Antimalarial Activity of Compounds Interfering with *Plasmodium falciparum* Phospholipid Metabolism: Comparison between Mono- and Bisquaternary Ammonium Salts‡

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On the basis of a previous structure−activity relationship study, we identified some essential parameters, e.g. electronegativity and lipophilicity, required for polar head analogues to inhibit *Plasmodium falciparum* phospholipid metabolism, leading to parasite death. To improve the in vitro antimalarial activity, 36 cationic choline analogues consisting of mono-, bis-, and triquaternary ammonium salts with distinct substituents of increasing lipophilicity were synthesized. For monoquaternary ammonium salts, an increase in the lipophilicity around nitrogen was beneficial for antimalarial activity: IC_{50} decreased by 1 order of magnitude from trimethyl to tripropyl substituents. Irrespective of the polar head substitution (methyl, ethyl, hydroxyethyl, pyrrolidinium), increasing the alkyl chain length from 6 to 12 methylene groups always led to increased activity. The highest activity was obtained for the *N*,*N*,*N*-tripropyl-*N*-dodecyl substitution of nitrogen (IC_{50} 33 nM). Beyond 12 methylene groups, the antimalarial activities of the compounds decreased slightly. The structural requirements for bisquaternary ammonium salts in antimalarial activity were very similar to those of monoquaternary ammonium salts, i.e. polar head steric hindrance and lipophilicity around nitrogen (methyl, hydroxyethyl, ethyl, pyrrolidinium, etc.). In contrast, with bisquaternary ammonium salts, increasing the lipophilicity of the alkyl chain between the two nitrogen atoms (from 5 to 21 methylene groups) constantly and dramatically increased the activity. Most of these duplicated molecules had activity around 1 nM, and the most lipophilic compound synthesized exhibited an IC_{50} as low as 3 pM (21 methylene groups). Globally, this oriented synthesis produced 28 compounds out of 36 with an IC_{50} lower than 1 μM, and 9 of them had an IC_{50} in the nanomolar range, with 1 compound in the picomolar range. This indicates that developing a pharmacological model for antimalarial compounds through choline analogues is a promising strategy.

Introduction

Malaria has reached epidemic proportions because of the increasing population at risk of the disease, difficulties in eradicating the mosquito vector in the tropics, and increased resistance to the most commonly used antimalarial drugs.[1,2] The high parasite cross-resistance to compounds that are not even structurally or pharmacologically related complicates the problem. New drugs with novel mechanisms of action are thus urgently required.[3]

In this context, only the identification of original target(s) could lead to novel antimalarial compounds which would a priori not allow cross-resistance with preexisting antimalarials. The phospholipid (PL) metabolism of infected erythrocytes is an effective pharmacological target for a new chemotherapy approach because of its specificity and importance for membrane biogenesis and parasite growth. More particularly, phosphatidylcholine (PC) constitutes the major PL of infected erythrocytes (about 50% of total PL). PC is synthesized from polar heads, mainly choline, drawn from plasma, and using the parasite enzymatic machinery.[4−6]

Systematic screening of 80 polar head analogues allowed us to demonstrate that interference with de novo PC biosynthesis from choline is lethal to the parasite, even against chloroquine-resistant parasites. In contrast, these compounds were weakly active in vitro against other eukaryotic cells, suggesting high parasite selectivity.[6] The probable target for these analogues is choline carrier which is a rate-limiting step in PC biosynthesis.[7] This is notably shown by a specific inhibition of de novo PC biosynthesis, as indicated by the early effect on PC biosynthesis and the very close correlation between PL antimetabolic and antimalarial activities. The compounds are specific to mature parasites (trophozoites), i.e. the most intense phase of PL biosynthesis during the erythrocytic cycle.[5,6]

Kinetic properties of the choline carrier have been investigated. It is asymmetric and functions according to a cyclic model with K_t for choline around 10 μM and

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‡ Abbreviations: PC, phosphatidylcholine; PL, phospholipid; (Q)-SAR, (quantitative) structure−activity relationship; IC_{50}, concentration resulting in 50% inhibition of parasite growth.

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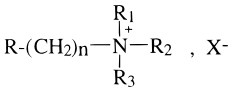
Table 1. Structures and Antimalarial Activities of Monoquaternary Ammonium Salts



