride (14) Treatment of 1,4-bis(6'-hydroxy-1'-hexynyl)benzene (0.22 g, 0.81 mmol) according to the general method for bromination gave **65** as a light yellow wax (0.28 g, 90 %) after flash chromatography Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.29 (4H, s), 3.47 (4H, t, *J* = 7.0 Hz), 2.46 (4H, t, *J* = 6.5 Hz), 2.02 (4H, m), 1.77 (4H, m). Treatment of the dibromide **65** (0.12 g, 0.30 mmol) and **6** (0.09 g, 0.63 mmol) according to the general method for bispyridinium formation gave **14** as a dark yellow oil (0.11 g, 60 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.89 (4H, d, *J* = 6.4 Hz), 7.94 (4H, d, *J* = 6.4 Hz), 7.26 (4H, s), 4.65 (4H, t, *J* = 7.5 Hz), 2.93 (4H, t, *J* = 7.3 Hz), 2.52 (4H, t, *J* = 7.0 Hz), 2.17 (4H, m), 1.71 (8H, m), 1.36 (8H, m), 0.90 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.9, 142.7, 129.8, 126.8, 122.0, 89.2, 79.6, 59.3, 34.8, 30.0, 29.2, 28.0, 23.9, 21.0, 17.2, 11.9; *m/z* ESI (positive ion) 232 $[M\text{-}2Cl^-\text{-}C_5H_{11}]^{2+}$ (100 %), 267 $[M\text{-}2Cl^-]^{2+}$ (50). Found $[M\text{-}2Cl^-]^{2+}$ 267.1983, $[C_{38}H_{50}N_2]^{2+}$ requires 267.1981. Anal. ($C_{38}H_{50}Cl_2N_2{\cdot}4.5H_2O$) calcd, C 66.45; H 8.6; N 4.1. Found C 66.6; H 8.4; N 4.0.

1,4-Bis[6'-(4''-pentylpyridinium)hexyl]benzene dichloride (15) Treatment of 1,4-bis(6'-bromohexyl)benzene (0.16 g, 0.40 mmol) and **6** (0.12 g, 0.84 mmol) according to the general method for bispyridinium formation gave **15** as a dark brown oil (0.14 g, 55 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.84 (4H, d, *J* = 6.5 Hz), 7.95 (4H, d, *J* = 6.5 Hz), 7.06 (4H, s, CH(2')), 4.57 (4H, t, *J* = 7.6 Hz), 2.95 (4H, t, *J* = 7.3 Hz), 2.56 (4H, t, *J* = 7.0 Hz), 2.00 (4H, m), 1.75 (4H, m), 1.62 (4H, m), 1.41 (16H, m), 0.90 (6H, t, *J* = 6.0 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.7, 142.6, 138.4, 126.8, 126.6, 59.7, 34.0, 33.7, 29.9, 29.8, 28.0, 27.1, 24.5, 20.9, 11.7, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 271 $[M\text{-}2Cl^-]^{2+}$ (100 %), 542 $[M\text{-}2Cl^-\text{-}H^+]^+$ (85), 577 $[M\text{-}^{37}Cl^-]^+$ (50), 579 $[M\text{-}^{35}Cl^-]^+$ (20). Found $[M\text{-}2Cl^-]^{2+}$ 271.2289, $[C_{38}H_{58}N_2]^{2+}$ requires 271.2294. Anal. ($C_{38}H_{58}Cl_2N_2{\cdot}3.5H_2O$) calcd, C 67.4; H 9.6; N 4.1. Found C 67.5; H 9.5; N 4.1.

1,3-Bis[6'-(4''-pentylpyridinium) hex-1'-ynyl]benzene dichloride (16) Treatment of **48** (0.14 g, 0.52 mmol) according to the general method for bromination gave **66** as a pale yellow solid (0.15 g, 72 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42 (1H, s), 7.30 (2H, d, *J* = 6.0 Hz), 7.18 (1H, t, *J* = 7.0 Hz), 3.47 (4H, t, *J* = 7.0 Hz), 2.45 (4H, t, *J* = 7.5 Hz), 2.05 (4H, m), 1.74 (4H, m). Treatment of the dibromide **66** (0.07 g, 0.18 mmol) and **6** (0.06 g, 0.39 mmol) according to the general method for bispyridinium formation gave **16** as a pale brown wax (0.05 g, 50 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.83 (4H, d, *J* = 6.5 Hz), 7.92 (4H, d, *J* = 6.5 Hz), 7.24 (1H, s), 7.22 (3H, m), 4.61 (4H, m), 2.90 (4H, t, *J* = 7.5 Hz), 2.48 (4H, t, *J* = 7.0 Hz), 2.18 (4H, m), 1.70 (8H, m), 1.35 (8H, m), 0.89 (6H, t, *J* = 6.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.9, 142.6, 132.7, 130.0, 127.4, 127.1, 122.8, 88.0, 79.0, 59.3, 34.1, 29.9, 29.1, 28.0, 23.8, 20.1, 17.0, 11.7; *m/z* ESI (positive ion) 267 $[M\text{-}2Cl^-]^{2+}$ (100 %), 534 $[M\text{-}2Cl^-\text{-}H^+]^+$ (50), 569 $[M\text{-}^{37}Cl^-]^+$ (30), 571 $[M\text{-}^{35}Cl^-]^+$ (15). Found $[M\text{-}2Cl^-]^{2+}$ 267.1975, $[C_{38}H_{50}N_2]^{2+}$ requires 267.1981. Anal. ($C_{38}H_{50}Cl_2N_2{\cdot}3H_2O$) calcd, C 71.1; H 8.5; N 4.4. Found C 71.2; H 8.7; N 4.3.

1,3-Bis[6'-(4''-pentylpyridinium)hexyl]benzene dichloride (17) Treatment of **56** (0.08 g, 0.28 mmol) according to the general method for bromination gave **67** as a pale yellow solid (0.11 g, 98 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.97 (4H, d, *J* = 6.0 Hz), 3.38 (4H, t, *J* = 6.6 Hz), 2.57 (4H, t, *J* = 7.8 Hz), 1.80 (4H, m), 1.52 (4H, m), 1.40 (8H, m). Treatment of the dibromide **67** (0.11 g, 0.29 mmol) and **6** (0.09 g, 0.61 mmol) according to the general method for bispyridinium formation gave **17** as a dark yellow oil (0.10 g, 57 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.86 (4H, d, *J* = 6.6 Hz), 7.96 (4H, d, *J* = 6.6 Hz), 7.10 (1H, t, *J* = 7.1 Hz), 6.95 (3H, m), 4.58 (4H, t, *J* = 7.6 Hz), 2.95 (4H, t, *J* = 7.4 Hz), 2.56 (4H, t, *J* = 7.4 Hz), 2.00 (4H, m), 1.75 (4H, m), 1.60 (4H, m), 1.40 (16H, m), 0.93 (6H, t, *J* = 6.5 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.7, 142.6, 141.2, 127.1, 126.8, 122.7, 59.7, 34.2, 34.1, 30.0, 29.8, 28.2, 28.0, 27.2, 24.6, 20.9, 11.8, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 271 $[M\text{-}2Cl^-]^{2+}$ (95 %), 542 $[M\text{-}2Cl^-\text{-}H^+]^+$ (100), 577 $[M\text{-}^{37}Cl^-]^+$ (70), 579 $[M\text{-}^{35}Cl^-]^+$ (25). Found $[M\text{-}2Cl^-]^{2+}$ 271.2289, $[C_{38}H_{58}N_2]^{2+}$ requires 271.2294. Anal. ($C_{38}H_{58}Cl_2N_2{\cdot}4H_2O$) calcd, C 66.5; H 9.7; N 4.1. Found C 66.4; H 9.5; N 4.1.

