Synthesis and Structure–Activity Relationships of Dequalinium Analogues as K^+ Channel Blockers. Investigations on the Role of the Charged Heterocycle

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Small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels occur in many cells but have been relatively little studied. Dequalinium, a bis-quinolinium compound, has recently been shown to be the most potent nonpeptidic blocker of this K^+ channel subtype. This paper examines the importance of the quinolinium rings for blocking activity. Analogues of dequalinium were synthesised in which one quinolinium group was removed (**1** and **2**) or replaced by a triethylammonium group (**3**). They have been assayed in vitro for their ability to block the after-hyperpolarization (mediated by the opening of SK_{Ca} channels) that follows the action potential in rat sympathetic neurones. The compound having one quinolinium and one triethylammonium group (**3**) showed reduced activity, and it is suggested that the stronger binding to the channel of the quinolinium relative to the triethylammonium group may be related to differences in their electrostatic potential energy maps. Two monoquaternary compounds (**1** and **2**) were tested, but they exhibited a different pharmacological profile that did not allow definite conclusions to be drawn concerning their potency as blockers of the SK_{Ca} channel. Replacement of both quinolinium groups by pyridinium, acridinium, isoquinolinium, or benzimidazolium reduced but did not abolish activity. These results show that compounds having a number of different heterocyclic cations are capable of blocking the SK_{Ca} channel. However, among the heterocycles studied, quinoline is optimal. Furthermore, charge delocalization seems to be important: the higher the degree of delocalization the more potent the compound.

Introduction

K^+ channels comprise the most diverse family of ion channels so far described with at least 20 subtypes.[1] The characterization of these subtypes has been based mainly on their electrophysiology and pharmacology. Despite the wealth of information that has emerged in recent years, the physiological role of many of the subtypes as well as their tissue distribution has still to be established. Furthermore, the structure of most K^+ channels (including the one which is the subject of the present work) is still unknown. The bulk of the structural information available concerns voltage-activated K^+ channels.[2] The latter are thought to have a tetrameric structure consisting of four identical subunits. Each subunit contains six (S1–S6) transmembrane domains and a hydrophobic part (H5), located between S5 and S6 in the primary sequence of the protein, which is tucked into the lipid bilayer forming a hairpin structure. It has been suggested that the pore of the channel is formed by four H5 segments, one being contributed from each of the four subunits, arranged in a cylindrical fashion. Recently, the structures of an inward rectifier[3] and an ATP-regulated[4] K^+ channel have been proposed, a gene encoding for high conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels has been cloned, sequenced, and expressed in *Xenopus* oocytes,[5] and a BK_{Ca} channel from smooth muscle has been characterized.[6,7]

The difficulties in studying K^+ channels are due in part to the lack of potent and selective blockers of the various subtypes. The existing K^+ channel blockers can be categorized into inorganic ions (e.g., Ba^{2+}), small organic compounds (e.g., tetraethylammonium, TEA), and peptide toxins. Of the second group, TEA has been used extensively for the characterization of K^+ channels. The mechanism of block of voltage-gated K^+ channels by TEA has been studied in some detail, and it has been suggested that the latter binds at a site formed by the side chains of four aromatic amino acids.[8,9] The binding site is situated inside the pore of the channel, and as a result, TEA physically obstructs the flow of K^+ ions through the pore. The usefulness of TEA is limited however by its lack of selectivity. On the other hand, some natural peptidic toxins that block K^+ channel subtypes[10] have been very helpful, but their use is also associated with problems arising from their limited supply and, in some instances (e.g., charybdotoxin), lack of selectivity. Since K^+ channels are involved in a variety of physiological and pathophysiological processes and since most of them are open to pharmacological exploration,[11,12] the discovery of novel, small, nonpeptidic blockers is of particular interest, as such compounds may lead to the development of novel therapeutically useful agents.

One of the least well-studied class of K^+ channels is that of small conductance Ca^{2+}-activated K^+ (SK_{Ca}) channels.[13,14] Apamin, an 18-amino acid neurotoxin isolated from the venom of the honey bee (*Apis mellifera*), has been shown to block SK_{Ca} channels potently ($IC_{50} \approx 1$ nM) and selectively.[15–17] Some efforts to elucidate its pharmacophore have been undertaken.[18] These channels are present in intestinal smooth muscle

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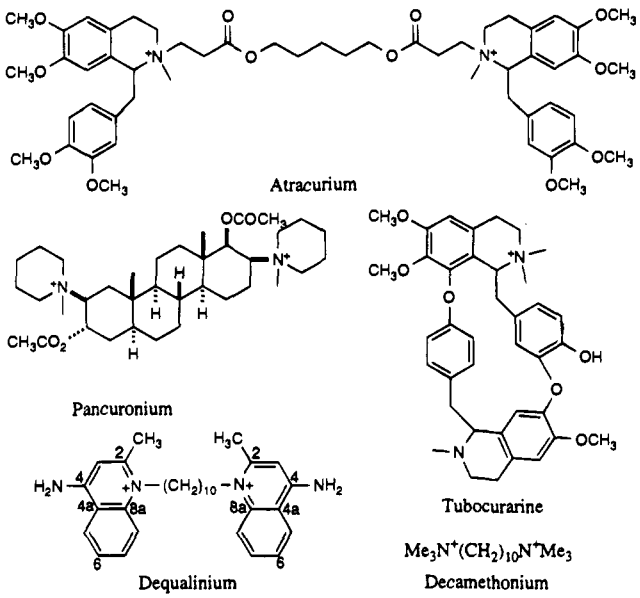
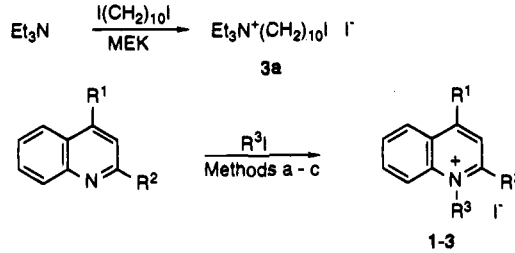


Figure 1. Structures of some SK_{Ca} channel blockers.

where their activation mediates the inhibitory action of α_1-adrenoceptors and the receptors for neurotensin and ATP.[19-21] In many neurones, including those of the sympathetic ganglia,[22,23] opening of SK_{Ca} channels mediates long hyperpolarizations that follow the action potential. That SK_{Ca} channels have a physiological role in the central nervous system is supported by the isolation of an endogenous ligand with apamin-like activity (see below) from pig brain.[24] Furthermore, SK_{Ca} channels have been implicated in myotonic muscular dystrophy, since the binding site for apamin is expressed in muscles of patients with this disease while it is completely absent in normal human muscle.[25] In addition, it has been suggested that a neurotrophic factor is involved in the regulation of the expression of apamin binding sites in skeletal muscle membranes.[26]

Apamin contains two contiguous arginine residues at positions 13 and 14. These positively charged amino acids are believed to be part of the pharmacophore, although alone they cannot account for the potency of apamin.[18] The presence of the bis-charged pharmacophore of apamin prompted tests of a number of bis-quaternary neuromuscular blockers, some of which were found to be effective blockers of the SK_{Ca} channel.[27-29] The three most potent are atracurium, tubocurarine, and pancuronium (Figure 1) having IC_{50}'s of 4.5, 7.5, and 6.8 μM, respectively. Furthermore, dequalinium (Figure 1), another bis-quaternary compound that has been used as an antiseptic,[30] is the most potent non-peptidic blocker of the SK_{Ca} channel so far described.[31,32] As nothing is known about the structure–activity relationships (SAR) of dequalinium, we have initiated studies toward identifying the pharmacophore of dequalinium for SK_{Ca} channel blockade.[33] Two of the structural features of dequalinium merit investigation, and these are the two quinolinium groups and the alkyl chain. The aim of the present work was to examine the role of the two quinolinium groups in an effort to gain some understanding of the interaction between the blocker and the channel, the ultimate objective being the design of more potent and selective nonpeptidic blockers. The investigation of the alkyl chain is currently under way in our laboratory.

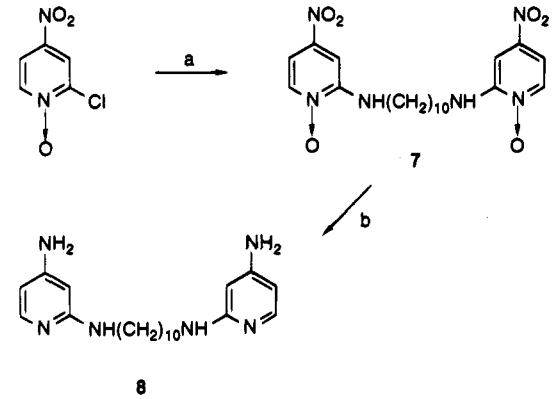
Scheme 1[a]



compd	R^1	R^2	R^3	method
1	NH_2	CH_3	$(CH_2)_9CH_3$	a
2	NH_2	CH_3	$(CH_2)_3CH_3$	b
3	NH_2	CH_3	$(CH_2)_{10}N^+Et_3$	c

[a] Methods: (a) 4-methylpentan-2-ol, reflux, 80 h; (b) MEK, reflux, 70 h; (c) 4-methylpentan-2-ol, 80 °C, 36 h.

Scheme 2[a]



[a] Methods: (a) $H_2N(CH_2)_{10}NH_2$, EtOH, reflux, 4 h; (b) H_2, Pd/C, MeOH.

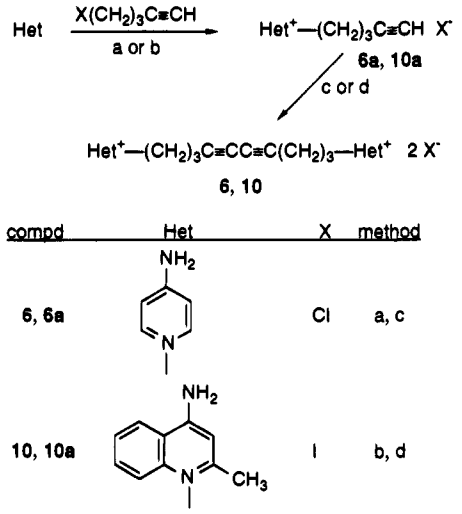
Chemistry

Compounds **1–3** were prepared by quaternization of the ring nitrogen of 4-amino-2-methylquinoline by the appropriate halide in 4-methylpentan-2-ol or methyl ethyl ketone (MEK) as described in Scheme 1. The (iododecanyl)ammonium salt **3a** was synthesized by quaternization of Et_3N with excess of 1,10-diiododecane. Scheme 2 shows the synthesis of compounds **7** and **8**. Displacement of the highly reactive chlorine atom of 2-chloro-4-nitropyridine *N*-oxide with 1,10-diaminodecane afforded **7**, hydrogenation of which reduced both the nitro group and the *N*-oxide to give **8**.