compd	R_1	R_2	R_3	*n*	R	X	IC_{50} (μM)
E2a	CH_3	CH_3	CH_3	8	H	Br	5
E70	CH_3	CH_3	CH_3	8	Φ	Cl	0.62
E6*	**CH_3**	**CH_3**	**CH_3**	**12**	**H**	**Br**	**0.5**
E8*	CH_3	CH_3	CH_3	16	H	Br	0.8
E20*	**CH_3**	**CH_3**	**C_2H_5**	**12**	**H**	**Br**	**0.11**
E21*	**CH_3**	**CH_3**	**nC_3H_7**	**12**	**H**	**I**	**0.15**
E22*	**CH_3**	**CH_3**	**nC_4H_9**	**12**	**H**	**I**	**0.26**
E23*	**CH_3**	**CH_3**	**CH_2-CH_2-Br**	**12**	**H**	**Br**	**0.21**
E25	CH_3		$-(CH_2)_4-$	6	H	Br	26
E24	**CH_3**		**$-(CH_2)_4-$**	**12**	**H**	**Br**	**0.22**
E27	CH_3		$-(CH_2)_4-$	14	H	Br	0.27
E26	CH_3		$-(CH_2)_4-$	16	H	Br	0.42
E60	C_2H_5	C_2H_5	C_2H_5	6	H	Br	9.7
E10*	**C_2H_5**	**C_2H_5**	**C_2H_5**	**12**	**H**	**Br**	**0.064**
E11	C_2H_5	C_2H_5	C_2H_5	16	H	Br	0.14
E12	C_2H_5	C_2H_5	C_2H_5	18	H	Br	0.27
E13*	**C_3H_7**	**C_3H_7**	**C_3H_7**	**12**	**H**	**Br**	**0.033**
E35	**CH_3**	**CH_3**	**CH_2-CH=CH_2**	**12**	**H**	**Br**	**0.27**
E36	**CH_3**	**CH_3**	**CH_2-C≡CH**	**12**	**H**	**I**	**0.36**
E34	**CH_3**	**CH_3**	**CH_2-CH_2-C≡CH**	**12**	**H**	**OTs**	**0.23**
E37	**CH_3**	**CH_2CH=CH_2**	**CH_2-CH=CH_2**	**12**	**H**	**Br**	**0.14**
E38	**CH_3**	**CH_2-C≡CH**	**CH_2-C≡CH**	**12**	**H**	**Br**	**0.38**
E30*	**CH_3**	**CH_3**	**$(CH_2)_{11}$-CH_3**	**12**	**H**	**Br**	**0.70**
F2a	CH_3	CH_3	CH_2-CH_2-OH	8	H	Br	3.85
F4*	**CH_3**	**CH_3**	**CH_2-CH_2-OH**	**12**	**H**	**Br**	**0.48**
F25	CH_3	CH_3	CH_2-CH_2-OH	16	H	Br	0.81
F32	CH_3	CH_3	CH_2-CH(CH_3)OH	5	H	Br	115
F30	**CH_3**	**CH_3**	**CH_2-CH(CH_3)OH**	**12**	**H**	**Br**	**0.43**
F50	**CH_3**	**CH_3**	**CH(CH_3)-CH(OH)Φ**	**12**	**H**	**Br**	**0.67**
F8*	**C_2H_5**	**C_2H_5**	**CH_2-CH_2-OH**	**12**	**H**	**Br**	**0.34**
F7*	**CH_3**	**CH_2-CH_2-OH**	**$(CH_2)_{11}$-CH_3**	**12**	**H**	**Br**	**0.84**
F11*	**C_2H_5**	**CH_2-CH_2-OH**	**$(CH_2)_{11}$-CH_3**	**12**	**H**	**Br**	**1.5**

*The activity of these compounds has already been reported.[8] The bold entries are those compounds possessing one or two dodecyl substituents.

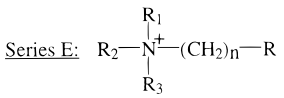
a V_m which is 10-fold increased after infection compared to the noninfected erythrocyte.[7] At the present time, information concerning protein sequence, structural nature, or subcellular localization is not available yet.

Ongoing structure−activity relationship (SAR) studies using autocorrelation vectors and multidimensional analysis indicated that among the relevant parameters for the antimalarial activity of these polar head analogues, the electronegativity and lipophilicity distribution in the molecular space were prominent.[8] *N*-Trimethyl compounds possessing a cationic nitrogen bearing a long lipophilic chain with 10−12 methylene groups were found to be active against *Plasmodium falciparum* in vitro (IC_{50} in the micromolar range).

The present studies were aimed at improving the antimalarial activity, notably by changing the lipophilicity and electronegativity of cationic compounds. For this purpose, 36 new compounds consisting of mono- or bisquaternary ammonium salts and one tri-ammonium with various lipophilic substituents around nitrogen or in the alkyl chain have been synthesized. Ten analogues out of 36 exhibited very potent in vitro antimalarial activity (IC_{50} in the nanomolar range), and one of them with high lipophilicity was active in the picomolar range. The structure of the most active compounds allowed us to develop a more accurate topographic model of the ligand binding site of the choline transporter, the probable target of these drugs, which provides the choline precursor for PC biosynthesis to the parasite.

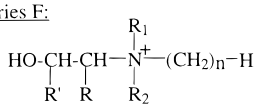
Results

A SAR study previously performed with 80 PL polar head analogues highlighted some essential parameters for in vitro antimalarial activity, i.e. essentially electronegativity and lipophilicity.[6,8] In the present work, to improve antimalarial activity, we thus designed, synthesized, and tested for in vitro antimalarial activity against *P. falciparum* 36 new quaternary ammonium salts with various degrees of lipophilicity. Nineteen compounds were monoquaternary ammonium salts, whose nitrogen atom bears one lipophilic chain (5−18 methylene groups) and three other small alkyl substituents (1−4 carbon atoms), saturated or unsaturated (series E, Table 1), or two small alkyls and one hydroxyalkyl group (series F, Table 1).



R_1, R_2, R_3 = alkyl or alkenyl or alkynyl or R_1= CH_3 and R_2-R_3= tetramethylene
R = H or phenyl
n = 5 to 18

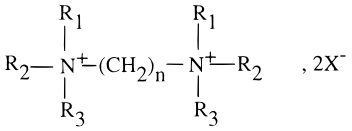
Series F:



R_1, R_2 = alkyl (CH_3 or C_2H_5)
R = H or CH_3; R'= H, CH_3 or C_6H_5
n = 5 to 18

Sixteen compounds were symmetrical bisquaternary ammonium salts, whose nitrogen atoms were linked by a chain of 3−21 methylene groups (Table 2). For 11 of them, other nitrogen substituents were alkyl (and/or

Table 2. Structures and Antimalarial Activities of Bis- and Triquaternary Ammonium Salts