1,2-Bis[6'-(4''-pentylpyridinium) hex-1'-ynyl]benzene dichloride (18) Treatment of **49** (0.10 g, 0.37 mmol) according to the general method for bromination gave **68** as a pale yellow solid (0.13 g, 90 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36 (2H, m), 7.19 (2H, m), 3.48 (4H, t, *J* = 6.6 Hz), 2.53 (4H, t, *J* = 6.6 Hz), 2.06 (4H, m), 1.78 (4H, m). Treatment of the dibromide **68** (0.13 g, 0.34 mmol) and **6** (0.14 g, 0.35 mmol) according to the general method for bispyridinium formation gave **18** as a dark yellow oil (0.09 g, 45 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.88 (4H, d, *J* = 6.5 Hz), 7.96 (4H, d, *J* = 6.5 Hz), 7.35 (4H, m), 4.67 (4H, t, *J* = 7.4 Hz), 2.96 (4H, t, *J* = 7.6 Hz), 2.58 (4H, m), 2.23 (4H, m), 1.68 (8H, m), 1.38 (8H, m), 0.93 (6H, t, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.9, 142.6, 130.4, 127.4, 126.7, 124.7, 91.0, 78.9, 59.2, 34.0, 29.8, 29.0, 28.0, 23.8, 20.9, 17.1, 11.7; *m/z* ESI (positive ion) 267 $[M\text{-}2Cl^-]^{2+}$ (53 %), 534 $[M\text{-}2Cl^-\text{-}H^+]^+$ (70), 569 $[M\text{-}^{37}Cl^-]^+$ (100), 571 $[M\text{-}^{35}Cl^-]^+$ (15). Found $[M\text{-}2Cl^-]^{2+}$ 267.1982, $[C_{38}H_{50}N_2]^{2+}$ requires 267.1981. Anal. ($C_{38}H_{50}Cl_2N_2{\cdot}3H_2O$) calcd, C 69.2; H 8.7; N 4.25. Found C 69.0; H 8.8; N 4.1.

1,2-Bis[6'-(4''-pentylpyridinium)hexyl]benzene dichloride (19) Treatment of **57** (0.10 g, 0.37 mmol) according to the general method for bromination gave **69** as a pale yellow solid (0.13 g, 90 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.12 (4H, s), 3.41 (4H, t, *J* = 6.8 Hz), 2.60 (4H, t, *J* = 7.3 Hz), 1.85 (4H, m), 1.52 (4H, m), 1.49 (8H, m). Treatment of the dibromide **69** (0.13 g, 0.35 mmol) and **6** (0.13 g, 0.33 mmol) according to the general method for bispyridinium formation gave **19** as a pale brown oil (0.08 g, 43 %). ^{1}H NMR (200 MHz, CD_3OD): δ 8.80 (4H, d, *J* = 6.6 Hz), 7.93 (4H, d, *J* = 6.6 Hz), 7.07 (4H, m), 4.57 (4H, t, *J* = 7.6 Hz), 2.94 (4H, t, *J* = 7.4 Hz), 2.59 (4H, t, *J* = 7.4 Hz), 1.98 (4H, m), 1.63 (8H, m), 1.39 (16H, m), 0.92 (6H, t, *J* = 7.4 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ 165.4, 145.0, 141.1, 130.3, 129.2, 127.0, 62.2, 36.5, 33.4, 32.4, 32.2, 30.5, 30.0, 27.2, 23.4, 14.2, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 271 $[M\text{-}2Cl^-]^{2+}$ (35 %), 542 $[M\text{-}2Cl^-\text{-}H^+]^+$ (100), 577 $[M\text{-}^{37}Cl^-]^+$ (70), 579 $[M\text{-}^{35}Cl^-]^+$ (20). Found $[M\text{-}2Cl^-]^{2+}$ 271.2289, $[C_{19}H_{29}N]^{2+}$ requires 271.2294. Anal. ($C_{38}H_{58}Cl_2N_2{\cdot}2H_2O$) calcd, C 68.3; H 9.7; N 4.2. Found C 67.9; H 9.6; N 4.1.

1,4-Bis[5'-(4''-pentylpyridinium) pent-1'-ynyl]benzene dichloride (20) Treatment of **54** (0.10 g, 0.42 mmol) according to the general method for bromination gave **70** as a pale colourless solid (0.14 g, 90 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.29 (4H, s), 3.56 (4H, t, *J* = 7.0 Hz), 2.60 (4H, t, *J* = 6.5 Hz), 2.12 (4H, m). Treatment of the dibromide **70** (0.14 g, 0.39 mmol) and **6** (0.14 g, 0.31

mmol) according to the general method for bispyridinium formation gave **20** as a dark yellow oil (0.07 g, 40 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.88 (4H, d, *J* = 6.5 Hz), 7.92 (4H, d, *J* = 6.4 Hz), 7.26 (4H, s), 4.74 (4H, t, *J* = 7.5 Hz), 2.90 (4H, t, *J* = 7.4 Hz), 2.63 (4H, t, *J* = 7.5 Hz), 2.37 (4H, m), 1.65 (4H, m), 1.36 (8H, m), 0.92 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.6, 144.4, 131.6, 128.1, 123.0, 89.0, 81.0, 60.6, 35.5, 31.4, 29.6, 29.3, 22.4, 16.1, 13.2; *m/z* ESI (positive ion) 253 $[M\text{-}2Cl^-]^{2+}$ (100 %), 506 $[M\text{-}2Cl^- - H^+]^+$ (60). Found $[M\text{-}2Cl^-]^{2+}$ 253.1820, $[C_{36}H_{46}N_2]^{2+}$ requires 253.1825. Anal. ($C_{36}H_{46}Cl_2N_2 \cdot 5.5H_2O$) calcd, C 63.9; H 8.4; N 4.0. Found C 63.7; H 8.2; N 4.0.