For the synthesis of the diacetylenes **6** and **10** (Scheme 3), we chose to make the quaternary ammonium monoacetylenes first and then couple these oxidatively to form the desired products. In the case of **6**, the method of Campbell and Eglinton[34] was employed. However, difficulties in the purification of the product were encountered, and the compound had to be treated with H_2S to remove Cu that was present in the sample. Therefore, it was decided to alter the method in the preparation of **10**, and in this case, the monoacetylene intermediate was efficiently coupled using the modification of Hay.[35] This method has the advantage of using milder conditions, while the CuCl·TMEDA (*N,N,N′,N′*-tetramethylethylenediamine) catalyst is capable of functioning in a variety of solvents (for a recent application, see ref 36). Indeed, no difficulties in the purification of **10** were encountered.

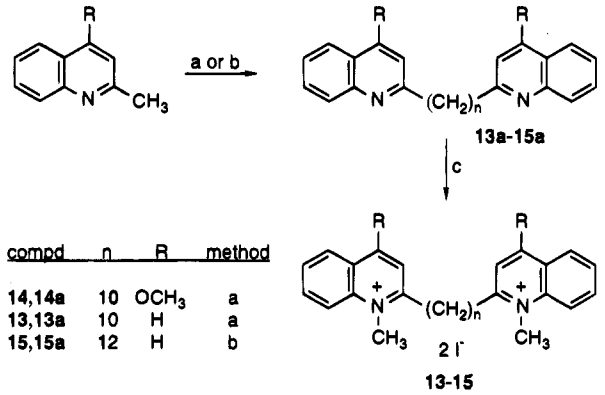
Compounds **13–15** were synthesized as shown in Scheme 4. The procedure using LDA to deprotonate the

Scheme 3[a]



[a] Methods: (a) MEK, reflux, 28 h; (b) iBu_2CO, 120–140 °C, 96 h; (c) i. MeOH/Py, $(AcO)_2Cu$, reflux, 24 h; ii. H^+, H_2S; (d) iPrOH:MeOH (4:1), CuCl, TMEDA, O_2.

Scheme 4[a]



[a] Methods: (a) i. Na/liq NH_3; ii. $I(CH_2)_8I$; (b) i. LDA/THF; ii. $Br(CH_2)_{10}Br$ −78 °C → room temperature; (c) MeI, MEK.

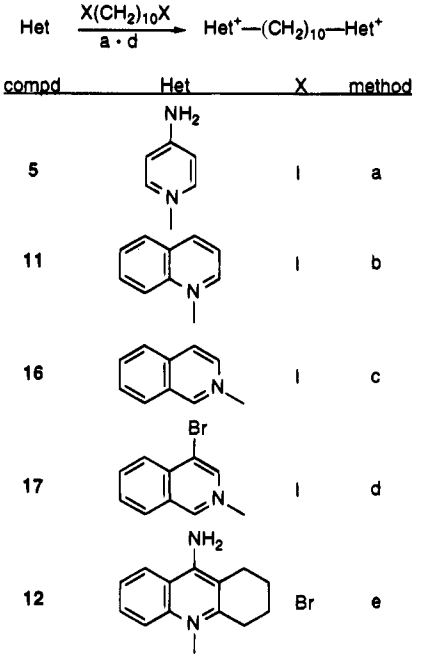
methyl of the quinoline was found to be advantageous both in terms of yield and ease of purification over the procedure using $NaNH_2$/liquid NH_3.

Quaternization of 4-aminopyridine, quinoline, isoquinoline, and 4-bromoisoquinoline with 1,10-diiododecane afforded compounds **5**, **11**, **16**, and **17**, respectively, as shown in Scheme 5.

The two benzimidazolium compounds **18** and **19** were synthesized by deprotonation of benzimidazole or 2-methylbenzimidazole with nBuLi in THF, alkylation with 1,10-diiododecane to yield the bis-benzimidazole compounds, and quaternization of these with MeI (Scheme 6). It should be noted that the anions of both benzimidazole and 2-methylbenzimidazole failed to attack 1,10-diiododecane at room temperature and reflux temperatures were required.

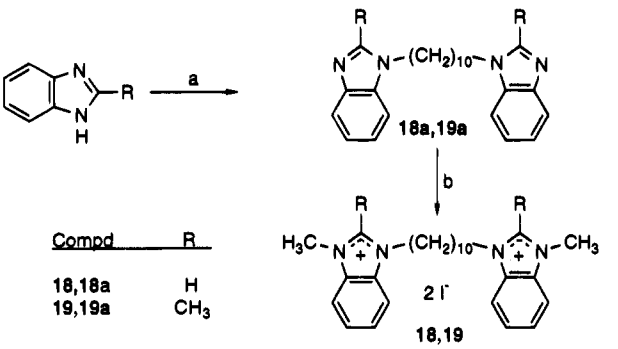
Reaction of 2-chloropyrimidine with 1,10-diaminodecane in ethanol afforded the corresponding bis-(pyrimidinylamino)decane, quaternization of which with MeI as usual gave compound **9** (Scheme 7). Similarly, displacement of the chlorine atom of 9-chloroacridine by 1,10-diaminodecane in phenol gave **20**. This, however, failed to react with MeI in a quaternization reaction and was, therefore, tested as the base (Scheme 7). The acridine groups of **20**, however, are basic enough to be protonated at physiological pH (cf. pK_a of 9-(methylamino)acridine[37] = 10.43). We were unable to quaternize 9-aminoacridine with 1,10-diiododecane to obtain a direct analogue of dequalinium, and this led to the decision of joining the two rings from the exocyclic rather than the endocyclic nitrogens. The other tricyclic compound, **12**, was synthesized by quaternization of

Scheme 5[a]



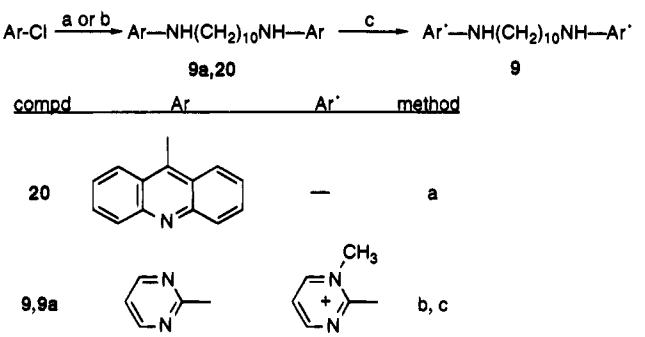
[a] Methods: (a) 4-methylpentan-2-ol, reflux, 1 h; (b) MEK, reflux, 99 h; (c) MEK, reflux, 23 h; (d) MEK, reflux, 96 h; (e) 155 °C, 1 h, no solvent.

Scheme 6[a]



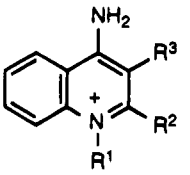
[a] Methods: (a) i. nBuLi/THF, −78 °C; ii. $I(CH_2)_{10}I$, reflux, 36 h; (b) MeI, MEK, 36 h.

Scheme 7[a]



[a] Methods: (a) $H_2N(CH_2)_{10}NH_2$, PhOH, 130–135 °C, overnight; (b) $H_2N(CH_2)_{10}NH_2$, Et_3N, EtOH, reflux, 30 h; (c) MeI, MEK, reflux, 14 h.

Table 1.



compd	R^1	R^2	R^3	$IC_{50} \pm SD$ (μM)	$EMR^a \pm SD$	n^b
1	$(CH_2)_9CH_3$	CH_3	H	5.5 ± 1.6	4.7 ± 1.7	3
2	$(CH_2)_3CH_3$	CH_3	H	12 ± 2	12 ± 5.9	3
3	$(CH_2)_{10}N^+Et_3$	CH_3	H	18 ± 3	17 ± 7.7	3
4	H	$CH_2CH_2-CH_2CH_2$		30[c]	30[c]	3

[a] Equieffective molar ratio: the ratio of the concentrations of the test compound and dequalinium that cause 50% inhibition, as determined in the same experiment. [b] Number of neurones tested. [c] Insufficient activity at this concentration to determine IC_{50}.

9-amino-1,2,3,4-tetrahydroacridine with 1,10-diiododecane. All compounds gave satisfactory analytical data, although in many cases the compounds tenaciously retained some water even after prolonged and thorough drying.

Biological Testing

The SK_{Ca} blocking action of the compounds was assessed from their ability to inhibit the after-hyperpolarization (AHP) in cultured rat sympathetic neurones as described previously.[32] Each compound was tested at two to four concentrations on at least three cells. Between three and eight compounds were examined at a time, and in each such series of experiments, dequalinium was also included as a reference compound. The Hill equation was fitted to the data to obtain estimates of the IC_{50}. However, because there was some variation in the potency of dequalinium during the course of the study, equieffective molar ratios (EMR; relative to dequalinium) were obtained by simultaneous nonlinear least squares fitting of the data with the Hill equation. It is these values which have been used for the comparison between compounds. It should be noted that the compounds were applied in a continuously flowing solution to isolated cells, so that differences in depletion as a concequence of variation in lipophilicity are unlikely to have been a complicating factor.

Although relatively simple, this assay relies on Ca^{2+} influx during the action potential to activate the SK_{Ca} channels, and the potency of any compound interfering with this influx may be overestimated. Dequalinium itself is a highly selective blocker of the SK_{Ca} channel, with no detectable effect on Ca^{2+} current even at the relatively high concentration of 10 μM.[32] As most of the compounds tested in the present work have a similar bis-cationic structure to dequalinium, an action on Ca^{2+} channels seems unlikely. Nevertheless, because of the indirectness of the assay, test concentrations of more than 10–30 μM were generally avoided. The time course of the onset of the blocking action provided an additional criterion since dequalinium and all but two of the compounds tested acted rapidly, within 90 s. The two exceptions were the amphipathic monocations **1** and **2** which were clearly less selective in their actions on sympathetic neurones.