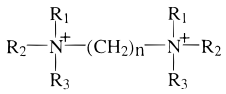


compd	R_1	R_2	R_3	*n*	X	IC_{50} (μM)
G1*	CH_3	CH_3	CH_3	6	Br	700
G4*	CH_3	CH_3	CH_3	12	Br	0.09
G5	**CH_3**	**CH_3**	**CH_3**	**16**	**Br**	**0.004**
G20*	CH_3		$-(CH_2)_4-$	5	$C_4H_5O_6$	650
G23*	CH_3		$-(CH_2)_4-$	10	Br	0.15
G24	CH_3		$-(CH_2)_4-$	12	Br	0.013
G22	CH_3		$-(CH_2)_4-$	14	Br	0.0011
G25	**CH_3**		**$-(CH_2)_4-$**	**16**	**Br**	**0.00064**
G12	C_2H_5	C_2H_5	C_2H_5	8	Br	0.54
G14	C_2H_5	C_2H_5	C_2H_5	12	Br	0.045
G15	**C_2H_5**	**C_2H_5**	**C_2H_5**	**16**	**Br**	**0.0016**
G19	**C_2H_5**	**C_2H_5**	**C_2H_5**	**21**	**Br**	**3×10^{-6}**
G94	CH_3	CH_3	CH_2-C≡CH	12	Br	0.0076
G74	CH_3	CH_3	CH_2-CH_2-C≡CH	12	OTs	0.044
G84	C_2H_5	C_2H_5	CH_2-CH=CH_2	12	Br	0.0053
H5	**C_2H_5**	**C_2H_5**	**CH_2-CH_2-OH**	**16**	**Br**	**0.0049**
G40	CH_3	CH_3	$(CH_2)_{11}$-CH_3	3	Br	10.8
G41	CH_3	CH_3	$(CH_2)_{11}$-CH_3	6	Br	0.22
G44	CH_3	CH_3	$(CH_2)_{11}$-CH_3	12	Br	0.18
G45	CH_3	CH_3	$(CH_2)_{11}$-CH_3	16	I	1.8
TA1	$(C_2H_5)_3N^+$-$(CH_2)_6$-$N^+(C_2H_5)_2$-$(CH_2)_6$-$N^+(C_2H_5)_3$				Br	800

*The activity of these compounds has already been reported.[8] The bold entries are the most active compounds in this series.

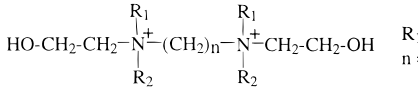
alkenyl) groups lower than 5 carbon atoms (**G5**−**G19, G24, G25, G22, G74**−**G94**). For four other compounds, each nitrogen atom bears a dodecyl substituent and two methyl groups (**G40**−**G45**), and for another each nitrogen atom bears a hydroxyethyl and two ethyl groups (**H5**).

Series G:



R_1, R_2, R_3 = alkyl or alkenyl or alkynyl or R_1= CH_3 and R_2-R_3= tetramethylene
n = 3 to 21

Series H:



R_1, R_2 = alkyl (CH_3 or C_2H_5)
n = 5 to 16

One compound is a triammonium salt (**TA1**, Table 2).

Monoquaternary Ammonium Salts. The electronegativity and lipophilicity distribution in the molecular space was modified in several ways, i.e. by lengthening one or several alkyl chains on the nitrogen atom, by increasing the bulk of the cationic head, by introducing various electronegative groups (bromide or hydroxyl), or by including nitrogen substituents with interesting electronic density, such as unsaturations.

All of the newly synthesized monoquaternary ammonium salts were lethal to *P. falciparum* in vitro, in a dose-dependent manner with IC_{50} values ranging from 115 μM (**F32**) to 0.14 μM (**E11**, **E37**) (Table 1).

(a) Chain-Length Modification. The effects of chain-length modification were studied via stepwise increases in methylene number (from 2 to 18) in one alkyl chain borne by the nitrogen atom. This was performed for six different polar heads: trimethylammonium (plot A), triethylammonium (plot B), *N*-methylpyrrolidinium (plot C), 2-hydroxyethyldimethylammonium (plot D), 2-hydroxyethyldiethylammonium (plot E), and 2-hydroxypropyldimethylammonium (plot F). IC_{50} values are plotted on a logarithmic scale against the lipophilic chain length on the nitrogen atom in Figure 1 for each polar head. Interestingly, irrespective of the cationic head, the plots showed a very close pattern when the alkyl chain length increased (from 2 to 18 carbon atoms), with a sharp decrease in the IC_{50} and maximal inhibitory effect occurring at a carbon chain length of 12, for all compounds. At this chain length, the IC_{50} values of these compounds ranged from 500 nM (for trimethylammonium, **E6**) to 64 nM (for triethylammonium, **E10**). Beyond a chain length of 12 methylene groups, the antimalarial activities of the compounds reached a plateau or slightly decreased, irrespective of the cationic head.

We also synthesized compound **E70** which possesses an electron-rich phenyl ring at the end of an octyl chain, making the substituent roughly the same length as a dodecyl. Interestingly, the antimalarial activity of this compound was very similar to that of the dodecyl analogue (**E70**, 0.62 μM/**E6**, 0.5 μM).

(b) Modification of the Bulk of the Cationic Head. We also studied the effects of varying the nitrogen substituents on antimalarial activity. First, these was a marked similarity in the activity patterns of compounds shown in Figure 1 when other substituents were trimethyl (plot A), dimethylhydroxyethyl (D), diethylhydroxyethyl (E), or dimethyl-2-hydroxypropyl (F). With an *N*-methylpyrrolidinium head (C), activities were slightly improved (at least for 12, 14, or 16 methylenes in the alkyl chain), whereas triethyl substitution (B) significantly increased the activity by 6−8-fold as compared to the trimethyl substitution.