1,4-Bis[5"-(4'-pentylpyridinium)pentyl]benzene dichloride (21) Treatment of **62** (0.11 g, 0.46 mmol) according to the general method for bromination gave **71** as a pale yellow solid (0.16 g, 92 %) after flash chromatography (Hex/EtOAc 16:1 → 16:2.4). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.08 (4H, s), 3.40 (4H, t, *J* = 6.8 Hz), 2.59 (4H, t, *J* = 7.5 Hz), 1.89 (4H, m), 1.60 (4H, m), 1.50 (4H, m). Treatment of the dibromide **71** (0.16 g, 0.42 mmol) and **6** (0.13 g, 0.88 mmol) according to the general method for bispyridinium formation gave **21** as a pale brown wax (0.08 g, 41 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.81 (4H, d, *J* = 6.5 Hz), 7.93 (4H, d, *J* = 6.5 Hz), 7.07 (4H, s), 4.55 (4H, t, *J* = 7.6 Hz), 2.93 (4H, t, *J* = 7.3 Hz), 2.57 (4H, t, *J* = 7.0 Hz), 2.03 (4H, m), 1.68 (8H, m), 1.39 (12H, m), 0.93 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.7, 142.5, 138.2, 126.9, 126.6, 59.6, 34.0, 33.5, 29.9, 29.6, 29.4, 28.0, 24.1, 20.9, 11.7; *m/z* ESI (positive ion) 257 $[M\text{-}2Cl^-]^{2+}$ (95 %), 514 $[M\text{-}2Cl^- - H^+]^+$ (100), 549 $[M\text{-}^{37}Cl^-]^+$ (80), 551 $[M\text{-}^{35}Cl^-]^+$ (25). Found $[M\text{-}2Cl^-]^{2+}$ 257.2133, $[C_{36}H_{54}N_2]^{2+}$ requires 257.2138. Anal. ($C_{36}H_{54}Cl_2N_2 \cdot 4H_2O$) calcd, C 65.7; H 9.4; N 4.3. Found C 65.65; H 9.2; N 4.2.

1,4-Bis[6'-(4"-pentylpyridinium) hex-1'-ynyl]-2,3,5,6-tetrafluorobenzene dichloride (22) Carbon tetrabromide (116 mg, 0.35 mmol) was added to a solution of **50** (46 mg, 0.13 mmol) in dry CH_2Cl_2 (12 mL) and the resulting mixture was stirred at RT for 10 min. Triphenylphosphine (92 mg, 0.35 mmol) in dry CH_2Cl_2 (4 mL) was added to the solution and the mixture was stirred for 24 h at 45 °C under a nitrogen atmosphere. The solvent was removed under reduced pressure then Hex/EtOAc (1:1, 20 mL) was added to the residue. The resulting solution was washed with $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was triturated with hexane then the filtrate was concentrated and removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 16:1 → 4:1) and the required fractions were concentrated to give **72** as a yellow oil (64 mg, 80 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.47 (4H, t, *J* = 7.0 Hz), 2.57 (4H, t, *J* = 6.5 Hz), 2.06 (4H, m), 1.81 (4H, m). Treatment of the dibromide **72** (65 mg, 0.14 mmol) and **6** (45 mg, 0.30 mmol) according to the general method for bispyridinium formation gave **22** as a pale brown wax (66 mg, 75 %). ^{1}H NMR (200 MHz, CD_3OD): δ 8.87 (4H, d, *J* = 6.4 Hz), 7.99 (4H, d, *J* = 6.4 Hz), 4.68 (4H, t, *J* = 7.0 Hz), 2.96 (4H, t, *J* = 7.4 Hz), 2.56 (4H, t, *J* = 7.5 Hz), 2.20 (4H, m), 1.75 (8H, m), 1.40 (8H, m), 0.94 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (50 MHz, CD_3OD): δ 164.7, 144.2, 128.4, 66.5, 60.8, 35.7, 31.5, 30.6, 29.7, 25.0, 22.5, 19.0, 13.3, not all aromatic signals were observed due to C-F coupling; *m/z* ESI (positive ion) 303 $[M\text{-}2Cl^-]^{2+}$ (100 %), 606 $[M\text{-}2Cl^- - H^+]^+$ (40), 641 $[M\text{-}^{37}Cl^-]^+$ (45), 643 $[M\text{-}^{35}Cl^-]^+$ (15). Found $[M\text{-}2Cl^-]^{2+}$ 303.1794, $[C_{38}H_{46}N_2F_4]^{2+}$ requires 303.1793. Anal. ($C_{38}H_{46}Cl_2N_2F_4 \cdot 3H_2O$) calcd, C 62.4; H 7.2; N 3.8. Found C 62.5; H 7.5; N 3.7.

1,4-Bis[6'-(4"-pentylpyridinium)hexyl]-2,3,5,6-tetrafluorobenzene dichloride (23) Carbon tetrabromide (95 mg, 0.28 mmol) was added to a solution of **56** (40 mg, 0.11 mmol) in dry CH_2Cl_2 (5 mL) and the resulting solution was stirred at RT for 10 min. Triphenylphosphine (75 mg, 0.28 mmol) in dry CH_2Cl_2 (2 mL) was added then mixture was stirred for 4 h at 45 °C under a nitrogen atmosphere. The solvent was removed under reduced pressure then Hex/EtOAc (1:1, 20 mL) was added to the residue, washed with $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was triturated with hexane and the filtrate was collected, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (2 x Hex/EtOAc 16:1 → 4:1) and the required fractions were concentrated to give **73** as a pale yellow wax (52 mg, 74 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.40 (4H, t, *J* = 6.8 Hz), 2.69 (4H, t, *J* = 7.4 Hz), 1.87 (4H, m), 1.61 (4H, m), 1.41 (8H, m). Treatment of the dibromide **73** (52 mg, 0.11 mmol) and **6** (34 mg, 0.23 mmol) according to the general method for bispyridinium formation gave **23** as a dark yellow wax (80 mg, 73 %). ^{1}H NMR (200 MHz, CD_3OD): δ 8.90 (4H, d, *J* = 6.6 Hz), 7.98 (4H, d, *J* = 6.6 Hz), 4.62 (4H, t, *J* = 7.4 Hz), 2.97 (4H, t, *J* = 7.5 Hz), 2.70 (4H, t, *J* = 6.6 Hz), 2.02 (4H, m), 1.77 (4H, m), 1.62 (4H, m), 1.41 (16H, m), 0.93 (6H, t, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.2, 144.1, 128.6, 61.1, 35.6, 31.4, 31.3, 29.5, 29.1, 28.6, 25.6, 22.4, 22.3, 13.3, not all aromatic signals were observed due to C-F coupling; *m/z* ESI (positive ion) 307 $[M\text{-}2Cl^-]^{2+}$ (100 %), 613 $[M\text{-}2Cl^- - H^+]^+$ (50), 649 $[M\text{-}^{37}Cl^-]^+$ (95), 651 $[M\text{-}^{35}Cl^-]^+$ (35). Found $[M\text{-}2Cl^-]^{2+}$ 307.2097, $[C_{38}H_{54}N_2F_4]^{2+}$ requires 307.2106. Anal. ($C_{38}H_{54}Cl_2N_2F_4 \cdot 3H_2O$) calcd, C 61.7; H 8.2; N 3.8. Found C 61.6; H 8.1; N 3.7.