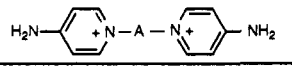
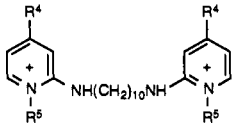
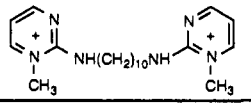
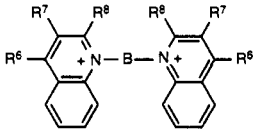
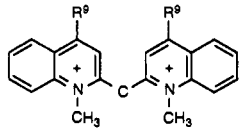
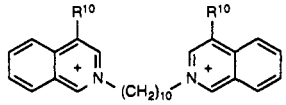
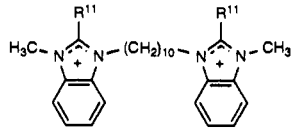
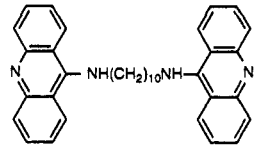
Results and Discussion

Despite being a relatively simple molecule, dequalinium is the most potent nonpeptidic blocker of the SK_{Ca} channel so far described.[31] It is more active than many other bis-quaternary compounds (such as atracurium, tubocurarine, and pancuronium; Figure 1) in which the positive charge is carried by alkylammonium groups.[27] It is also approximately 500 times more potent than a simple structural analogue, decamethonium (Figure 1), in which the positive charges are carried by two trimethylammonium groups.[27]

In view of these findings, it seemed important to address the questions of the need for two quinolinium groups and of their role. The answer to the first question would establish whether our structural analogues should have one or two quinolinium groups, and the answer(s) to the second should reveal the explanation for the importance of the charge being carried by a quinolinium rather than an alkylammonium group and should also guide our thinking in designing more potent blockers of the SK_{Ca} channel.

We therefore started by removing one quinolinium group from the molecule of dequalinium to provide compound **1** (Table 1). Although the drop in activity that results from this change appears rather small to account for the loss of a binding site, the biological test result for **1** does not reflect pure SK_{Ca} channel blocking activity, for at least two reasons. Firstly, **1** slowed the rising phase of the action potential of the neurone, and this in itself would reduce the AHP by lowering Ca^{2+} entry. Secondly, in contrast to dequalinium, the onset of the action of **1** was slow (many minutes rather than seconds), suggesting a different mechanism of action. Shortening the chain of **1** from 10 to 4 carbons provided **2** which, although it did not block the action potential of the neurone, again had a slow onset of action. It is important to note that, as for dequalinium,[32] analogues **3**, **5**, **6**, **8**, and **10**–**20** (Table 2) have a fast onset of action (complete within 90 s). It seems that **1** and **2** either bind at a different site on the SK_{Ca} channel or act via a different mechanism. However, the finding that replacement of the aminoquinolinium groups of dequalinium by the 9-amino-1,2,3,4-tetrahydroacridine group affords an equipotent compound (**12**; see below) prompted the testing of 9-amino-1,2,3,4-tetrahydroacridine (**4**) itself as another example of a compound having one instead of two positive charges. As can be seen from Tables 1 and 2, **12** is substantially more potent than **4**. It should be noted that the inactivity of **4** can neither be attributed to the lack of a positive charge, since the compound is protonated at physiological pH as its pK_a is 9.95,[38] nor to the lack of a quaternary nitrogen, since it is shown later in the discussion that nonquaternary compounds (**8** and **20**) are active as blockers of the SK_{Ca}

Table 2.

Compd	Structure					$IC_{50} \pm SD$¶	EMR†±SD	n*
				A				
5				$(CH_2)_{10}$		35 ± 6	33 ± 13	7
6				Acetylene#		6 ± 0.8	4.7 ± 1.6	4
				R⁴	R⁵			
7				NO_2	O⁻	>> 30	>> 30	4
8				NH_2	H	5.5 ± 0.7	5 ± 1.8	3
9						>> 10	>> 10	5
				R⁶	R⁷	R⁸	B	
Deq				NH_2	H	CH_3	$(CH_2)_{10}$	0.74 ± 0.05	1	18
10				NH_2	H	CH_3	Acetylene#	1.6 ± 0.3	2.4 ± 0.9	3
11				H	H	H	$(CH_2)_{10}$	21 ± 5	15 ± 7.9	5
12				NH_2	CH_2CH_2—CH_2CH_2		$(CH_2)_{10}$	1 ± 0.2	0.9 ± 0.7	5
				R⁹	C			
13				H	$(CH_2)_{10}$	72 ± 15	130 ± 34	6
14				OCH_3	$(CH_2)_{10}$	9.5 ± 3.5	27 ± 9	3
15				H	$(CH_2)_{12}$	21 ± 15	33 ± 10	4
				R¹⁰				
16				H		25 ± 5	80 ± 42	5
17				Br		4.7 ± 0.9	7.6 ± 3.1	5
				R¹¹				
18				H		16 ± 5.6	77 ± 23	4
19				CH_3		> 10	> 10	4
20						6.7 ± 0.4	12.2 ± 4.9	3

¶ In μM.

† For definition of EMR see footnote to Table I.

* Number of neurones tested.

Acetylene = $-(CH_2)_3C{\equiv}CC{\equiv}C(CH_2)_3-$

> Insufficient activity at this concentration to determine IC_{50}.

>> No activity up to this concentration.

channel. However, some ambiguity concerning the validity of the comparison of **4** with **12** may arise from the absence of an alkyl chain R^1 in compound **4**. This, combined with the uncertain mechanism of action of the other monoquaternary compounds (**1** and **2**), suggested that attention should be focused on bis-charged compounds for potency, consistency, and obtaining comparable and interpretable results.

Replacement of one quinolinium group of dequalinium by the triethyl ammonium group to give **3** resulted in a 20-fold drop in potency (relative to dequalinium), confirming that the properties of both of the charged groups are critical. It could also be concluded on comparing **3** with **1** that the introduction of a second charge to the latter restores the dequalinium-type pharmacological profile, as **3** has a fast onset of action and does not interfere with the action potential of the neurone. Focusing on those differences between the triethylammonium and quinolinium groups which might account for the superiority of the latter, it is evident that the charge distribution is very different and that enhanced delocalization via the resonance effect of the amino group in the case of dequalinium may be of significance (Figure 2). The flatness of the ring could also contribute through, for example, π-stacking if there were appropriately situated side chains of aromatic amino acids in the channel. Indeed, quinolinium cations have been shown to bind more strongly than alkylammonium cations to artificial receptors containing aromatic rings.[39] Although the structure of the SK_{Ca} channel is not known, there appears to be remarkable conservation of aromatic amino acids close to or in the

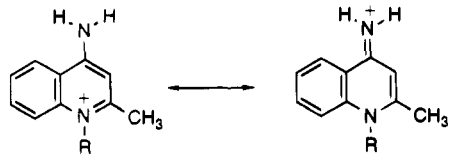


Figure 2. Two of the canonical structures contributing to aminoquinolinium resonance.

pore-forming areas of the proteins of the structures which have been proposed for the ATP-regulated,[4] inward rectifier,[3] voltage-dependent,[40] and high-conductance Ca^{2+}-activated[5] K^+ channels. Further evidence on the importance of aromatic amino acids in the binding of K^+ channel blockers is provided by the finding that TEA blocks tetrameric voltage-dependent K^+ channels by binding at a site consisting of four aromatic amino acids, one being contributed from each of the four subunits.[8,9]

On the other hand, the preference for the quinolinium over the triethylammonium group could arise from a more favorable electrostatic interaction with anionic sites on the channel, and this in turn may be due to a more favorable charge distribution. Although, conventionally, the positive charge is shown localized on the quaternary nitrogen in the structure of the quinolinium and triethylammonium groups, it is actually mainly distributed over the hydrogen atoms. To identify any differences in charge distribution between the two groups, the partial charges for two model compounds, namely, 4-amino-1,2-dimethylquinolinium and methyltriethylammonium, were examined (Figure 3). The choice of the two model compounds was based on the assumption that the two charged groups in the bis-quaternary blockers, which are separated by 10 carbons and cannot therefore interact inductively or mesomerically, do not interact through space i.e., the two charged groups are treated as being isolated. To simplify the problem, the aliphatic chain was replaced by methyl. Although the former has slightly different electronic properties in terms of its inductive and hyperconjugation effects compared with the latter, the errors introduced are expected to be largely the same in the case of the quinolinium and triethylammonium groups so that the results should be comparable. The charges were obtained by a semiempirical molecular orbital calculation using the MOPAC package, with the AM1 Hamiltonian[41] and performing Mulliken population analysis.

It can be seen in Figure 3 that in the quinolinium group the positive charge is mainly distributed over carbon atoms 2, 4, and 8a and the hydrogen atoms of the two methyl and amino groups, with all aromatic hydrogen atoms carrying substantial positive charges. The fact that the carbon atoms of positions 2, 4, and 8a are positively charged is in qualitative agreement with the conventional depiction of delocalization that results from resonance structures for the quinolinium group. On the other hand, the positive charge of the triethylammonium group is distributed over its hydrogen atoms, with the methylene groups directly attached to the quaternary nitrogen atom being more positively charged than the three terminal methyl groups. The charge distribution differences observed between the two groups may account for the difference in the potencies of dequalinium and **3**, but it is not clear that this is necessarily so.

Alternatively, the electrostatic interaction may involve matching parts of the electric fields of the two interacting species, which have oposite signs, rather than matching point charges. It is likely that the interaction with the receptor is at an anionic site, in which case the electrostatic potential maps of the molecules should provide a better representation of what the receptor actually "sees" of the molecule. These are shown in Figure 4. The electrostatic potential energy map of the triethylammonium group corresponds to one of the low-energy conformations of the group. Clearly, there are considerable differences in the two maps. The field around the methyltriethylammonium is more spherical in shape, while the one around the quinolinium group is more ring shaped and arises from the positively charged hydrogen atoms of the heterocycle. There is no buildup of positive charge above and below the plane of the quinolinium ring. It should also be noted that the shape of the positive field around the quinolinium group is "fixed" since all atoms that give rise to the field occupy strictly defined positions relative to each other. This is not the case with the field of the triethylammonium group because of the conformational mobility of the three ethyl groups.