We then synthesized various analogues of monoammonium salts possessing one *N*-dodecyl substituent which was found to be optimal for antimalarial activity (as indicated above). The three other substituents were

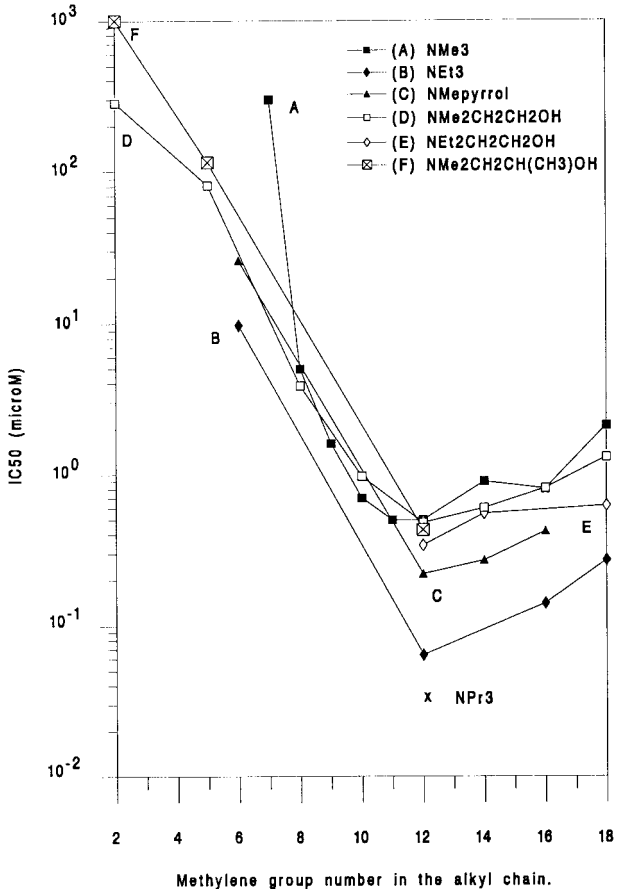


Figure 1. Antimalarial activity of alkyltrimethylammonium salts (plot A), alkyltriethylammonium salts (plot B), *N*-alkyl-*N*-methylpyrrolidinium salts (plot C), alkyl-2-hydroxyethyldimethylammonium salts (plot D), alkyl-2-hydroxyethyldiethylammonium salts (plot E), and alkyl-2-hydroxypropyldimethylammonium salts (plot F) as a function of alkyl chain length. IC_{50} values against in vitro growth of *P. falciparum*, expressed as μM, are from Table 1 and ref 8.

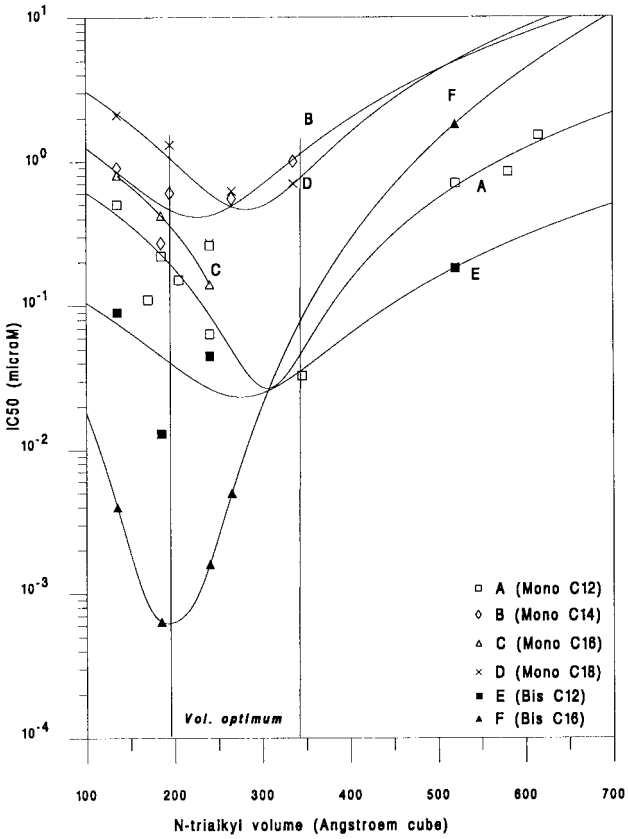


Figure 2. Antimalarial activity of *N*-dodecylammonium salts (plot A), *N*-tetradecylammonium salts (plot B), *N*-hexadecylammonium salts (plot C), *N*-octadecylammonium salts (plot D), 1,12-dodecamethylenebisammonim salts (plot E), and 1,16-hexadecamethylenebisammonim salts (plot F) as a function of the polar head volume. The polar head volume was calculated using a molecular modeling software program (TSAR, Oxford Molecular). The best volume fitting the active site was between 200 and 350 Å^3. The IC_{50} values against in vitro growth of *P. falciparum*, expressed as μM, are from Tables 1 and 2 and ref 8.

either alkyl (**E20**−**E22, E13**) and/or alkenyl (**E35**, **E37**) or alkynyl (**E36**, **E34**, **E38**) of various sizes (1−4 carbon atoms). We also synthesized bulkier ammonium salts bearing two dodecyl groups, with the two other substituents being two methyl groups (**E30**) or one hydroxyethyl group and one short alkyl group (methyl, **F7**, or ethyl, **F11**), or bearing one dodecyl group and one arylalkyl group, with the two other substituents being two methyl groups (**F50**). All of these compounds are shown in bold in Table 1. The IC_{50} values ranged from 1.5 μM (**F11**) to 33 nM (**E13**) and closely depended on the size of the polar head. It should be noted that among these ammonium salts bearing a dodecyl group the activity was not linearly correlated with the volume of the polar head, since both dodecyltrimethylammonium (**E6**, 0.5 μM), bearing the smallest substituents, and compounds bearing the bulkiest substituents (two dodecyl groups for **E30**: 0.7 μM, **F7**: 0.84 μM, and **F11**: 1.5 μM; and a phenyl cycle for **F50**: 0.67 μM) were the least active molecules.

