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The antibacterial activity of 4,4′-bipyridinium amphiphiles with conventional, bicephalic and gemini architectures

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ABSTRACT

Dialkyl 4,4′-bipyridinium compounds are widely employed for their useful redox properties, and are commonly known as viologens due to their intense coloration upon reduction. Despite their prevalence and amphiphilic nature, the antibacterial activity of these compounds remains largely unreported. We have thus prepared a series of mono- and bis-alkylated analogs of 4,4′-bipyridine to investigate structure–activity relationships in their inhibition of a battery of Gram positive and Gram negative bacteria. The prepared cationic compounds were conventional (one cationic head, one non-polar tail), bicephalic (two heads, one tail), or gemini (two heads, two tails) in their amphiphilic structure. Additionally, an isomeric series of six bis-alkylated compounds ranging from symmetric (PQ-11,11) to highly asymmetric (PQ-20,2) were prepared. Four themes of bioactivity emerged: (1) the most bioactive compounds were gemini in structure; (2) 22 carbons in the alkyl chains, with little to modest asymmetry, led to optimal activity; (3) bicephalic compounds were generally comparable to conventional amphiphiles, though only about 12 carbons in the alkyl chains were solubilized in water by each cationic nitrogen; (4) the effects of counterion identity were not evident between chlorides and bromides; however, the presence of the iodide counterion inhibited dissolution in all compounds tested. Three isomeric compounds with little to no asymmetry in tail length, PQ-11,11, PQ-12,10, and PQ-14,8, prepared as the bromide salts, showed comparable and highly potent activity, with MIC levels around 2 μM against 3 of 4 bacteria tested. The simple (one- to two-step) syntheses of potent antimicrobials portend well for future optimization.

Amphiphiles have long served as a privileged platform for antimicrobial agents, from simple soaps and benzalkonium chlorides, to more complex antimicrobial peptides and dendrimeric architectures. While disruption of intracellular processes is key to the bioactivity of some amphiphiles,[1,2] membrane disruption is often the primary pathway leading to microbial death. Membrane targeting can be advantageous in terms of reduced development of bacterial resistance.[3] Our research program aims to develop and understand the physical and biological characteristics of novel cationic polycephalic (multiheaded) amphiphiles—amphiphiles with multiple polar headgroups and a single alkyl chain (Fig. 1). This class of amphiphiles has recently emerged as a particularly efficient group of membrane disruptors, killing bacteria at low concentrations through a mechanism of action that may depend on their cone-shaped structure.[4–8] Mechanistically, the Coulombic attraction of the cationic groups to the anionic surface of bacterial cells likely allows hydrophobic alkyl groups to insert into and disrupt bacterial cell membranes.[1,4,9–11] A recent review points to cationic amphiphiles as a promising motif for antibacterial lead structures.[12]

In 2011, our laboratories reported a series of compounds with strong and broad antibacterial activity, based on a bicephalic amphiphilic arene platform (**1**, Fig. 2).[5] Correlations were drawn between antimicrobial activity and structural characteristics including proximity of the cations, number of cations, and length of chain. Longer alkyl chains corresponded to lower MIC values and shorter time required to kill bacteria; 3,5-substitution patterns on the arene (relative to the alkoxy group) led to optimal activity. Subsequent to this report, we have observed a significant increase in activity when switching the cationic headgroups to pyridinium analogs (manuscript in preparation); this is consistent with numerous reports of the antibacterial activity of pyridinium-based amphiphiles.[6,10,13–17] In order to expand on our preliminary observation that bispyridinium compounds may display strong antimicrobial activity,[18] particularly in the context of amphiphilic

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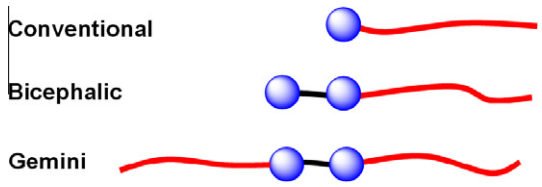


Figure 1. Comparison of amphiphile architectures. Blue sphere = polar head; red line = non-polar tail; black line = linker. Conventional amphiphiles in this work have one cationic head and one tail, bicephalic amphiphiles have two cations and one tail, and gemini amphiphiles have two cations and two tails.

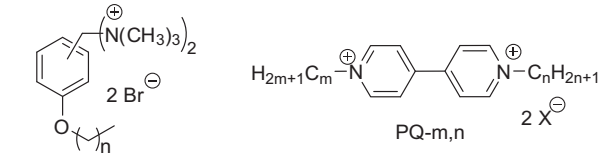


Figure 2. Aryl-based bicephalic amphiphiles (left) and paraquats (right).

structure, we turned our attention towards bisalkyl 4,4′-bipyridinium compounds (**2**). Such 'paraquats' (PQs) are commonly known as viologens[19] due to their tendency to turn violet in color when reduced. Scores of paraquats have been studied in electrochromic and photochromic applications, and are as such exploited in biological systems as sensors of membrane transport and other biological events.[20,21] Amphiphilic paraquats, despite their prevalence, have scarcely been reported for antimicrobial applications. Heptyl viologen (1,1′-diheptyl-4,4′-bipyridinium dibromide, or PQ-7,7)[22] and decyl viologen (PQ-10,10)[23] are reported to exhibit micromolar antibacterial activity; Kourai et al. reported micromolar bioactivity of a series of alkyl bipyridinium diiodides, which are significantly less soluble than salts bearing alternate counterions (vide infra), against a single *Escherichia coli* strain.[24] Intrigued by this pervasive and promising class of amphiphiles, we embarked on a systematic investigation of analogs of the paraquat backbone, to correlate the effects of chain length, counterion, and amphiphilic structure (conventional, bicephalic, or gemini) with bioactivity against Gram positive and Gram negative bacteria.