2,5-Bis[6'-(4"-pentylpyridinium) hex-1'-ynyl]thiophene dichloride (24) A solution of **51** (0.07 g, 0.28 mmol) in CH_2Cl_2 (8 mL) was cooled to 0 °C then NEt_3 (0.10 mL, 0.72 mmol), DMAP (4 mg, cat.) and *p*-TsCl (0.11 g, 0.61 mmol) were added. The resulting solution was stirred under a nitrogen atmosphere at 4 °C for 7 h, then poured onto a mixture of ice (8 g) and HCl (1 M, 20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL), then dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was further purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) and the required fractions were concentrated to give the tosylate **74** as a brown oil (0.11 g, 70 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.77 (4H, d, *J* = 6.6 Hz), 7.32 (4H, d, *J* = 6.6 Hz), 6.89 (2H, s), 4.08 (4H, t, *J* = 6.0 Hz), 2.43 (6H, s), 2.38 (4H, t, *J* = 6.1 Hz), 1.79 (4H, m), 1.63 (4H, m); ^{13}C NMR (200 MHz, $CDCl_3$): δ 145.4, 133.6, 131.5, 130.5, 128.4, 124.6, 94.3, 74.8, 70.5, 28.6, 24.9, 22.2, 19.6. Treatment of the ditosylate **74** (93 mg, 0.19 mmol) and **6** (56 mg, 0.37 mmol) according to the general method for bispyridinium formation gave **24** as a dark yellow oil (68 mg, 70 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.90 (4H, d, *J* = 6.4 Hz), 7.97 (4H, d, *J* = 6.4 Hz), 6.95 (2H, s), 4.66 (4H, t, *J* = 7.5 Hz), 2.95 (4H, t, *J* = 7.3 Hz), 2.55 (4H, t, *J* = 7.0 Hz), 2.16 (4H, m), 1.71 (8H, m), 1.37 (8H, m), 0.92 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 163.4, 143.7, 130.4, 128.9, 124.3, 94.9, 73.9, 60.7, 35.6, 31.4, 30.4, 29.5, 25.2, 22.4, 18.8, 13.3; *m/z* ESI (positive ion) 270 $[M\text{-}2Cl^-]^{2+}$ (100 %), 540 $[M\text{-}2Cl^- - H^+]^+$ (25), 575 $[M\text{-}^{37}Cl^-]^+$ (45), 577 $[M\text{-}^{35}Cl^-]^+$ (18). Found $[M\text{-}2Cl^-]^{2+}$ 270.1765, $[C_{36}H_{48}N_2S]^{2+}$ requires 270.1764. Anal. ($C_{36}H_{48}Cl_2N_2S \cdot 3.5H_2O$) calcd, C 64.1; H 8.1; N 4.15. Found C 64.4; H 7.9; N 4.2.

2,5-Bis[6'-(4"-pentylpyridinium)hexyl]thiophene dichloride (25) A solution of **59** (0.05 g, 0.20 mmol) in CH_2Cl_2 (7 mL) was cooled to 0 °C then NEt_3 (0.07 mL, 0.53 mmol), DMAP (3 mg, cat.) and *p*-TsCl (0.09 g, 0.45 mmol) were added. The resulting solution was stirred under a nitrogen atmosphere at 4 °C for 10 h, then poured onto a mixture of ice (8 g) and HCl (1 M, 20 mL). The mixture was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL), then dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was further purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) and the required fractions were concentrated to give the tosylate **75** (0.12 g, 56 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (4H, d, *J* = 6.3 Hz), 7.34 (4H, d, *J* = 6.5 Hz), 6.52 (4H, s), 4.02 (4H, t, *J* = 7.2 Hz), 2.69 (4H, t, *J* = 7.5 Hz), 2.44 (6H, s), 1.63 (8H, m), 1.30 (8H, m). Treatment of the ditosylate **75** (90 mg, 0.15 mmol) and **6** (50 mg, 0.33 mmol) according to the general method for bispyridinium formation gave **25** as a pale brown oil (67 mg, 72 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.85 (4H, d, *J* = 6.5 Hz), 7.96 (4H, d, *J* = 6.5 Hz), 6.54 (4H, s), 4.58 (4H, t, *J* = 7.6 Hz), 2.96 (4H, t, *J* = 7.3 Hz), 2.74 (4H, t, *J* = 7.0 Hz), 2.01 (4H, m), 1.78 (4H, m), 1.65 (4H, m), 1.41 (16H, m), 0.93 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 162.7, 142.6, 141.4, 126.7, 123.3, 59.7, 34.1, 30.1, 29.9, 29.8, 28.3, 28.0, 26.9, 24.4, 20.9, 11.8; *m/z* ESI (positive ion) 274 $[M\text{-}2Cl^-]^{2+}$ (50 %), 548 $[M\text{-}2Cl^- - H^+]^+$ (45), 583 $[M\text{-}^{37}Cl^-]^+$ (100), 585 $[M\text{-}^{35}Cl^-]^+$ (40). Found $[M\text{-}2Cl^-]^{2+}$ 274.2078, $[C_{36}H_{56}N_2S]^{2+}$ requires 274.2077. Anal. ($C_{36}H_{56}Cl_2N_2S \cdot 4.5H_2O$) calcd, C 61.6; H 9.3; N 4.0. Found C 61.5; H 9.1; N 4.0.

4,4'-Bis[5''-(4'''-pentylpyridinium) pent-1''-ynyl]biphenyl dichloride (26) Carbon tetrabromide (0.59 g, 1.77 mmol) was added to a solution of **52** (0.22 g, 0.81 mmol) in dry CH_2Cl_2 (8 mL) and the resulting solution was stirred at RT for 10 min. Triphenylphosphine (0.46 g, 1.77 mmol) in dry CH_2Cl_2 (4 mL) was added then the mixture was stirred for 9 h at 50 °C under a nitrogen atmosphere. The solvent was removed under reduced pressure then Hex/EtOAc (1:1, 20 mL) was added to the residue. The resulting solution was washed with $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was triturated with hexane then filtrates were collected, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6), and the required fractions were concentrated to give **76** as a yellow wax (80 mg, 36 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.48 (8H, m), 3.60 (4H, t, *J* = 6.5 Hz), 2.64 (4H, t, *J* = 6.7 Hz), 2.17 (4H, m). Treatment of the dibromide **76** (6 mg, 0.01 mmol) and **6** (5 mg, 0.03 mmol) according to the general method for bispyridinium formation gave **26** as a dark yellow oil (6 mg, 76%). ^{1}H NMR (300 MHz, CD_3OD): δ 8.90 (4H, d, *J* = 6.6 Hz), 7.92 (4H, d, *J* = 6.6 Hz), 7.59 (4H, d, *J* = 6.0 Hz), 7.37 (4H, d, *J* = 6.0 Hz), 4.77 (4H, t, *J* = 7.0 Hz), 2.80 (4H, t, *J* = 7.5 Hz), 2.66 (4H, t, *J* = 6.5 Hz), 2.34 (4H, m), 1.61 (4H, m), 1.31 (8H, m), 0.86 (6H, t, *J* = 6.8 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.6, 144.4, 139.9, 132.2, 128.1, 126.8, 123.0, 88.2, 81.7, 70.7, 35.5, 31.4, 29.5, 29.2, 22.3, 16.2, 13.2; *m/z* ESI (positive ion) 291 $[M\text{-}2Cl^-]^{2+}$ (100 %), 582 $[M\text{-}2Cl^-\text{-}H^+]^+$ (25). Found $[M\text{-}2Cl^-]^{2+}$ 291.1972, $[C_{42}H_{50}N_2]^{2+}$ requires 291.1981. Anal. ($C_{42}H_{50}Cl_2N_2{\cdot}4H_2O$) calcd, C 69.5; H 8.1 N 3.9. Found C 69.2; H 8.3; N 4.1.