Simultaneous substitution of the three methyl groups (**E6**, 0.5 μM) by three ethyl (**E10**, 64 nM) or three propyl (**E13**, 33 nM) groups markedly improved the activity (10-fold), with the latter being the most active in the monoquaternary ammonium series. Progressive substitution of one methyl group (**E6**, 0.5 μM) by an ethyl (**E20**, 0.11 μM) or a propyl (**E21**, 0.15 μM) group slightly improved the activity. This was also the case when two of the methyl groups were substituted by a cyclic tetramethylene (**E24**, 0.22 μM), which sterically roughly corresponds to the diethyl analogue. However, beyond a certain limit, increasing the nitrogen substituent length decreased the antimalarial activity [0.26 μM for butyl (**E22**), 0.67 μM for **F50**, which roughly corresponds to a hexyl, 0.7 μM for a dodecyl substituent (**E30**); see also **F7,** 0.84 μM/**F4,** 0.48 μM]. A further increase in the steric hindrance at the nitrogen level also led to an increase in the IC_{50} (see **F11**, 1.5 μM/**F7,** 0.84 μM).

Hence, for *N*-dodecyl-substituted monoquaternary ammonium salts, the presence of alkyl groups bulkier than methyl on the nitrogen atom was favorable for the activity up to three methylenes (**E24, E10, E13,** or **E37** compared to **E6**). This was also the case for tetradecyl,[8] hexadecyl (**E8/E11**), and octadecyl[8] substituted monoquaternary ammonium salts.

It thus appears that the volume of the cationic head meets very strict requirements to adapt to the active site. Interestingly, plotting IC_{50} as a function of the polar head volume (Figure 2) revealed a very similar pattern with dodecyl (plot A), tetradecyl (plot B), hexadecyl (plot C), or octadecyl (plot D) trialkylammonium. There was

a sharp decrease in the IC_{50} between 100 and 250 Å^3, and then, the IC_{50} increased again. Clearly, in this context, an additional dodecyl chain could not fit within the active site and was thus excluded (see Discussion). This active site can then be schematized as a sphere whose volume is similar to that of an *N*-tripropyl head (i.e. ~350 Å^3) and therefore whose radius is ~4 Å.

(c) Introduction of Electronegative or Electron-Rich N-Substitutions. Finally, we modified the lipophilicity and electronic density distribution of the *N*-dodecyl-substituted compounds by introducing unsaturations or electronegative groups (hydroxyl or bromine atom). Both modifications decreased the lipophilicity and modified the polarity of compounds relative to the corresponding saturated carbon groups.

The presence of unsaturations in N-substituents, consisting of short alkenyl or alkynyl groups (i.e. containing 3 or 4 carbon atoms) did not significantly change the activity (by less than 3-fold), regardless of whether they were present in one or two nitrogen substituents (compare **E21/E35/E36**, **E37/E38,** or **E22/E34**).

Introduction of a hydroxyl function in one N-substituent decreased the activity by 3−5-fold when dealing with compounds whose polar heads were weakly hindered (up to propyl) (see **E20**, 0.11 μM/**F4**, 0.48 μM; **E10**, 64 nM/**F8**, 0.34 μM; **E21**, 0.15 μM/**F30**, 0.43 μM). In contrast, this had no significant effect for compounds possessing two long *N*-alkyl chains (e.g. dodecyl, see **E30/F7**). In this latter case, it seemed that the deleterious effect of the second dodecyl chain hid other possible adverse effects on the activity.

Finally, in the alkyl chain, the presence of a bromine atom that was less electronegative than a hydroxyl group was only slightly detrimental (see **E23/E21**).

Bisquaternary Ammonium Salts. The pharmacological concept of molecule duplication is often an efficient way to further improve the biological activity of active compounds. We thus designed twin drugs containing two identical pharmacophoric groups, i.e. two quaternary ammonium heads combined covalently in a single molecule.

Bisquaternary ammonium salts, whose chemical structures and IC_{50} values against *P. falciparum* are reported in Table 2, exhibited a wide range of antiplasmodial activities, with IC_{50} values ranging from 7×10^{-4} M (**G1**) to as low as 3×10^{-12} M (**G19**), i.e. up to 4 orders of magnitude lower than the monoquaternary ammonium salts (see above). As for monoquaternary ammonium salts, we investigated the possible beneficial effects of various modifications such as lipophilicity by increasing the inter-nitrogen chain length or by adding electronegative or electron-rich N-substitutions, i.e. introducing unsaturations or hydroxyl groups on the N-substituents of cationic heads.

(a) Chain-Length Modification. As for monoquaternary ammonium salts, we synthesized five series of compounds: trimethylammonium, triethylammonium, *N*-methylpyrrolidinium, 2-hydroxyethyldiethylammonium, and dimethyldodecylammonium. These polar heads were linked by an alkyl chain of 3−21 methylenes. Figure 3 summarizes the present results (Table 2), supplemented with other data obtained previously with compounds with short alkyl chains (less than 12 methylenes) and weaker antimalarial activity.[8] Plots A (trimethylammonium), B (triethylammonium), C (*N*-methylpyrrolidinium), and D (2-hydroxyethyldiethylammonium) show a significant and continuous steep decrease in IC_{50} values, when the methylene group number between the two nitrogen atoms increased. This indicated that the antimalarial activity was closely correlated with the chain length separating the two ammonium groups. Even up to 21 carbon atoms, these plots did not exhibit any plateau, contrary to the corresponding monoquaternary ammoniums (see Figure 1). In fact, with up to 12 carbon atoms in the lipophilic chain, the mono- and bisammonium activity plots merged, but above, the IC_{50} values of monoammonium salts reached a plateau, while the bisammonium IC_{50} values continued to decrease constantly, reaching 3×10^{-12} M for 21 carbon atoms, i.e. for *N*,*N*,*N*,*N*′,*N*′,*N*′-hexaethyl-1,21-heneicosanediaminium (**G19**).