The advantageous interaction resulting from a ring-shaped charge distribution might be related to the existence of rings of negative charge in the pore region of many ion channels.[42] These result from the negatively charged side chains of amino acids, one being contributed from each subunit of the channel, arranged in a circular fashion, pointing toward the center of the pore, thus forming a negative "wall" at that point. One can envisage the positive ring of the quinolinium group complementing the negative ring of the channel. In this way, the plane of the quinoline would be perpendicular to the longitudal axis of the channel pore and the compound would obstruct the flow of K^+ through the channel, thus acting as a blocker. On the other hand, the spherical (and smaller) field of the triethylammonium group would be a poor match to the ring-shaped field of the channel, yielding an interaction of lower strength. The conformational mobility of the ethyl groups could further weaken the interaction either directly, by varying the electrostatic field of the molecule, or indirectly, by introducing unfavorable entropic factors. Although, as mentioned in the introductory section, the structure of the SK_{Ca} channel is not known, this represents a resonable hypothesis explaining the observed more favorable binding of the quinolinium compared with the triethylammonium groups, particularly since all K^+ channel types that have been cloned so far have negatively charged amino acids in the sequence of the putative pore-forming region of the protein.[3-5,40]

Removal of the fused benzene ring to provide a pyridinium compound (**5**; Table 2) results in substantial loss of potency, and this may be due to reduction in the area of the flat π-electron system and/or altered charge distribution. As far as the latter is concerned, the size of the positively charged ring in the electrostatic potential energy map of the compound is smaller than in the case of the quinoline. To increase rigidity of the linking aliphatic chain of this compound in order to force the charged rings to occupy more strictly defined positions in space relative to each other, triple bonds were

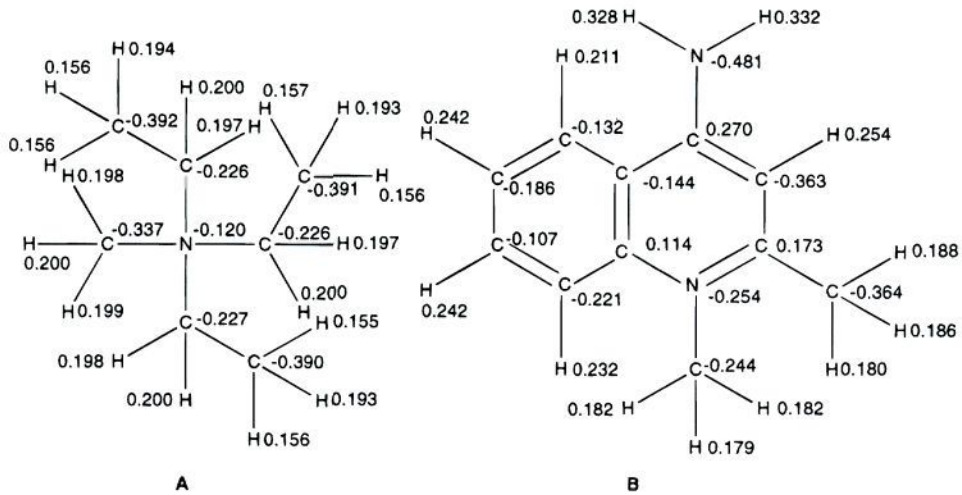


Figure 3. Partial charges on the atoms of two model compounds: methyltriethylammonium (A) and 4-amino-1,2-dimethylquinolinium (B).

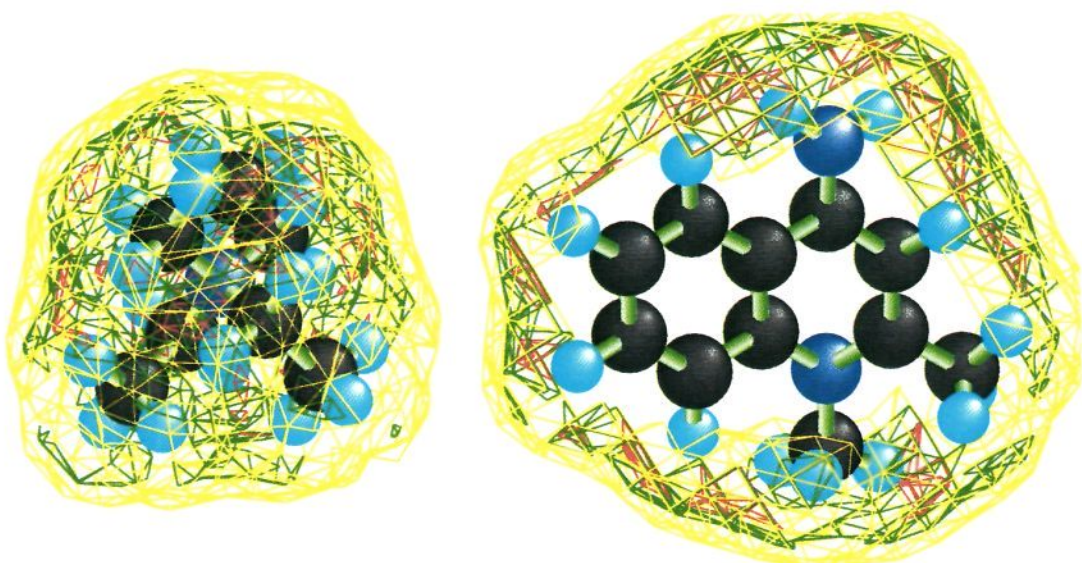


Figure 4. Electrostatic potential energy maps of the two model compounds of Figure 3. Contours were calculated at 30 (yellow), 40 (green), and 50 (red) kcal/mol using Sybyl 6.0. The molecules were minimized with AM1. Atoms are colored black (carbon), ultramarine (nitrogen), and turquoise (hydrogen).

introduced (**6**; Table 2), and this resulted in a 6-fold increase in potency. Motivated by this finding, the chain of dequalinium was analogously constrained by making **10**. However, this did not provide the expected increase in activity but rather a small decrease.

Experimenting further with the pyridine compound **5**, a second amino group at position 2 was introduced and the two rings were linked via this group (**8**). This compound is not a quaternary ammonium salt, but its p*K*a is sufficiently high (p*K*a of 2,4-diaminopyridine[43] = 9.52) for the rings to be protonated and, hence, charged at physiological pH. The resulting 6-fold increase in activity may be due to the greater delocalization effected by the second amino group, suggesting once again that charge delocalization is an important feature for potent blockade of the channel. As a speculative structural modification, **7** (an intermediate in the synthesis of **8**) was also tested, but it proved to be completely inactive at concentrations up to 30 μM.

As mentioned in the introductory section, it is believed that the arginine residues of apamin and in particular the guanidinium groups are crucial for activity. To bridge apamin with the dequalinium-like compounds, **9** was synthesized in which a guanidinium-type group is partially incorporated into a heterocyclic ring to provide an aminopyrimidinium structure. This compound was less potent than **5**. Since there seemed to be no advantage with the pyridine compounds, attention was refocused on the quinoline series.

Since charge delocalization appears to be an important factor, its contribution was tested by reducing its extent and removing the NH_2 group of dequalinium to give **11**; this resulted in considerable loss of potency, adding further evidence in support of the argument. To investigate an alternative position for the aliphatic chain in joining the two quinoline rings, some representative compounds were made. Moving the linking point of **11** from position 1 to position 2 (**13**) resulted in further loss of potency, possibly due to changes in conformation. Introduction of a methoxy group at position 4 of the quinoline rings of **13** improved activity by a factor of 5 (**14**), possibly due to increased delocal-

ization of the charge. Adding two more methylene groups to the 10-methylene chain of **13** also produced a similar increase in potency (**15**).

Replacement of the quinoline ring of **11** by the isoquinoline ring results in **16** which is 5 times less potent than **11**. Introduction of a bromine atom at position 4 of the isoquinoline (**17**) provided almost an order of magnitude increase in activity compared with **16**.

Assuming that the nitrogen of the amino group of dequalinium serves to delocalize the positive charge, it seemed appropriate to examine the importance of it being exocyclic. Hence, the benzimidazole compound **18** was synthesized, in which the exocyclic nitrogen of dequalinium has been incorporated into the ring, while keeping its ability to delocalize the charge. This compound is considerably less potent than dequalinium. It is even 5 times less potent than **11** and half as potent as **5**. Adding a methyl group at position 2 of the benzimidazole (**19**) did not alter potency significantly.

To explore further the nature of the charged heterocycle, two acridine analogues (**20** and **12**) were synthesized. In the case of **20**, we were unable to obtain a direct comparison with dequalinium because 9-aminoacridine failed to react with 1,10-diiododecane, as mentioned in the Chemistry section. However, **20**, in which the rings are joined by the exocyclic rather than the endocyclic nitrogens, is active but some 10 times less potent than dequalinium. On the other hand, **12** is equipotent with dequalinium, suggesting the binding site can accommodate a tricyclic structure and the reduced potency of **20** is not due to the size of the heterocycle.

Conclusion

From the results presented above, a number of conclusions can be drawn. It seems that the presence of two charged groups and the properties of both these groups are important for SK_{Ca} channel blocking activity in the dequalinium analogues. The present results have allowed us to develop a hypothetical model for the interaction of the charged part of the molecules examined with the channel. It is based on the differences in electrostatic potential energy maps, which could account for the tighter binding of the quinolinium compared with the triethylammonium groups. In particular, it seems that there is a need for a ring-shaped positive electrostatic field around the charged part of the molecule for blockade of the SK_{Ca} channel.

Furthermore, it appears that the blocking potency of the compounds correlates qualitatively with the degree of the delocalization of the positive charge within a series having the same heterocycle and also between compounds bearing different heterocycles. There may also be some dependence of activity on the extent of the flat π-electron system of the heterocyclic ring. Furthermore, it has been demonstrated that the quinolinium rings of dequalinium can be replaced by other heterocyclic cations to give active compounds. The binding site must therefore possess steric tolerance since compounds having monocyclic, bicyclic, or tricyclic heterocycles can be accommodated. In addition to the above, it has been shown that the rings do not necessarily have to be permanently charged, since non-quaternary compounds, basic enough to be protonated at physiological pH, are relatively potent blockers.