4,4'-Bis[5''-(4'''-pentylpyridinium)pentyl]biphenyl dichloride (27) Carbon tetrabromide (0.59 g, 1.77 mmol) was added to a solution of **60** (0.22 g, 0.81 mmol) in dry CH_2Cl_2 (8 mL) and the resulting solution was stirred at RT for 10 min. Triphenylphosphine (0.46 g, 1.77 mmol) in dry CH_2Cl_2 (4 mL) was added and the mixture was stirred for 9 h at 50 °C under a nitrogen atmosphere. The solvent was removed under reduced pressure and Hex/EtOAc (1:1, 20 mL) was added to the residue. The resulting solution was washed with $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was triturated with hexane then filtrates were collected, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6), and the required fractions were concentrated to give **77** (0.10 g, 95 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.52 (4H, m), 7.25 (4H, m), 3.44 (4H, t, *J* = 6.8 Hz), 2.68 (4H, t, *J* = 7.5 Hz), 1.93 (4H, m), 1.61 (8H, m). Treatment of the dibromide **77** (105 mg, 0.23 mmol) and **6** (73 mg, 0.49 mmol) according to the general method for bispyridinium formation gave **27** as a dark yellow oil (95 mg, 80 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.80 (4H, d, *J* = 6.5 Hz), 7.89 (4H, d, *J* = 6.5 Hz), 7.50 (4H, d, *J* = 6.0 Hz), 7.24 (4H, d, *J* = 6.4 Hz), 4.57 (4H, t, *J* = 7.6 Hz), 2.88 (4H, t, *J* = 7.3 Hz), 2.67 (4H, t, *J* = 7.0 Hz), 2.04 (4H, m), 1.75 (8H, m), 1.33 (12H, m), 0.90 (6H, t, *J* = 6.5 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.4, 144.2, 141.5, 138.9, 129.3, 128.3, 126.8, 61.2, 35.7, 35.1, 31.6, 31.1, 30.8, 29.7, 25.5, 22.5, 13.4; *m/z* ESI (positive ion) 295 $[M\text{-}2Cl^-]^{2+}$ (100 %), 589 $[M\text{-}2Cl^-\text{-}H^+]^+$ (25), 625 $[M\text{-}^{37}Cl^-]^+$ (24), 627 $[M\text{-}^{35}Cl^-]^+$ (10). Found $[M\text{-}2Cl^-]^{2+}$ 295.2285, $[C_{42}H_{58}N_2]^{2+}$ requires 295.2294. Anal. ($C_{42}H_{58}Cl_2N_2{\cdot}2.5H_2O$) calcd, C 71.5; H 8.9; N 4.0. Found C 71.8; H 8.9; N 3.95.

2,7-Bis[5'-(4''-pentylpyridium)pent-1'-ynyl]fluorene dichloride (28) Carbon tetrabromide (0.59 g, 1.77 mmol) was added as to a solution of **53** (0.22 g, 0.81 mmol) in dry CH_2Cl_2 (8 mL) and stirred at RT for 10 min. Triphenylphosphine (0.46 g, 1.77 mmol) in dry CH_2Cl_2 (4 mL) was added to the solution and the mixture was stirred for 9 h at 50 °C under a nitrogen atmosphere. The solvent was removed under reduced pressure then Hex/EtOAc (1:1, 20 mL) was added to the residue. The resulting solution was washed with $NaHCO_3$ (2 x 30 mL) and brine (30 mL), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was triturated with hexane then filtrates were collected, and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) the required fractions were concentrated to give **78** (30 mg, 44 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.66 (2H, d, *J* = 6.5 Hz), 7.56 (2H, s), 7.41 (2H, d, *J* = 6.5 Hz), 3.84 (2H, s), 3.61 (4H, t, *J* = 6.5 Hz), 2.64 (4H, t, *J* = 6.7 Hz), 2.17 (4H, m). Treatment of the dibromide **78** (30 mg, 0.06 mmol) and **6** (22 mg, 0.14 mmol) according to the general method for bispyridinium formation gave **28** as a pale brown wax (37 mg, 85 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.95 (4H, d, *J* = 6.0 Hz), 7.91 (4H, d, *J* = 6.5 Hz), 7.74 (2H, d, *J* = 6.5 Hz), 7.50 (2H, s), 7.40 (2H, d, *J* = 6.7 Hz), 4.78 (4H, t, *J* = 7.0 Hz), 2.71 (8H, m), 3.87 (2H, s), 2.37 (4H, m), 1.52 (4H, m), 1.23 (8H, m), 0.81 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ 164.5, 144.4, 143.9, 141.1, 130.7, 128.2, 128.1, 122.2, 121.3, 87.5, 82.5, 60.8, 36.3, 35.5, 31.5, 29.6, 29.0, 22.3, 16.3, 13.2; *m/z* ESI (positive ion) 297 $[M\text{-}2Cl^-]^{2+}$ (100 %), 593 $[M\text{-}2Cl^-\text{-}H^+]^+$ (100). Found $[M\text{-}2Cl^-]^{2+}$ 297.1973, $[C_{43}H_{50}N_2]^{2+}$ requires 297.1981. Anal. ($C_{43}H_{50}Cl_2N_2{\cdot}5H_2O$) calcd, C 68.3; H 7.9; N 3.7. Found C 68.3; H 7.6; N 3.8.

2,7-Bis[5'-(4''-pentylpyridium)pentyl]fluorene dichloride (29) NEt_3 (0.03 mL, 0.23 mmol), DMAP (3 mg, cat.) and *p*-TsCl (0.03 g, 0.17 mmol) were added to a solution of **61** (0.02 g, 0.06 mmol) in CH_2Cl_2/DMF (4 mL, 1:1). The resulting solution was stirred under a nitrogen atmosphere at RT for 24 h. The mixture was diluted with hexane (20 mL) and washed with H_2O (4 x 20 mL) and HCl (1 M, 2 x 20 mL) then the organic phase was died ($MgSO_4$), and the solvent removed under reduced pressure. The residue was purified by flash chromatography (Hex/EtOAc 4:0.3 → 4:0.6) and the required fractions were concentrated to give the tosylate **79** (0.11 g, 70 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (4H, d, *J* = 6.5 Hz), 7.63 (2H, d, *J* = 6.5 Hz), 7.30 (6H, m), 7.15 (2H, d, *J* = 6.5 Hz), 4.02 (4H, t, *J* = 6.5 Hz), 3.82 (2H, s), 2.62 (4H, t, *J* = 6.5 Hz), 2.42 (6H, s), 1.64 (4H, m), 1.37 (8H, m). Treatment of the ditosylate **79** (27 mg, 0.04 mmol) and **6** (14 mg, 0.09 mmol) according to the general method for bispyridinium formation gave **29** as a dark yellow oil (25 mg, 90 %). ^{1}H NMR (300 MHz, CD_3OD): δ 8.75 (4H, d, *J* = 6.4 Hz), 7.86 (4H, d, *J* = 6.4 Hz), 7.65 (2H, d, *J* = 6.0 Hz), 7.33 (2H, s), 7.13 (2H, d, *J* = 6.1 Hz), 4.55 (4H, t, *J* = 7.3 Hz), 3.77 (2H, s), 2.89 (4H, t, *J* = 7.5 Hz), 2.71 (4H, t, *J* = 7.5 Hz), 2.06 (4H, m), 1.75 (8H, m), 1.33 (12H, m), 0.92 (6H, t, *J* = 7.7 Hz); ^{13}C NMR (300 MHz, CD_3OD): δ 164.2, 144.0, 143.7, 140.8, 139.8, 128.0, 127.3, 125.2, 119.4, 60.9, 36.4, 35.4, 31.4, 30.9, 30.7, 29.4, 25.1, 22.3, 13.2, 1 signal obscured or overlapping; *m/z* ESI (positive ion) 301 $[M\text{-}2Cl^-]^{2+}$ (100 %), 602 $[M\text{-}2Cl^-\text{-}H^+]^+$ (18), 637 $[M\text{-}^{37}Cl^-]^+$ (20), 639 $[M\text{-}^{35}Cl^-]^+$ (10). Found $[M\text{-}2Cl^-]^{2+}$ 301.2294, $[C_{43}H_{58}N_2]^{2+}$ requires 301.2285. Anal. ($C_{43}H_{58}Cl_2N_2{\cdot}0.5H_2O$) calcd, C 75.4; H 8.7; N 4.1. Found C 75.2; H 9.2; N 3.9.