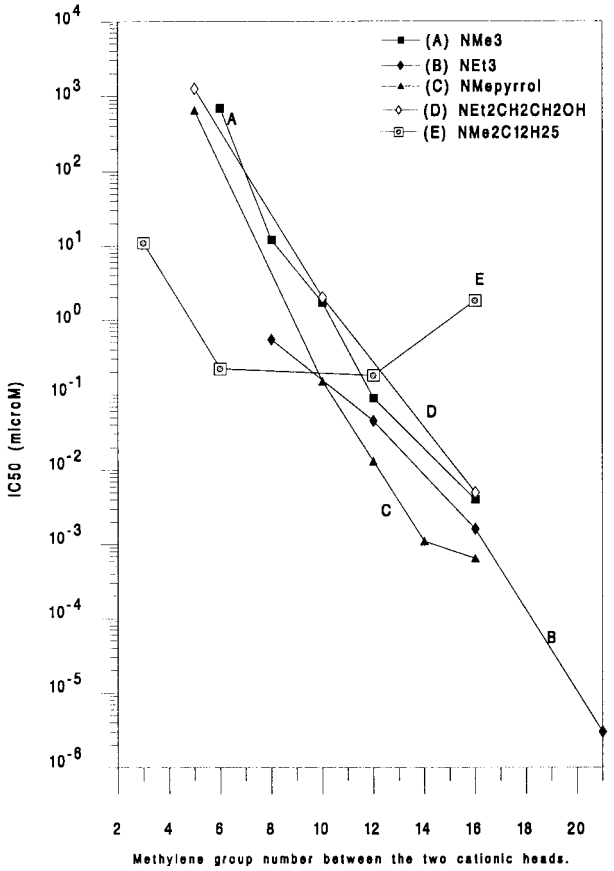


Figure 3. Antimalarial activity of alkylbis(trimethylammonium) salts (plot A), alkylbis(triethylammonium) salts (plot B), alkylbis(*N*-methylpyrrolidinium) salts (plot C), alkylbis(2-hydroxyethyldiethylammonium) salts (plot D), and alkylbis-(dodecyldimethylammonium) salts (plot E) as a function of the methylene group number between the two cationic heads. IC_{50} values against *P. falciparum*, expressed as μM, are from Table 2 and ref 8.

On the other hand, more hindered bisammonium compounds whose nitrogen atoms each bear a second long lipophilic chain (dodecyl) (**G40**−**G45**, Figure 3, plot E) behaved differently. Up to 6 carbon atoms between the two cationic heads, they were more active than other analogues by 3 orders of magnitude (**G41**, 1.22 μM/**G1**, 700 μM). From 12 carbon atoms in the inter-nitrogen alkyl chain, the activity decreased, whereas the activity of compounds with three small substituents on the nitrogen greatly increased (**G45**, 1.8 μM/**G5**, 4 nM).

Hence, for bisdodecyl compounds, the IC_{50} was minimal roughly between 6 and 12 carbon atoms in the interchain, followed by a slight increase, instead of a decrease in the nanomolar range of activity. In fact, for short polymethylene interchains ($n = 6$, **G41**), the compounds roughly behaved as the corresponding monoquaternary ammonium (**E6**, trimethyldodecylammonium). This was probably also the case for shorter lengths ($n = 3$, **G40**), but in this case the IC_{50} was slightly higher than that of **E6**, probably due to mutual steric hindrance of the polar heads which were too close.

Finally, we tested the antimalarial activity of one triquaternary ammonium salt (**TA1**) in which each nitrogen atom was separated by 6 methylene groups. It was found to be far less active (IC_{50} 800 μM) than the bisquaternary ammonium which contained 12 methylenes (**G4**, 0.09 μM). In contrast, the **TA1** IC_{50} was very close to that of the bisquaternary ammonium whose interchain between the two nitrogens possessed 6 methylenes (**G1**, 700 μM).

(b) Modification of the Bulk of the Cationic Head. When we compare bisammonium salts with the same inter-nitrogen atom but different cationic heads, i.e. trimethyl, methylpyrrolidinium, triethyl, diethylhydroxyethyl, or dimethyldodecyl (**G4/G24/G14/G44** or **G5/G25/G15/H5/G45**), we noticed a similar correlation between antimalarial activity and cationic head size, as observed with monoammonium salts (Table 1), but the order changed according to the polar head. For the same inter-nitrogen atom chain (either C_{12} or C_{16}), compounds with a triethylammonium head were about twice as active as their trimethyl analogues (**G14/G4** and **G15/G5**). This factor was around 7 for monoammonium salts (**E10/E6** and **E11/E8**, see also Figures 1 and 3). An *N*-methylpyrrolidinium head instead of a trimethylammonium improved the activity by 6−7-fold (**G24/G4** and **G25/G5**), whereas it slightly increased the activity (by 2-fold) for monoammonium (**E24/E6** or **E26/E8**).

The presence of an additional lipophilic chain ($C_{12}H_{25}$) on each nitrogen atom slightly decreased the activity (**G4/G44**), as observed with monoammonium salts. In contrast, when the interchain had 16 methylenes, the presence of $C_{12}H_{25}$ caused a dramatic decrease in activity (by 450-fold, **G5/G45**). With 1,12-dodecane and 1,16-hexadecane diaminium, plots of IC_{50} as a function of the polar head volume (Figure 2) were similar to those obtained with monoammonium salts, i.e. minimums between 200 and 350 Å^3.

(c) Introduction of Hydroxyl Groups and Unsaturations in N-Substituents. We also investigated the effects of introducing a hydroxyl function or unsaturations in the nitrogen substituents of these duplicated molecules on the antimalarial activity. The presence of a hydroxyl group on one short nitrogen substituent led to a 3-fold reduction of antimalarial activity (**G15/H5**, see also Figure 3), as with monoammonium salts (**E10/F8**, see also Figure 1).