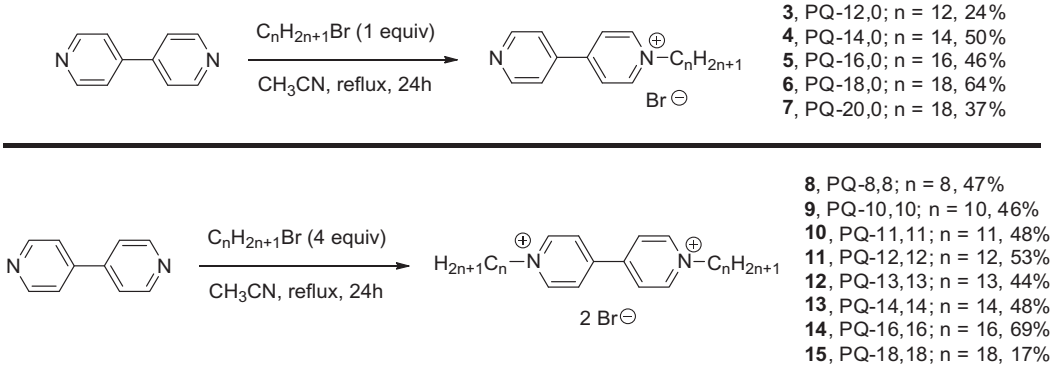
To prepare a substantial set of compounds for bioactivity analysis, we began with the alkylation of the inexpensive 4,4′-bipyridine. Monoalkylation was accomplished via exposure to the corresponding alkyl bromide (1 equiv) in acetonitrile at reflux for 24 h (Scheme 1, top). While minor amounts of the starting materials and bis-alkylated products were generally observed, compounds **3** through **7** were purified by simple vacuum filtration, rinsing, and recrystallization. After purification, yields ranging from 24% to 64% were obtained. Bisalkylation (Scheme 1, bottom) required an increase of equivalents of the alkyl bromide (4 equiv); other conditions remained unchanged and yields were comparable (17–69%). In our hands, microwave conditions led to variable and somewhat diminished yields for these compounds, though reaction times were shorter.[25] Compounds are designated by the shorthand PQ-n,n to indicate the chain lengths of any alkyl groups attached; full synthetic detail for all compounds is presented in the Supplementary data.

In order to prepare bicephalic amphiphilic compounds that had two cationic nitrogens but only one long alkyl chain (and one short chain of 1–2 carbons), we set out to prepare the PQ-n,1 and PQ-n,2 series, where n is the number of carbons in the alkyl group and the second alkyl group is methyl or ethyl, respectively. This began with the monomethylation of 4,4′-bipyridine, which was accomplished by heating to reflux in methyl iodide for one hour (**16**, 99% yield, Scheme 2). Subsequent alkylation with the corresponding longer chain alkyl bromides (C_{12}–C_{20}, 48 h, CH_3CN, Δ) prepared the PQ-n,1 series (**17**–**21**), all of which contain one bromide and one iodide counterion. For the preparation of the PQ-n,2 series, it was found to be more convenient to begin with the alkylated compounds **3**–**6**; exposure of each to bromoethane led to the corresponding PQ-n,2 compounds in 16–60% yields. Microwave irradiation (130–150 °C, 30 min) was employed for the preparation of compounds **23**–**25**.

For preparation of compounds with alternate halogen counterions, 4 equiv of the corresponding alkyl halide were used, as indicated in Scheme 3. As expected, a distinct difference in reactivity was noted between the chloride and iodide. Whereas the alkyl iodides led to alkylation in 63–74% isolated yields in 24 h, the less-reactive chlorides required 7 days to react, and provided the products in very low yields (⩽10%). Alternate solvents (DMF) and heating regimes (microwave) did not improve purified yields, though standard purification conditions provided ample quantities for biological assessment.

Finally, in order to investigate the effect of asymmetry on bioactivity, we prepared a series of isomers with a total of 22 carbons in the alkyl chains: PQ-12,10 (**34**), PQ-14,8 (**35**), PQ-16,6 (**36**), PQ-18,4 (**37**), and PQ-20,2 (**38**). Synthesis of these compounds began with the corresponding monoalkylated species PQ-12,0 through PQ-20,0 (**3**–**7**), and yields ranged from 10% to 68% (Scheme 4).

The compounds prepared above were tested for biological activity against a panel of four bacteria, including both Gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram negative (*Pseudomonas aeruginosa* and *E. coli*) strains. The broth microdilution for determining the MIC of the compounds was performed as previously reported[5]; details are reported in the Supplementary data.



Scheme 1. Synthesis of conventional amphiphiles (PQ-n,0) and gemini amphiphiles (PQ-n,n).