Experimental Section

Melting points (mp) were obtained on an Electrothermal melting point apparatus and are uncorrected. Infrared (IR) spectra were run on a Perkin-Elmer 983 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200 (200 MHz) or VXR-400 (400 MHz) spectrometer, and chemical shifts (ppm) are reported relative to the solvent peak ($CHCl_3$ in $CDCl_3$ at 7.24 ppm and DMSO in DMSO-d_6 at 2.49 ppm) or relative to TMS. Signals are designated as follows: s, singlet; s_{br}, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quadruplet; quint, quintet; m, multiplet. Mass spectra were run on a ZAB SE or VG 7070H spectrometer. Analytical reverse phase high-performance liquid chromatography (HPLC) was performed on either a Gilson or Shimadzu HPLC apparatus with a UV detector at 215 or 254 nm and a Kromasil C18 7 μm (K) or Lichrosorb RP SELECT B 7 μm (L) column. Isocratic elutions using solvent mixtures of A = water + 0.1% TFA and B = MeOH + 0.1% TFA or C = water + 0.5% sodium salt of hexanesulfonic acid + 0.5% orthophosphoric acid and D = MeOH + 0.5% sodium salt of hexanesulfonic acid + 0.5% orthophosphoric acid were performed unless otherwise stated. The ratio of A:B or C:D is indicated for each individual compound. The flow rate was 1 mL/min.

1-Decanyl-2-methyl-4-aminoquinolinium Iodide (1). A solution of 4-aminoquinaldine (1 g, 6.3 mmol) and 1-iododecane (1.66 g, 6.3 mmol) in 4-methyl-2-pentanol (30 mL) was heated under reflux for 80 h. After cooling the solvent was removed in vacuo, and the resulting red oil was purified by column chromatography on silica gel using 10% MeOH in EtOAc. This gave a solid which was recrystallized from hot water to give fine white needles (0.12 g, 4.5%): mp = 196.1–196.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (m, 2 H, $-CH_2-$), 1.25 (m, 13 H, $-CH_2-$), 1.43 (m, 2 H, $-CH_2-$), 1.47 (m, 2 H, $-CH_2-$), 2.73 (s, 3 H, quinoline-CH_3), 4.45 (t, J = 8 Hz, 2 H, $-CH_2-N$), 6.73 (s, 1 H, quinoline-H_3), 7.72 (t, J = 9 Hz, 1 H, quinoline-H_6 or $-H_7$), 8.01 (t, J = 9 Hz, 1 H, quinoline-H_7 or $-H_6$), 8.14 (d, J = 9 Hz, 1 H, quinoline-H_5 or $-H_8$), 8.44 (d, J = 9 Hz, 1 H, quinoline-H_8 or $-H_5$), 8.78 (s_{br}, 2 H, NH_2); HPLC column L, flow 0.75 mL/min, C:D = 20:80, major peak at 9.26 min representing 96.9% of the absorption at 254 nm. Anal. ($C_{20}H_{31}N_2I\cdot H_2O$) C, H, N.

1-Butyl-2-methyl-4-aminoquinolinium Iodide (**2**). 4-Aminoquinaldine (0.5 g, 3.2 mmol) was dissolved in MEK (20 mL) and treated with 1-iodobutane (5 mL, 40 mmol) under reflux for 70 h. This gave a creamy white precipitate which was collected by vacuum filtration, washed with MEK and then EtOAc, and dried. Recrystallization from a mixture of iPrOH and MeOH afforded creamy colored platelet crystals (0.51 g, 47%): mp 151 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 1.05 (t, J = 7.4 Hz, 3 H, $-CH_2CH_3$), 1.58 (sextet, J = 7.6 Hz, 2 H, $-CH_2CH_3$), 1.84 (quint, J = 7.7 Hz, 2 H, $-CH_2CH_2CH_3$), 2.78 (s, 3 H, quinoline-CH_3), 4.52 (t, J = 7.9 Hz, 2 H, $-CH_2N$), 6.79 (s, 1 H, quinoline-H_3), 7.70 (t, 1 H, quinoline-H_6 or $-H_7$), 8.03 (t, 1 H, quinoline-H_7 or $-H_6$), 8.11 (d, 1 H, quinoline-H_5 or $-H_8$), 8.33 (d, 1 H, quinoline-H_8 or $-H_5$); HPLC column L, C:D = 50:50, major peak at 8.38 min representing 99.4% of the absorption at 215 nm. Anal. ($C_{14}H_{19}N_2I$) C, H, N, I.

1,1,1-Triethyl-1-(10-iododecan-1-yl)ammonium Iodide (**3a**). 1,10-Diiododecane (1 g, 2.54 mmol) was dissolved in MEK (20 mL) and treated with triethylamine (0.175 mL, 1.25 mmol) under reflux for 24 h. The mixture was allowed to cool and a further portion of triethylamine (0.175 mL, 1.25 mmol) added; the mixture was heated under reflux for a further 72 h. The white precipitate was removed by vacuum filtration and shown to be 1,1′-(decane-1,10-diyl)bis(1,1,1-triethylammonium) diiodide by NMR, MS, and elemental analysis (data not shown). The filtrate was evaporated in vacuo to yield a yellow oil (0.9 g) which was purified by column chromatography on silica gel using EtOAc to remove the unreacted 1,10-diiododecane and 1% aqueous NH_3 in MeOH to give the product as a pale yellow oil (0.252 g, 24%): MS (FAB, MNOBA matrix) $(M + 1)^+$ 494, M^+ 368, fragments at m/z 240, 226, 212, 198, 184, 170, 156, 142, 128, 114, 100.

1-[10-(*N,N,N*-Triethylammonium-1-yl)decan-1-yl]-2-methyl-4-aminoquinolinium Diiodide (3). 1,1,1-Triethyl-1-(10-iododecan-1-yl)ammonium iodide (0.252 g, 0.685 mmol) was dissolved in 4-methyl-2-pentanol (25 mL) and treated with 4-aminoquinaldine (0.15 g, 0.95 mmol) at 80 °C for 36 h. The temperature was then raised to 120 °C for a further 90 h. The solid precipitate was collected by vacuum filtration, washed with MEK, and dried to give the product which was characterized as a white powder (0.03 g, 6.7%): mp 226–229 °C dec; ^{1}H NMR (400 MHz, CD_3OD) δ 1.34–1.90 (m, 23 H, $-CH_2-$ and $-CH_3$), 2.82 (s, 3 H, quinoline-CH_3), 3.24 (m, 2 H, $Et_3N^+CH_2$), 3.36 (m, 8 H, $-CH_2-$), 4.56 (t, J = 7.7 Hz, 2 H, $-CH_2-N_{quinoline}$), 6.82 (s, 1 H, quinoline-H_3), 7.75 (t, 1 H, quinoline-H_6 or $-H_7$), 8.07 (t, 1 H, quinoline-H_7 or $-H_6$), 8.16 (d, 1 H, quinoline-H_5 or $-H_8$), 8.38 (d, 1 H, quinoline-H_8 or $-H_5$); HPLC column L, C:D = 40:60, major peak at 4.48 min representing 98.7% of the absorption at 215 nm. Anal. ($C_{26}H_{45}N_3I_2$) H, N; C: calcd, 47.78; found, 47.07. I: calcd, 38.83; found, 40.16.

1,1′-(Decane-1,10-diyl)bis(4-aminopyridinium) Diiodide[44] (5). 4-Aminopyridine (4 g, 42 mmol) was dissolved in 4-methyl-2-pentanol (75 mL) and treated with 1,10-diiododecane (8.7 g, 22 mmol) under reflux with stirring for 1 h. After cooling to room temperature, a creamy white solid precipitated from the solution. This was collected by vacuum filtration and washed thoroughly with water and then acetone. After drying, the solid was recrystallized from hot MeOH to yield creamy colored microneedles of product (7.09 g, 55.4% yield): mp = 249–251 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 1.20 (s, 12 H, $-CH_2-$), 1.70 (m, 4 H, $-CH_2-$), 4.10 (t, J = 5 Hz, 4 H, $-CH_2-N$), 6.79 (d, J = 7 Hz, 4 H, pyridine-H_3 and $-H_5$), 8.17 (d, J = 7 Hz, 4 H, pyridine-H_2 and $-H_6$), 7.95 (s, 4 H, NH_2, disappears with D_2O); HPLC column L, linear gradient elution with C:D = 80:20 at 0 min to C:D = 40:60 at 20 min, major peak at 30.11 min representing 98.6% of the absorption at 254 nm. Anal. ($C_{20}H_{32}N_4I_2$) C, H, N; I: calcd, 43.60; found, 45.40.

1-(4-Pentyn-1-yl)-4-aminopyridinium Chloride (6a). 4-Aminopyridine (0.5 g, 5.3 mmol) was dissolved in MEK (20 mL) and treated with 5-chloro-1-pentyne (1.06 mL, 10 mmol) under reflux for 28 h. After the mixture had cooled to room temperature, the precipitate was collected by vacuum filtration and then purified by column chromatography on silica gel using 50% MeOH in EtOAc. This gave a colorless gum which solidified on standing (0.151 g, 15% yield): mp = 139.5–141 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.00 (m, 2 H, $-CH_2-$), 2.25 (m, 2 H, $-CH_2-$), 2.88 (t, J = 2 Hz, 1 H, C≡CH), 4.08 (t, J = 6 Hz, 2 H, N-CH_2), 6.63 (d, J = 7 Hz, 2 H, pyridine-H_3 and $-H_5$), 7.79 (d, J = 7 Hz, 2 H, pyridine-H_2 and $-H_6$), 8.11 (s, 2 H, NH_2).