The presence of an unsaturated group (allyl in **G84**, propargyl in **G94**, or but-3-ynyl in **G74**) on the nitrogen atom led to very active compounds (IC_{50} in the nanomolar range). A slight improvement in the activity was noted in comparison to trimethyl (**G4**, 90 nM) or triethyl (**G14**, 45 nM), but in this case the degree of improvement was difficult to accurately determine since the exact saturated counterpart with similar polar head size was not synthesized. Note also that the propargyl substituent (**G94**, 7.6 nM) led to a 6-fold lower activity than butynyl (**G74**, 44 nM), probably due to a higher steric hindrance at the nitrogen level in the latter. Nevertheless, this effect was not observed for monoammonium salts (compare **E36** and **E34**, IC_{50} 0.36 and 0.23 μM, respectively).

Discussion

Extensive fundamental research on intraerythrocytic *Plasmodium* PL metabolism revealed potential targets for chemotherapeutic interference. The most effective interference now appears to be blockage of the choline carrier, a limiting step which gives the parasite the precursor required for the synthesis of PC, the major PL of *Plasmodium*.[4,5] This pharmacological interference was previously validated with a very close correlation between antimalarial activity and inhibition of PL metabolism.[6]

QSAR analyses have indicated that lipophilicity is a major relevant parameter for antimalarial activity.[8] In the present study, 36 original compounds, mono- or biscationic compounds, with various lipophilicities were synthesized to improve in vitro activity. The present data revealed a dramatic improvement in IC_{50}, from 10^{-8} to 10^{-12} M.

The most active monoquaternary monoammonium salts were triethyldodecylammonium bromide (**E10**, 6.4 $\times$ 10^{-8} M) and tripropyldodecylammonium bromide (**E13**, 3.3 $\times$ 10^{-8} M). Bisquaternary ammonium salts exhibited far higher activity than monoammoniums, especially those with a long intercationic chain of more than 12 carbon atoms (10^{-9}−10^{-12} M). It is noteworthy that our oriented chemical syntheses led to eight novel compounds (out of 36 newly synthesized) whose IC_{50} values were lower than 10 nM. They are therefore more active than most current antimalarial compounds, i.e. antifolic/antifolinic or lysosomotropic compounds (e.g. quinine or chloroquine).[9] Only halofantrine[9] and artemisinin analogues[10] exhibited IC_{50} values in a low nanomolar range. Furthermore, some of our analogues in the monoammonium (e.g. **E10, E30, F8**) and bisammonium (e.g. **G5** and **G25**) series were tested against various chemoresistant *P. falciparum* strains or isolates. They showed the same high activity as against sensitive strains. More specifically, **G25** was equally active against the two chloroquine-resistant strains FCR3 and L1 (IC_{50} of chloroquine is 0.8 and 0.9 μM, respectively) with IC_{50} of 3.6 and 1.4 nM, respectively. **G25** was also very active (nanomolar range) against various human isolates, as they are cycloguanil-, chloroquine-, or mefloquine-resistant (data not shown).

This study also provided additional information regarding the active site of the pharmacological target. Our previous study suggested that potent antimalarials are cationic substances with a "small" (not precisely defined) quaternary ammonium head and a long hydrophobic chain (roughly corresponding to 10−12 methylenes), which could combine with an anionic site and a long hydrophobic adjacent region of the target.[8] The results of the present study extend these data and specify the optimum volume of the anionic pocket in which the quaternary ammonium polar head can fit and

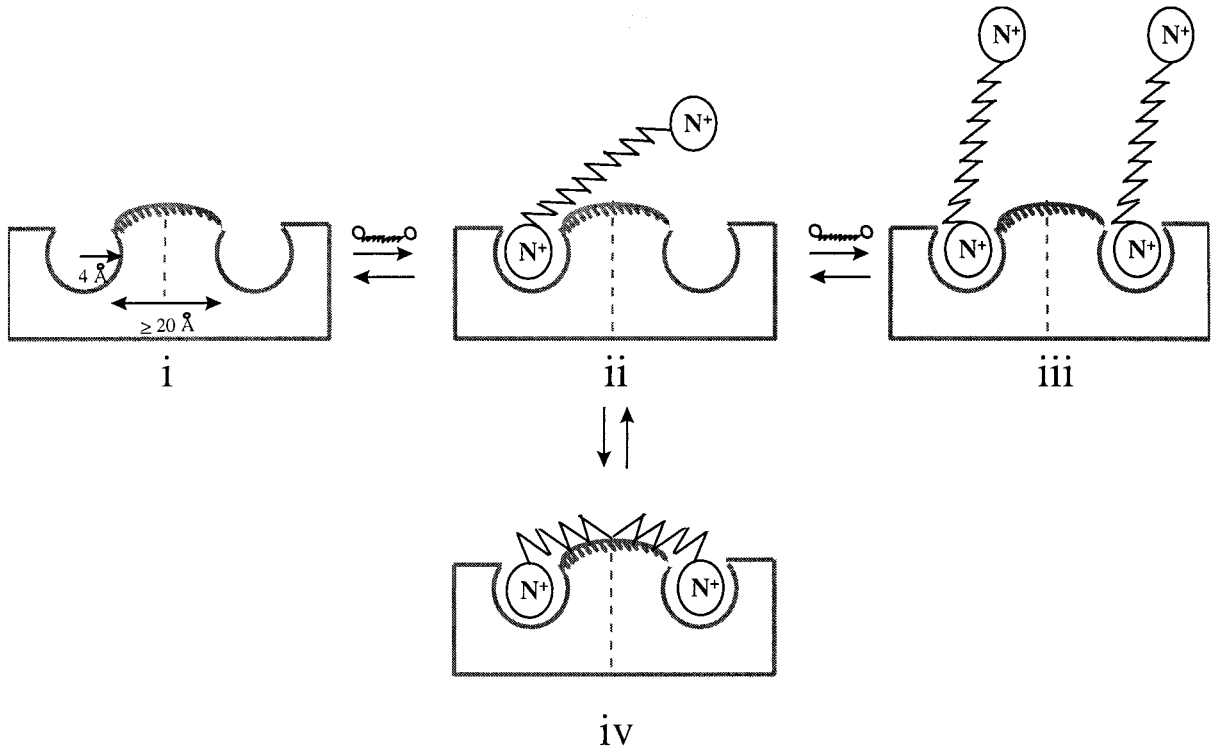


Figure 4. Schematic description of steps involved in the binding of a bivalent ligand to vicinal recognition sites: (i) two unoccupied vicinal sites, (ii) univalent binding of a bivalent ligand, (iii) occupation of vicinal recognition sites by individual bivalent ligands, (iv) bridging of vicinal sites. The sites may or may not be identical. This scheme is adapted from ref 17. For the choline carrier, the active site is schematized by a sphere whose radius is 4 ± 0.4 Å (capable of accommodating up to 3 propyl groups) and the length of the hydrophobic domain (hatched) between the two sites is 20 Å (corresponding to 14 methylene groups).