Fungal isolates and media. A virulent clinical isolate of *C. neoformans* var. *grubii* (serotype A), H99, was kindly supplied by Dr. Gary Cox (Duke University Medical Center, Durham, NC, USA) and subcultured onto Sabouraud dextrose agar (SDA) at 30°C. H99 secretes high levels of phospholipase B activity attributable to the phospholipase B1 (PLB1) enzyme, which was used for the phospholipase inhibition assays. The *PLB1* deletion mutant (Δ*plb1)* and reconstituted strain (Δ*plb1::REC15)* were created from strain H99 as described in Cox *et al*.[9] The Δ*sec14-1* deletion mutant was also created from strain H99 as described in Chayakulkeeree et al.[31]

Antifungal susceptibility testing. The antifungal activity of the compounds was measured using the standard broth microdilution methods of the US National Committee for Clinical Laboratory Standards (NCCLS) for yeasts[26] and filamentous fungi.[27] All compounds were prepared as stock solutions (generally 700 μM; in some cases lower concentrations were used) in water and diluted in RPMI medium according to NCCLS methodology. Two drug-free controls were included in each test run, one contained medium alone (sterile control) and the other, medium plus the inoculum (growth control). The minimal inhibitory concentration (MIC) was defined as that which produced no visible growth after 48, or 72 hours, of culture at 35 °C.[26,27] All tests were performed in duplicate in at least two independent experiments and the MICs were within ± one dilution step in all cases (values are given as single number where all four replicates were identical and as a range when replicates varied).

Haemolytic activity assay. Human blood was collected in 10 mL Vacutainer tubes containing potassium-EDTA as anticoagulant. The blood

from each Vacutainer was transferred to a 50 mL centrifuge tube and the cells washed three times with 30 mL of calcium- and magnesium-free phosphate-buffered saline (PBS; Gibco). Cells were collected by centrifugation at 2,000 × g for 10 mins in a Beckman TJ-6 centrifuge. The third supernatant was clear and colourless. Cells were stored in PBS (20 mL) for up to two weeks. Haemolysis was assessed by mixing 0.5 mL of the cell suspension with 0.5 ml of test substance from stock solutions of 700, 350, 175, 70 and 7 μM in PBS (final erythrocyte concentration around 0.5×10^9 per mL). The mixtures were incubated with gentle shaking at 37 ºC for 1 h, centrifuged at 2,000 × g for 10 mins, the supernatant diluted 10-fold with PBS, and the optical density measured at 540 nm. The values for 0% and 100% lysis were determined by incubating cells with PBS or 0.1 % (w/v) Triton X-100 (in water), respectively. Assays were carried out in triplicate and the difference between runs was < 5% in all cases. The concentration of test compounds in the assays was 350, 175, 87.5, 35 and 3.5 μM. The haemolytic concentration, HC_{50}, was defined as the concentration at which there was 50% lysis determined by plotting % lysis against concentration of drug.

Cytotoxicity. Cytotoxicity was assessed in a human lung epithelial cell line (A549) and in Madin-Darby canine kidney (MDCK) cells which were maintained in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS). 3 x 10^4 cells were seeded onto each well of a 96 well plate, and incubated in supplemented DMEM at 37º C for 24 h. 50 μL of the supernatant medium was removed and replaced with 50 μL of test compound at various concentrations. All drugs were prepared as stock solutions of 700 μM in DMEM. The mixture was incubated for 3 h at 37º C. Negative and positive controls contained equivalent volumes of DMEM and 0.1% (w/v) Triton X-100 in DMEM, respectively. Cell viability was determined by the MTS tetrazolium method[28] and the dose at which cell viability was reduced by 50% (CC_{50}) is reported (values determined by plotting % viability against concentration of drug (tested at 350, 175, 87.5, 44, 17.5 and 3.5 μM). Assays were carried out in triplicate and mean values ± SD are reported. Data analysis was performed using Graphpad Prism software.[41]

Preparation of supernatants containing secreted phospholipase activities. This was carried out essentially as described previously.[40] Briefly, WT *C. neoformans* strain H99 was grown to confluence on SDA in 16 cm diameter Petri dishes for 72 h at 30 °C in air. Cells scraped from 10-20 dishes were washed sequentially in 50 ml Falcon tubes with isotonic saline and imidazole buffer (10 mM imidazole, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 56 mM D-Glucose, made up in isotonic saline, pH 5.5). The final cell pellet was resuspended in a volume of imidazole buffer equivalent to about 10 % of the cell volume, and incubated for 24 h at 37 °C. The cell-free supernatant was collected by centrifugation and stored at -70 °C.