1,1′-(Deca-4,6-diyne-1,10-diyl)bis(4-aminopyridinium) Dichloride Dihydrate (6). 1-(4-Pentyn-1-yl)-4-aminopyridinium chloride (1.3 g, 6.63 mmol) and a saturated solution of finely powdered $(AcO)_2Cu$ (1.725 g, 9.48 mmol) in 60 mL of pyridine:MeOH (1:1) were heated under reflux for 24 h. The solvents were removed in vacuo, and the green solid was dried in vacuo. This was purified by column chromatography on silica gel using 50% MeOH in EtOAc which was increased to 80% toward the end of the procedure. The product was isolated as a yellow-green needle-like material. This was dissolved in the minimum amount of MeOH, and iPrOH was added until the solution turned cloudy. After filtration, Et_2O was added dropwise to the filtrate to precipitate the product. The solvents were removed in vacuo, and the resultant pale yellow solid rigorously was dried under vacuum at 70 °C and then dissolved in water and treated with H_2SO_4 (a few drops) and H_2S gas for 30 min. Decolorizing charcoal was added, and the mixture was stirred for 10 min. After filtration the filtrate was neutralized with Na_2CO_3 and the solvents were removed in vacuo. This gave a solid material which was twice dissolved in iPrOH and concentrated to dryness to azeotropically remove water and then dissolved in hot iPrOH, and the insoluble Na_2SO_4 and Na_2CO_3 were removed by filtration. The solvents were removed in vacuo from the filtrate to give a sticky gum. This was treated with dry Et_2O and scratched until a powder formed. The Et_2O was removed in vacuo and the proceedure repeated with fresh Et_2O until all the gum had solidified (0.2 g, 19%): mp 115 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.00 (quint, 4 H, $-CH_2-$), 2.38 (t, 4 H, $-CH_2-$), 4.05 (t, 4 H, N-CH_2-), 6.52 (d, 4 H, pyridine-H_3 and $-H_5$), 7.72 (d, 4 H, pyridine-H_2 and $-H_6$), 8.00 (s_{br}, 4 H, NH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.4, 28.5, 56.1, 65.5, 76.7, 109.3, 142.0, 158.8; HPLC column L, linear gradient elution with C:D = 70:30 at 0 min to C:D = 20:80 at 30 min, major peak at 18.09 min representing 99.2% of the absorption at 254 nm. Anal. ($C_{20}H_{24}N_4Cl_2 \cdot 2H_2O$) C, H; N: calcd, 13.11; found, 12.57.

2,2′-*N,N′*-(Decane-1,10-diyl)bis(4-nitro-2-aminopyridine 1-oxide) (7). A solution of 2-chloro-4-nitropyridine 1-oxide[45] (0.5 g, 0.28 mmol) in EtOH (50 mL) containing 1,-10-diaminodecane (0.25 g, 0.14 mmol) was heated under reflux for 4 h. The EtOH was removed in vacuo to yield an orange solid which was a mixture of products and starting materials (TLC). This was purified by column chromatography on silica gel using EtOAc until the starting materials had been eluted and then using MeOH to give two compounds. The first was identified as the desired product and obtained as a mixture of the free base and the dihydrochloride salt. Total conversion to the free base was achieved by basification of a solution of the mixture in water with aqueous NH_3 and extraction with EtOAc. The yield of the pure free base was 0.192 g (31%): mp 126.5–127 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35–1.75 (m, 16 H, $-CH_2-$), 3.36 (q, J = 7 Hz, 4 H, N-CH_2-), 6.97 (t_{br}, 2 H, NH), 7.39 (m, 4 H, pyridine-H_5 and $-H_6$), 8.26 (m, 2 H, pyridine-H_3); HPLC column L, MeOH:EtOAc = 20:80, major peak at 6.87 min representing 99.6% of the absorption at 254 nm. Anal. ($C_{20}H_{28}N_6O_6$) C, H; N: calcd, 18.75; found, 18.00.

2,2′-*N,N′*-(Decane-1,10-diyl)bis(2,4-diaminopyridine) (8). 2,2′-*N,N′*-(Decane-1,10-diyl)bis(4-nitro-2-aminopyridine 1-oxide) (0.26 g) was dissolved in MeOH (100 mL) and treated with 10% Pd/C (0.5 g) in a Parr low-pressure hydrogenation apparatus at 50 psi of hydrogen for 11.5 h. The catalyst was filtered off, and the filtrate was evaporated in vacuo to give a colorless gum. This was purified by column chromatography on silica gel using 1% NH_4OH in MeOH. The product (0.061 g, 30% yield) was recrystallized from MeOH to yield a white, microcrystalline material: mp 140–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (m, 12 H, $-CH_2-$), 1.45 (quint, J = 6 Hz, 4 H, $-CH_2-$), 3.03 (q, J = 7 Hz, 4 H, N-CH_2-), 5.46 (s, 4 H, NH_2), 5.50 (d, J = 2 Hz, 2 H, pyridine-H_3), 5.74 (m, 4 H, pyridine-H_5 and NH), 7.45 (d, J = 6 Hz, 2 H, pyridine-H_6); HPLC column L, flow 0.75 mL/min, C:D = 35:65, major peak at 5.91 min representing 93.5% of the absorption at 254 nm. Anal. ($C_{20}H_{32}N_6 \cdot H_2O$) H; C: calcd, 64.14; found, 65.41. N: calcd, 22.44; found, 21.84.

2,2′-*N,N′*-(Decane-1,10-diyl)bis(2-aminopyrimidine) (9a). 2-Chloropyrimidine (1 g, 8.73 mmol), 1,10-diaminodecane (0.752 g, 4.37 mmol), and Et_3N (2 mL, 14.35 mmol) were dissolved in 40 mL of absolute EtOH and heated under reflux for 30 h. After the solution had cooled to room temperature, MeOH was added to the solution and the white precipitate formed was collected by vacuum filtration and washed with MeOH to yield pure product. The filtrate was concentrated to dryness, and the resulting solid formed was basified with dilute KOH solution until the solution was strongly alkaline and extracted with 5 × 50 mL CH_2Cl_2. After drying (Na_2SO_4) the CH_2Cl_2 was removed in vacuo to give a redish oil which was recrystallized from MeOH to yield white crystals (total: 0.973 g, 68%): mp 107–108 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 1.29 (m, 12 H, $-CH_2-$), 1.62 (quint, 4 H, $-CH_2-$), 3.39 (td, J_1 = 5.8 Hz, J_2 = 7.2 Hz, 4 H, N-CH_2-), 5.18 (s_{br}, 2 H, NH), 6.50 (t, J = 4.8 Hz, 2 H, pyrimidine-H_5), 8.27 (d, J = 4.7 Hz, 4 H, pyrimidine-H_4 and $-H_6$); HPLC column K, A:B = 50:50, major peak at 12.12 min representing 97.7% of the absorption at 215 nm. Anal. ($C_{18}H_{28}N_6 \cdot 0.2CH_3OH$) C, H, N.

2,2′-*N,N′*-(Decane-1,10-diyl)bis(1-methyl-2-aminopyrimidinium) Diiodide (9). 2,2′-*N,N′*-(Decane-1,10-diyl)bis(2-aminopyrimidine) (0.2 g, 0.61 mmol) and MeI (3 mL, 48.2 mmol) were dissolved in MEK and heated under reflux under argon for 14 h. A creamy precipitate formed which was collected by vacuum filtration and washed extensively with MEK (0.328 g, 88%): mp 182–184 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ 1.26 (m, 12 H, $-CH_2-$), 1.58 (m, 4 H, $-CH_2-$), 3.50 (q, 4 H, N-CH_2-), 3.72 (s, 6 H, $-CH_3$), 7.08 (dd, J_1 = 4.4 Hz, J_2 = 6.5 Hz, 2 H, pyrimidine-H_5), 8.61 (dd, J_1 = 2.1 Hz, J_2

= 6.5 Hz, 2 H, pyrimidine-H_4), 8.74 (t_{br}, 2 H, NH), 8.88 (dd, J_1 = 4.4 Hz, J_2 = 2.1 Hz, 2 H, pyrimidine-H_6); HPLC column K, linear gradient elution with A:B = 45:55 at 0 min to A:B = 10:90 at 15 min, major peak at 4.46 min representing 100% of the absorption at 254 nm. Anal. ($C_{20}H_{34}N_6I_2$) C, H, N.

1-(4-Pentyn-1-yl)-2-methyl-4-aminoquinolinium Iodide (10a). 4-Aminoquinaldine (0.7 g, 4.42 mmol) and 5-iodo-1-pentyne[48] were dissolved in 25 mL of hot diisobutyl ketone, and the solution was heated to 120–140 °C under argon for 96 h. The reaction mixture was cooled to room temperature and the dark solid collected by filtration and dried under vacuum. This was dissolved in MeOH, adsorbed onto silica, and chromatographed using 10% MeOH in EtOAc. The fractions containing the product were combined, and the solvents removed in vacuo to yield a dark red oil (1.1 g, 70.6%) which was recrystallized from MeOH–Et_2O to yield a pink solid: mp = 193–195 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.92 (quint, 2 H, -CH_2-), 2.42 (t_{br}, 2 H, -CH_2-), 2.73 (s, 3 H, -CH_3), 2.97 (s, 1 H, ≡CH), 4.52 (t, J = 8.2 Hz, 2 H, N^+-CH_2-), 6.71 (s, 1 H, quinoline-H_3), 7.72 (t, J = 7.6 Hz, 1 H, quinoline-H_6 or -H_7), 8.02 (t, J = 7.6 Hz, 1 H, quinoline-H_7 or -H_6), 8.19 (d, J = 8.9 Hz, 1 H, quinoline-H_5 or -H_8), 8.43 (d, J = 8.1 Hz, 1 H, quinoline-H_8 or -H_5), 8.84 (d_{br}, 2 H, NH_2). Anal. ($C_{15}H_{17}N_2I$·0.25 H_2O) C, H, N; I: calcd, 35.57; found, 34.98.