Radiometric assay for fungal phospholipase activity. The three enzyme activities associated with the multifunctional enzyme, PLB1 and present in the cryptococcal supernatant (PLB: hydrolyses both fatty acyl chains from a phospholipid; LPL: hydrolyses the remaining fatty acyl chain on a lysophospholipid and LPTA: transfers a fatty acid onto a lysophospholipid to reconstitute a phospholipid) were measured as described previously[8,11,40] in a final volume of 125 μL at 37° C. For the determination of secreted PLB activity, carrier dipalmitoyl phosphatidylcholine (DPPC, final concentration 800 μM) and 1,2-di[1-^{14}C] palmitoyl phosphatidylcholine (20,000 dpm) were dried under nitrogen and suspended in 125 mM imidazole acetate buffer pH 4.0 (assay buffer) by sonication using a Branson 450 sonifier. Each reaction contained 1 μg of total protein and the reaction time was 22 min. PLB activity was determined by the rate of decrease of the radiolabelled PC substrate, with appearance of the label in free fatty acid. Secreted LPL and LPTA activities were measured simultaneously in a reaction mixture containing 1-[^{14}C]palmitoyl lyso-PC (25,000 dpm) and carrier lyso-PC (final concentration 200 μM) in assay buffer. Each reaction contained 1 μg of total protein and the reaction time was 15 sec. LPL activity was measured by the rate of loss of 1-[^{14}C]palmitoyl lyso-PC, with appearance of the label in free fatty acid. LPTA activity was estimated from the rate of formation of radiolabelled DPPC. All reactions were terminated by adding 0.5 mL of chloroform:methanol (2:1 v/v). The reaction products were extracted by the method of Bligh and Dyer,[41] separated by TLC using chloroform: methanol:water (65:25:4; v/v/v) as the eluting solvent, detected by iodine staining and quantified by beta counting as previously described.[40]

Testing of bis(alkylpyridinium)s as inhibitors of fungal PLB1. Solutions of bis(alkylpyridinium)s were prepared as stock solutions of 700 μM in assay buffer containing 5 mM EDTA, which was then diluted serially with buffer to give solutions with concentrations of 70, 7 and 0.7 μM. In each assay, 45 μL of the stock or diluted solutions was used, and the final volume of 125 μL was made up of substrate, enzyme and buffer. This gave solutions with compound concentrations of 250, 25, 2.5 and 0.25 □M. The radiometric assay was carried out as above. Inhibition was calculated as the percent of substrates (DPPC or Lyso-PC) remaining in the case of PLB and LPL activities, respectively, or of DPPC produced, in the case of the LPTA activity, measured against inhibitor-free controls, the results of which had been normalised to 100 %. All assays were performed in triplicate; the variation between runs was < 10 % in all cases. Compounds which showed inhibition were then assayed over a narrower concentration range to determine the IC_{50}.[42]

Porcine pancreatic PLA_2 activity. Porcine pancreatic phospholipase A_2 was suspended in 3.2 M ammonium sulfate (2.9 mg protein/mL, Sigma St.Louis MO, USA). One part of well-mixed enzyme suspension was added to 4 parts of buffer [10 mM Tris/HCl, 10 mM $CaCl_2$, pH 8.2].[30] Activity and inhibition by test compounds were then measured by the radiometric method described above, except that 25 μL of enzyme solution was used, and the reaction time was 1 hour. These conditions resulted in ~ 60% substrate conversion in the inhibitor-free control, which was normalised to 100% and inhibition in the presence of compounds calculated against it. All assays were performed in triplicate; the variation between runs was < 10 % in all cases. Compounds which showed inhibition were then assayed over a narrower concentration range to determine the IC_{50}.[42]

Protein assays. Total protein estimations were performed using a Coomassie Blue binding assay with BSA as standard (Pierce Chemical Co., IL, USA).

Assessment of fungal mitochondrial function by $DiOC_6$ staining. *C. neoformans* cells were grown in YPD broth overnight and resuspended in fresh YPD medium at OD_{600}=0.1. Cultures were supplemented with compounds **1** or **8** at 2-4 μg/ml or FCCP at 3 μM. For the flow cytometry, the cultures were incubated for 3.5 hours and the OD of each culture was adjusted to 0.06 using the same (used) medium. The cells were then stained with 0.3 μM $DiOC_6$ for 20 min and analyzed using BD FACSCanto II. The assay was performed in triplicate. For microscopy, similarly treated cultures were incubated for 7 hours and their OD adjusted to 0.5 using the same (used) medium. The cells were then stained with $DiOC_6$ (2.5 μM) and DAPI (1 μg/ml) for 15 min. The cells were visualized using DeltaVision RT deconvolution microscope (Applied Precision, Inc., Issaquah, WA) fitted with a 100 oil/1.40 objective lens (Olympus, Tokyo, Japan).

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Keywords: medicinal chemistry • structure-activity relationships • antifungal agent • bispyridinium • haemolytic activity

References:

[1] a) A. Chakrabati, M. R. Shivaprakash, *J. Postgrad. Med.* **2005***, 51*, 16-20; b) F. Zand, M. Moghaddami, M. A. Davarpanah, M. Masjedi, R. Nikandish, A. Amanati, M. Afarid, L. Nafarieh, M. Nami, *Biosci. Biotech. Res. Comm.* **2016,** *9*, 371-381; c) K. V. Ramana, S. Kandi, V. Bharatkumar, C.H. V. Sharada, R. Rao, R. Mani, S. D. Rao, *Am. J. Infect. Dis. and Microbiol.,* **2013**, *1*, 64-69.

[2] R. Rajasingham, R. M. Smith, B. J. Park, J. N. Jarvis, N. P. Govender, T. M. Chiller, D. W. Denning, A. Loyse, D. R. Boulware, *Lancet Infect. Dis.*, **2017**, *17*, 873-881.

[3] E. L. Berkow, S. R. Lockhart, *Infect. Drug Resist.*, **2017**, *10*, 237-245.

[4] a) M. Nucci, E. Anaissie, *Clin. Microbiol. Rev*., **2007**, *20*, 695-704;b) D. L. Horn, A. G. Freifeld, M. G. Schuster, N. E. Azie, B. Franks, C. A. Kauffman. *Mycoses*, **2014**, *57*, 652–658. c) A. Pellon, A. Ramirez-Garcia I. Buldain, A. Antoran, A. Rementeria, F. L. Hernando, PloSOne, 2017, 12, e0174885.

[5] J. T. Djordjevic, *Frontiers in Microbiology*, **2010**, *1*, 1-13.

[6] F. Mirbod, Y. Banno, M. A. Ghannoum, A. S. Ibrahim, S. Nagashima, Y. Kitajhima, G. T. Gole, Y. Nozawa, *Biochim. Biophys. Acta* **1995**, *1257*, 181-188.

[7] R. A. Calderone, W. A.; Fonzi, *Trends Microbiol.* **2001**, *9*, 327-335.

[8] S. C. A. Chen, M. Muller, J. Z. Zhou, L. C. Wright, T. C.; Sorrell, T. C. *J. Infect. Dis*. **1997**, *175*, 414-420.

[9] G. M. Cox, H. C. McDade, S. C. A. Chen, S. C. Tucker, M. Gottfredsson, L. C. Wright, T. C. Sorrell, S. D. Leidich, A. Casadevall, M. A. Ghannoum, J. R. Perfect, *J. R. Mol. Microbiol.* **2001**, *39*, 166-175.

[10] D. K. Shen A. D. Noodeh A. Kazemi, R. Grillot, G. Robson, J. F. Brugère, *FEMS Microbiol. Lett.,* **2004**, *239*, 87-93.

[11] S. C. Chen, L. C. Wright, J. C., Golding, T. C. Sorrell, *Biochem. J.,* **2000**, *347*, 431–439.

[12] C. K. L. Ng, D. Obando, F. Widmer, L. Wright, T. C. Sorrell and K. A. Jolliffe, *J. Med. Chem.*, **2006***, 49*, 811-816.

[13] C. K. L. Ng, V. Singhal, F. Widmer, L. C. Wright, T. C. Sorrell and K. A. Jolliffe, *Bioorg. Med. Chem*., **2007**, *15*, 3422-3429.