1,1′-(Deca-4,6-diyne-1,10-diyl)bis(4-amino-2-methylquinolinium) Diiodide Hydrate (10). TMEDA (0.5 g, 4.3 mmol) and CuCl (0.42 g, 4.25 mmol) were dispersed with vigorous stirring in 100 mL of iPrOH. The solution turned deep blue and some solid remained undissolved. After the solid had precipitated, 2 mL of the supernatant solution were added to a solution of 1-(4-pentyn-1-yl)-2-methyl-4-aminoquinolinium iodide (0.1 g, 0.284 mmol) in a mixture of iPrOH:MeOH 4:1. The solution was rapidly decolourized. O_2 was bubbled through with vigorous stirring for 2 h while the reaction mixture was kept in a water bath at 40 °C. The bubbling of O_2 was stopped, and the solution was stirred vigorously for another 48 h. The reaction mixture was concentrated to a small volume; the creamy precipitate formed was collected, washed with MeOH, and dried in vacuo (0.07 g, 70%): mp 295–297 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.95 (q, 4 H, -CH_2-), 2.58 (t, J = 6.9 Hz, 4 H, -CH_2-), 2.73 (s, 6 H, -CH_3), 4.50 (t, J = 8.1 Hz, 4 H, N^+-CH_2-), 6.71 (s, 2 H, quinoline-H_3), 7.72 (t, J = 7.6 Hz, 2 H, quinoline-H_6 or -H_7), 8.01 (t, J = 8.5 Hz, 2 H, quinoline-H_7 or -H_6), 8.18 (d, J = 9.0 Hz, 2 H, quinoline-H_5 or -H_8), 8.43 (d, J = 8.4 Hz, 2 H, quinoline-H_8 or -H_5), 8.85 (d, 2 H, NH_2); HPLC column K, A:B = 55:45, major peak at 13.67 min representing 95% of the absorption at 215 nm. Anal. ($C_{30}H_{32}N_4I_2$·1.25H_2O) C, H, N; I: calcd, 35.01; found, 33.44.

1,1′-(Decane-1,10-diyl)bis(quinolinium) diiodide[46] (11): HPLC column L, C:D = 40:60, major peak at 5.26 min representing 99.5% of the absorption at 220 nm.

1,1′-(Decane-1,10-diyl)bis(9-amino-1,2,3,4-tetrahydroacridinium) dibromide[47] hydrate (12): HPLC column L, C:D = 25:75, major peak at 6.61 min representing 98.2% of the absorption at 254 nm.

2,2′-(Decane-1,10-diyl)bis(quinoline) (13a). Na (1.966 g, 85.52 mmol) was dispersed in liquid NH_3 containing a catalytic amount of $Fe(NO_3)_3$ under argon. When the dark blue color of the suspension turned gray, quinaldine (11.638 g, 81.28 mmol) was added and the reaction mixture was stirred for 1 h. 1,8-Diiodooctane (14.87 g, 40.63 mmol) was then gradualy added, and the NH_3 was allowed to evaporate overnight. Water was added to the residue and the aqueous phase extracted with Et_2O. The extracts were combined, dried (Na_2SO_4), and rotay evaporated to dryness to yield an oil which was dissolved in the minimum ammount of petroleum ether at 40–60 °C and kept at −20 °C overnight. The solid mass that had formed was allowed to warm to room temperature, filtered, and the yellow solid obtained was washed with the solvent and dried (1.984 g, 12.3%). This contained some quinaldine (TLC); hence it was recrystallized from MeOH. Large, orange crystals came out of solution, which were collected and dried. However, the compound was still impure; therefore, it was purified by column chromatography on silica gel using petroleum ether:EtOAc (3:1). It was isolated as yellow crystals which were dried in vacuo (1.03 g): mp 96–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$, TMS) δ 1.27–1.42 (m, 12 H, -CH_2-), 1.80 (quint, 4 H, -CH_2-), 2.96 (t, J = 7.9 Hz, 4 H, quinoline-CH_2-), 7.29 (d, J = 8.4 Hz, 2 H, quinoline-H_3), 7.48 (t, J = 6.9 Hz, 2 H, quinoline-H_6 or -H_7), 7.68 (t, J = 7.0 Hz, 2 H, quinoline-H_7 or -H_6), 7.77 (d, J = 8.1 Hz, 2 H, quinoline-H_5 or -H_8), 8.03–8.07 (d, d, 4 H, quinoline-H_8 or -H_5, quinoline-H_4); HPLC column K, A:B = 50:50, major peak at 14.76 min representing 100% of the absorption at 215 nm. Anal. ($C_{28}H_{32}N_2$·0.1H_2O) C, H, N.

2,2′-(Decane-1,10-diyl)bis(1-methylquinolinium) Diiodide Hydrate (13). 2,2′-(Decane-1,10-diyl)bis(quinoline) (0.3 g, 0.757 mmol) and MeI (2 mL, 32.13 mmol) were dissolved in MEK, and the solution was heated under reflux for 33 h. More MeI was added after 6 h (1 mL, 16.07 mmol) and after 16 h (2 mL, 32.13 mmol). The reaction mixture was filtered hot, and the yellow solid collected was dried. Four recrystallizations from MeOH were needed to get an analyticaly pure sample for testing. At the last two, care was taken to allow the material to crystallize only for 30 min at room temperature. The crystals collected were washed with hot MeOH (0.119 g, 23%): mp = 234 °C dec; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) 1.31 (m, 8 H, -CH_2-), 1.45 (m, 4 H, -CH_2-), 1.76 (quint, 4 H, -CH_2-), 3.36 (t, J = 7.9 Hz, 4 H, quinoline-CH_2-), 4.48 (s, 6 H, N^+-CH_3), 8.00 (t, J = 7.5 Hz, 2 H, quinoline-H_6 or -H_7), 8.11 (d, J = 8.7 Hz, 2 H, quinoline-H_3), 8.23 (td, J_1 = 1.5 Hz, J_2 = 8.1 Hz, 2 H, quinoline-H_7 or -H_6), 8.40 (d, J = 8.1 Hz, 2 H, quinoline-H_5 or -H_8), 8.57 (d, J = 9.1 Hz, 2 H, quinoline-H_8 or -H_5), 9.11 (d, J = 8.7 Hz, 2 H, quinoline-H_4); HPLC column K, A:B = 50:50, major peak at 4.92 min representing 97.3% of the absorption at 215 nm. Anal. ($C_{30}H_{38}N_2I_2$·H_2O) C, H, N.

2,2′-(Decane-1,10-diyl)bis(4-methoxyquinoline) (14a). Na (0.42 g, 18.27 mmol) was dispersed in aproximately 150 mL of liquid NH_3, under argon, containing a catalytic ammount of ferric nitrate. When the initially dark blue suspension had turned gray, 4-methoxy-2-methylquinoline[49] (3 g, 17.32 mmol) was added and the dark red reaction mixture was stirred for 1 h. Then, 1,8-diiodooctane (3.128 g, 8.54 mmol) was added slowly. After 1 h of stirring, no reaction had taken place (TLC); therefore, 90 mL of dry DMF was added, and the solution was stirred overnight. The reaction was quenched by adding water, and the solvents were removed in vacuo at 35 °C. Water was added to the residue, and it was extracted with Et_2O. The extracts were combined and dried (Na_2SO_4) and the solvent removed in vacuo. The residue consisted mainly of product and 4-methoxyquinaldine, which were separated by column chromatography on silica gel using CH_2Cl_2:EtOAc (2:1). The product was recrystallized from MeOH to yield small, white-grayish crystals (1.1 g, 28%): mp 88–89 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29–1.42 (m, 12 H, -CH_2-), 1.80 (quint, J = 7.4 Hz, 4 H, -CH_2-), 2.94 (t, J = 7.9 Hz, 4 H, quinoline-CH_2-), 4.04 (s, 6 H, OCH_3), 6.64 (s, 2 H, quinoline-H_3), 7.44 (t, J = 7.1 Hz, 2 H, quinoline-H_6 or -H_7), 7.66 (t, J = 7.1 Hz, 2 H, quinoline-H_7 or -H_6), 7.99 (d, J = 8.5 Hz, 2 H, quinoline-H_5 or -H_8), 8.14 (dd, J_1 = 1.5 Hz, J_2 = 8.3 Hz, 2 H, quinoline-H_8 or -H_5); HPLC column K, A:B = 45:55, major peak at 18.02 min representing 98.8% of the absorption at 215 nm. Anal. ($C_{30}H_{36}N_2O_2$·4H_2O) C, H, N.

2,2′-(Decane-1,10-diyl)bis(1-methyl-4-methoxyquinolinium) Diiodide (14). 2,2′-(Decane-1,10-diyl)bis(4-methoxyquinoline) (0.2 g, 0.44 mmol) and MeI (2 mL, 32.13 mmol) were dissolved in 20 mL of MEK, and the solution was heated under reflux for 12 h under argon. The white precipitate that formed was collected by filtration, washed with solvent, and dried in vacuo. A single recrystallization from CH_2Cl_2 afforded pure material (0.167 g, 51.5%): mp 186–188 °C; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) 1.33 (m, 8 H, -CH_2-), 1.47 (m, 4 H, -CH_2-), 1.74 (m, 4 H, -CH_2-), 3.28 (t, J = 8.1 Hz, 4 H, quinoline-CH_2), 4.30 (s, 12 H, OCH_3 and NCH_3), 7.62 (s, 2 H, quinoline-H_3), 7.91 (t, J = 7.2 Hz, 2 H, quinoline-H_6 or -H_7), 8.17 (t, J = 7.1 Hz, 2 H, quinoline-H_7 or -H_6), 8.38–8.45 (d, d, 4 H, quinoline-H_5, and -H_8); HPLC column K, A:B = 45:55, major peak at 8.16 min representing 100% of the absorption at 215 nm. Anal. ($C_{32}H_{42}N_2O_2I_2$) H, N; C: calcd, 51.90; found, 51.44.

2,2′-(Dodecane-1,12-diyl)bis(quinoline) Hemihydrate (15a). To a solution of LDA (23.05 mmol) in THF at −78 °C

was added quinaldine (3 g, 20.95 mmol) over a period of 15 min. The solution was stirred at −78 °C for 1 h. 1,10-Diiododecane (3.144 g, 10.47 mmol) was then added, and the solution was allowed to warm gradually to room temperature and stirred for 18 h. The reaction was quenched with MeOH, and the mixture was concentrated to dryness, diluted with water, and extracted with $CHCl_3$. The extracts were dried (Na_2SO_4) and rotary evaporated to dryness to yield a yellow solid. This was recrystallized from MeOH to give pale yellow crystals (3.46 g, 78%): mp 98−100 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (m, 16 H, -CH_2-), 1.80 (quint, 4 H, -CH_2-), 2.96 (t, 4 H, quinoline-CH_2), 7.30 (d, J = 8.5 Hz, 2 H, quinoline-H_3), 7.47 (t, J = 7.5 Hz, 2 H, quinoline-H_6 or -H_7), 7.66 (t, J = 7.6 Hz, 2 H, quinoline-H_7 or -H_6), 7.77 (d, J = 8.1 Hz, 2 H, quinoline-H_5 or -H_8), 8.05 (d, d, 4 H, quinoline-H_4, -H_8 or -H_5); HPLC column K, A:B = 35:65, major peak at 6.32 min representing 100% of the absorption at 215 nm. Anal. ($C_{30}H_{36}N_2$·0.5H_2O) C, H, N.

2,2′-(Dodecane-1,12-diyl)bis(1-methylquinolinium) Diiodide (15). 2,2′-(Dodecane-1,12-diyl)bis(quinoline) (0.5 g, 1.18 mmol) and MeI (2 mL, 32.13 mmol) were dissolved in MEK, and the solution was heated under reflux for 72 h under argon, more portions of MeI (2 mL) being added after 12, 24, 36, 48, and 60 h. The yellow solid formed was collected by filtration and washed with the solvent and Et_2O. This was recrystallized twice from absolute EtOH to yield yellow crystals (0.7 g, 84%): mp = 172−174 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.28 (m, 12 H, -CH_2-), 1.46 (m, 4 H, -CH_2-), 1.77 (quint, 4 H, -CH_2-), 3.38 (t, J = 8 Hz, 4 H, quinoline-CH_2), 4.52 (s, 6 H, -CH_3), 8.01 (td, J_1 = 7.6 Hz, J_2 = 0.7 Hz, 2 H, quinoline-H_6 or -H_7), 8.12 (d, J = 8.6 Hz, 2 H, quinoline-H_3), 8.24 (td, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 2 H, quinoline-H_7 or -H_6), 8.41 (d, J = 8.1 Hz, 2 H, quinoline-H_5 or -H_8), 8.59 (d, J = 9 Hz, 2 H, quinoline-H_8 or -H_5), 9.13 (d, J = 8.6 Hz, 2 H, quinoline-H_4); HPLC column L, A:B = 50:50, major peak at 17.42 min representing 100% of the absorption at 215 nm. Anal. ($C_{32}H_{42}N_2I_2$) C, H, N.

2,2′-(Decane-1,10-diyl)bis(isoquinolinium) Diiodide[46] (16): HPLC column K, A:B = 50:50, major peak at 5.2 min representing 98.9% of the absorption at 254 nm.

1,1′-(Decane-1,10-diyl)bis(4-bromoisoquinolinium) Diiodide (17). A solution of 4-bromoisoquinoline (3 g, 14.4 mmol) in MEK (100 mL) was treated with 1,10-diiododecane (2.84 g, 7.2 mmol) and heated under reflux with stirring for 96 h. After cooling the precipitate was collected by vacuum filtration and recrystallised twice from MeOH to give golden yellowish crystals (2 g, 2.5 mmol, 34.7%): mp 241−243 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26−1.31 (m, 12 H, -CH_2-), 2.03 (m, 4 H, -CH_2-), 4.69 (t, J = 7.5 Hz, 4 H, N-CH_2), 8.18 (ddd, J_1 = 1.4 Hz, J_2 = 6.7 Hz, J_3 = 8.1 Hz, 2 H, isoquinoline-H_6 or -H_7), 8.38−8.44 (m, 4 H, isoquinoline), 8.57 (d, J = 8.3 Hz, 2 H, isoquinoline-H_5 or -H_8), 9.34 (d, J = 1 Hz, 2 H, isoquinoline-H_3), 10.23 (s, 2 H, isoquinoline-H_1); HPLC column K, A:B = 50:50, major peak at 7.1 min representing 96.8% of the absorption at 254 nm. Anal. ($C_{28}H_{32}N_2Br_2I_2$) C, H, N.

1,1′-(Decane-1,10-diyl)bis(1*H*-benzimidazole) (18a). To a suspension of benzimidazole (1 g, 8.47 mmol) in THF at −78 °C, was added 5.3 mL (8.48 mmol) of a 1.6 M solution of nBuLi in hexanes. The resultant solution was stirred for 30 min and then allowed to warm to room temperature. 1,10-Diiododecane (1.668 g, 4.23 mmol) was added, and the solution was heated under reflux for 36 h under argon. MeOH was then added, and the solvents were removed in vacuo to yield an oil. This was purified by column chromatography on silica gel using EtOAc:MeOH (10:1). The product (R_f = 0.3) was obtained as a yellow solid. This was dispersed in 50 mL of hot water, and EtOH was added until the solution was clear. On cooling to room temperature, a yellow oil came out of solution. The supernatant was decanted into another flask, excess of water was added dropwise to it while scratching the walls of the flask, and the suspension was placed in the refridgerator for 5 h. The white precipitate was collected by filtration, washed well with water, and dried in vacuo at 40 °C (60% yield): mp 87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22−1.29 (m, 12 H, -CH_2-), 1.86 (m, 4 H, -CH_2-), 4.16 (t, J = 7 Hz, 4 H, NCH_2), 7.26−7.31 (m, 4 H, benzimidazole-H_5, H_6), 7.40 (dd, J_1 = 6.9 Hz, J_2 = 2.5 Hz, 2 H, benzimidazole-H_4 or -H_7), 7.81 (dd, J_1 = 6.8 Hz, J_2 = 2 Hz, 2 H, benzimidazole-H_7 or -H_4), 7.89 (s, 2 H, benzimidazole-H_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.7, 29.0, 29.2, 29.7, 45.1, 109.6, 120.3, 122.0, 122.8, 133.7, 142.9, 143.8; MS (FAB, MNOBA matrix) $[M + H]^+$ 375, fragments at m/z 257, 243, 229, 215, 201, 187, 173, 159, 145, 131, 119; HPLC column K, A:B = 55:45, major peak at 10.44 min representing 99.9% of the absorption at 215 nm. Anal. ($C_{24}H_{30}N_4$·0.2H_2O) C, H, N.

1,1′-(Decane-1,10-diyl)bis(3-methylbenzimidazolium) Diiodide Hemihydrate (18). 1,1′-(Decane-1,10-diyl)bis(1*H*-benzimidazole) (0.2 g, 0.53 mmol) and MeI (1 mL, 16.06 mmol) were dissolved in 15 mL MEK and heated under reflux for 36 h, more MeI (1 mL) being added after 12 h. The white solid that formed was collected by filtration, washed extensively with solvent, and dried (0.332 g, 95%): mp 204−205 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.24−1.30 (m, 12 H, -CH_2-), 1.88 (m, 4 H, -CH_2-), 4.08 (s, 6 H, CH_3), 4.47 (t, J = 7.1 Hz, 4 H, N-CH_2), 7.69−7.73 (m, 4 H, benzimidazole), 8.02−8.04 (m, 2 H, benzimidazole), 8.08−8.10 (m, 2 H, benzimidazole), 9.72 (s, 2 H, benzimidazole-H_2); HPLC column K, A:B = 55:45, major peak at 11.91 min representing 99.2% of the absorption at 215 nm. Anal. ($C_{26}H_{36}N_4I_2$·0.5H_2O) C, H, N.

1,1′-(Decane-1,10-diyl)bis(2-methylbenzimidazole) (19a). To a suspension of 2-methylbenzimidazole (1 g, 7.57 mmol) in THF (70 mL) at −78 °C was added a 1.6 M solution of nBuLi in hexanes (5 mL, 7.95 mmol) dropwise, and the mixture was stirred at −78 °C for 30 min. Then, a solution of 1,10-diiododecane (1.49 g, 3.78 mmol) in THF was added, and the mixture was warmed to room temperature and then heated under reflux for 36 h under Ar. After the solution had cooled to room temperature, MeOH was added and the solvents were removed in vacuo to yield an oil. This was partitioned between water (50 mL) and $CHCl_3$ (40 mL), and the water phase was further extracted with 40 mL $CHCl_3$. The extracts were combined and dried (Na_2SO_4), and the solvent was removed in vacuo to yield an oil. This was purified by column chromatography on silica gel using petroleum ether:EtOAc (10:1). The product (R_f = 0.25) was isolated as a white solid which was dissolved in $CHCl_3$ and filtered to remove any silica and the solvent removed in vacuo to yield a white solid (1.444 g, 95%): mp 101−102 °C; ^{1}H NMR (200 MHz, $CDCl_3$, TMS,) δ 1.23−1.31 (m, 12 H, CH_2), 1.78 (m, 4 H, CH_2), 2.60 (s, 6 H, CH_3), 4.09 (t, J = 7.1 Hz, 4 H, N-CH_2), 7.20−7.30 (m, 6 H, Ar), 7.67−7.72 (m, 2 H, Ar). Anal. ($C_{26}H_{34}N_4$·0.2H_2O) C, H, N.

1,1′-(Decane-1,10-diyl)bis(2,3-dimethylbenzimidazolium) Diiodide (19). 1,1′-(Decane-1,10-diyl)bis(2-methylbenzimidazole) (0.2 g, 0.497 mmol) and MeI (1 mL, 16.06 mmol) were dissolved in MEK and heated under reflux for 36 h under Ar, more MeI (1 mL) being added after 24 h. After cooling to room temperature, the solid formed was collected by filtration and washed extensively with MEK. This was recrystallized from MeOH, but the sample obtained was less pure (by HPLC). It was suspected that it decomposed on heating; therefore, it was recrystallized again from approximately 25 mL of MeOH with gentle heating (0.2 g, 59%): mp 274−276 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23−1.32 (m, 12 H, CH_2), 1.74 (quint, 4 H, CH_2), 2.88 (s, 6 H, CH_3), 3.98 (s, 6 H, N-CH_3), 4.47 (t, J = 7.4 Hz, 4 H, N-CH_2), 7.62−7.64 (m, 4 H, Ar), 7.98−8.02 (m, 4 H, Ar); HPLC column K, A:B = 50:50, major peak at 4.62 min representing 97.8% of the absorption at 215 nm. Anal. ($C_{28}H_{40}N_4I_2$·0.2H_2O) C, H, N.

1,10-Bis[*N*-(Acridin-9-yl)amino]decane dihydrochloride dihydrate[50] (20): HPLC column L, flow 0.75 mL/min, A:B = 35:65, major peak at 10 min representing 96% of the absorption at 215 nm.

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