[14] D. Obando, N. Pantarat, R. Handke, Y. Koda, F. Widmer, J. Djordjevic, D. H. Ellis, T. C. Sorrell and K. A. Jolliffe, *Bioorg. Med. Chem.*, **2009**, *17*, 6329-6339.

[15] S. C.-A. Chen, C. Biswas, R. Bartley, F. Widmer, N. Pantarat, D. Obando, J. T. Djordjevic, D. H. Ellis, T. C. Sorrell, K. A. Jolliffe, *Antimicrob. Agents Chemother*., **2010**, *54*, 3233-3240.

[16] S. Gunzenhauser, E. Biala, P. Strazewski, *Tetrahedron Lett.*, **1998**, *39*, 6277-6280.

[17] J. F. W. Keana, G. S. Heo, J. S. Mann, F. L. Van Nice, L. Lex, V. S. Prabhu, G. Ferguson, *J. Org. Chem.*, **1988**, *53*, 2268-2274.

[18] E. C. Hass, P. G. Mezey, S. R. Abrams, *J. Comput. Chem.*, **1982**, *3*, 185-190.

[19] D. Ma, X. Lu, *Tetrahedron*, **1990**, *46*, 6319-6330.

[20] S. W. Baldwin, J. D. Wilson, J. Aube, *J. Org. Chem.*, **1985**, *50*, 4432-4439.

[21] L. Brandsma, W. F. Nieuwenhuizen, J. W. Zwikker, U. Mäeorg. *Eur. J. Org, Chem*., **1999**, *5*, 775-779.

[22] J. M. Montierth, D. R. DeMario, M. J. Kurth, N. E. Schore, *Tetrahedron*, **1998**, *54*, 11741-11748.

[23] A. Lei, M. Srivastava, X. Zhang, *J. Org. Chem.*, **2002**, *67*, 1969-1971.

[24] R. Chinchilla, C. Nájera, *Chem. Soc. Rev*., **2011**, *40*, 5084-5121.

[25] T. Ueda, N. Kanomata, H. Machida, *Org. Lett*., **2005**, *7*, 2365-2368.

[26] Reference method for broth dilution susceptibility testing of yeasts: Approved Standard. NCCLS document M27-A (ISBN 1-56238-186-5). National Committee for Clinical Laboratory Standards, Pennsylvania, USA, 1997.

[27] Reference method for broth dilution susceptibility testing of filamentous fungi: Approved Standard. NCCLS document M38-A (ISBN 1-56238-186-5). National Committee for Clinical Laboratory Standards, Pennsylvania, USA, 2002.

[28] G. Malich, B. Markovic, C. Winder, *Toxicology* **1997**, *124*, 179-192.

[29] R. Ganendren, F. Widmer, V. Singhal, C. Wilson, T. C. Sorrell, L. Wright, *Antimicrob. Agents Chemother.* **2004**, *48*, 1561-1569.

[30] G. H. De Haas, M. G. Van Oort, R. Dijkman, R. Verger, R. *Biochem. Soc. Trans*. **1989**, *17*, 274-276.

[31] M. Chayakulkeeree, S. A. Johnston, J. Bijosono Oei, S. Lev, P. R. Williamson, C. F. Wilson, X. Zuo, A. L. Leal, M. H. Vainstein, W. Meyer, T. C. Sorrell, R. C. May, J. T. Djordjevic, *Mol. Microbiol*. **2011**, *80*, 1088–1101.

[32] F. Wang, M. A. Ogasawara, P. Huang, *Mol. Aspects Med*. **2010**, 31, 75–92.

[33] a) M. Shingu-Vazquez, A. Traven, *Eukaryotic Cell*, **2011**, *10*, 1376–1383; b) R. Calderone, D. Li, A. Traven, *FEMS Yeast Res.*, *2015*, **15**, fov027; doi: 10.1093/femsyr/fov027

[34] a) X. Zuo, J. T. Djordjevic, J. Bijosono Oei, D. Desmarini, S. D. Schibeci, K. A. Jolliffe, T. C. Sorrell, *Mol. Pharmacol*., **2011**, *80*, 476-485; b) J. H. Kim, R. P. Haff, N. C. G. Faria, M. de L. Martins, K. L. Chan, B. C. Campbell. *Molecules*, **2013**, *18*, 8873-8894; c) M. A. Ansari, Z. Fatima, S. Hameed, *Open Microbiol. J*., **2016**, *10*, 12-22; d) J. H. Kim, H.-O. Lee, Y.-J. Cho, J. Kim, J. Chun, J. Choi, Y. Lee, W. H. Jung, *PLOS ONE*, **2014**, 9(2): e89122.

[35] H. Chang, H. Huang, T. Huang, P. Yang, Y. Wang, H. Juan, *Bio-protocol*, **2013**, *3* e430.

[36] P. Stöckl, C. Zankl, E. Hütter, H. Unterluggauer, P. Laun, G. Heeren, E. Bogengruber, D. Herndler-Brandstetter, M. Breitenbach, P. Jansen-Dürr, *Free Radic. Biol. Med*., **2007**, *43*, 947-958.

[37] M. Korchak, A. M. Rich, C. Wilkenfield, L. E. Rutherford, G. Weissmann, *Biochem. Biophys. Res. Comm*., **1982**, *108*, 1495-1501.

[38] M. Kimara, I. Kimura, M. Muroi, K. Tanaka, H. Nojima, T. Uwano, T. Koizumi *Biol. Pharm. Bull.*, **1994**, *17*, 1224-1231.

[39] R. Hulst, I. Muizebelt, P. Oosting, C. Van der Pol, A. Wagenaar, J. Smisterova, E. Bulten, C. Driessen, D. Hoekstra, J. B. F. N. Engberts, *Eur. J. Org. Chem.*, **2004**, *4*, 835-849.

[40] S. C. Chen, L. C. Wright, R. T. Santangelo, M. Muller, V. R. Moran, P. W. Kuchel, T. C. Sorrell, *Infect. Immun*. **1997**, *65*, 405-411.

[41] E. C. Bligh, W. J. Dyer, *Can. J. Biochem. Physiol*. **1959**, *37*, 911-917.

[42] GraphPad Prism version 4.02 for Windows, GraphPad Software, San Diego California, USA, www.graphpad.com

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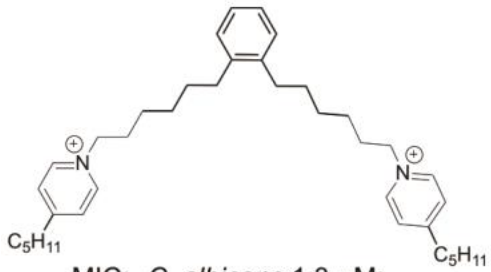
Entry for the Table of Contents



A series of bis(4-pentylpyridinium) compounds with a variety of linkers between the two head groups have been synthesised and their antifungal activity evaluated, together with their haemolytic activity and mammalian cell cytotoxicity. An *ortho*-dihexylbenzene spacer provided good antifungal activity and an improved therapeutic index over the dodecanyl and tetradecanyl derivatives.