the nature of the hydrophobic domain. The possible nature of the target is also discussed in the light of data obtained with the most active bisquaternary ammonium salts, thus providing a more accurate definition of the active site.

1. Active Site(s) Accommodate Cationic Ligands (Choline or Quaternary Ammonium). Concerning the strict requirement of a cationic headgroup of our compounds,[8] it is likely that the target has a negative subsite that could involve either carboxylate anions of Asp or Glu or aromatic rings of some amino acids. The presence of aromatic side chains (also called "aromatic basket") in the binding sites for quaternary ammonium ligands appears to be a common property shared by several acetylcholine binding proteins, acetylcholinesterase, and acetylcholine muscarinic and nicotinic receptors and also in the potassium channels which bind tetraethylammonium.[11]

In both mono- and bisquaternary ammonium salts, a moderate increase in lipophilicity around nitrogen was quite beneficial for antimalarial potency, indicating that this pocket could be large enough to accommodate a polar head bigger than a trimethylammonium, e.g. bearing up to three propyl groups (**E13**), corresponding to a globular pocket whose volume ranges from 200 to 350 Å^3 and therefore whose mean radius is ∼4 ± 0.4 Å (see Figure 4).

When steric hindrance was further increased, e.g. presence of a second long lipophilic group (butyl and especially equivalent to hexyl or dodecyl) on the nitrogen atom, one of the chains is probably forced to extend out of the pocket which is energetically more unfavorable, thus leading to decreased affinity. This was especially notable for bisquaternary ammonium with a hexadecylmethylene inter-nitrogen chain, for which the presence of a dodecyl substituent decreased by 450-fold the activity (**G45/G25**).

Hydrogen bonds are probably not required to enable the cationic portion of active molecules to come within the anionic pocket, since long active molecules devoid of a hydroxyl group have the same or better activity than molecules possessing one.

In fact, the affinities of corresponding trimethyl and triethyl (or *N*-methylpyrrolinium) analogues (between 12 and 16 methylenes in the lipophilic chain) almost always differed throughout both series. In the monoammonium series, the improvement was constantly higher when the polar head was an *N*-methylpyrrolidinium (2.5-fold compared with trimethyl) and particularly for a triethyl (8-fold increase) (see Figure 1). This consistency indicated a highly specific binding site and that all the analogues are bound at this site. Affinities are thus not simply determined by the hydrophobic chain length of the analogues, and inhibitors containing the same number of methylene groups may have distinct affinities. For the bisammonium series, a significant difference was also noted, but in this series *N*-methylpyrrolidinium derivatives were constantly more active (11-fold higher compared to trimethyl), whereas *N*-triethyl analogues were only 2-fold more active (see Figure 3). The reason the target better accommodates triethyl groups for monoammonium salts and pyrrolidinium groups for bisammonium salts remains unclear but probably reflects the presence of other constraints imposed by additional interactions of bisquaternary ammonium salts with the target.

The distinct pattern of activity as a function of polymethylene chain length of mono- and bisquaternary ammonium compounds, along with the high potency improvement, may indicate the presence of two active anionic (or electron-rich) pockets in the target (see below). These two active sites could be identical or distinct and located either in two regions of a single protein or on two separate protein subunits. Indeed, some divalent pharmacological ligands can recognize distinct regions in a target,[12] as demonstrated with symmetrical polymethylene tetraamines, with respect to the M2 muscarinic receptor[12] or to the acetylcholine nicotinic receptor protein with quaternary ammonium salts.[11,13]

2. Bridging Two Proximal Active Sites. Dimerization of the quaternary ammonium produced effectors with enhanced antimalarial activity relative to the monomeric congeners, indicating increased affinity. This is a result of the presence of the second nitrogen atom in the molecule, not a nonspecific effect of the hydrophobic spacer.

This huge increase in activity is not unusual for bivalent ligands able to bind adjacent target proteins or subsites,[14−17] regardless of whether they are identical or not (see above). Binding of a bivalent ligand to vicinal recognition sites may involve distinct steps: (i) two unoccupied vicinal sites, (ii) univalent binding of a bivalent ligand via only one pharmacophore, (iii) occupation of vicinal recognition sites by individual bivalent ligands, or (iv) bridging, i.e. simultaneous occupation of vicinal sites (which may or may not be identical) by one bivalent ligand[16−18] (see Figure 4). Proceeding from state (ii) to state (iv) would be more likely than univalent binding of a second bivalent ligand (iii), if the spacer length permits bridging.[17] In this case, a bivalent ligand should, at most, possess an affinity constant equal to the product of the binding contributions of the individual recognition units,[16] i.e. for symmetrical bivalent compounds, the square of the affinity constant of the monoligand if the two sites are identical.

Our results with bisammonium salts are consistent with bridging between proximal sites at a specific spacer length. Bivalent ligands containing 10−12 methylenes had antimalarial potency equivalent to monovalent analogues with the same number of methylene groups, i.e. around 10^{-7}−10^{-8} M (see Tables 1 and 2). With successive additions of methylene units to the spacer (from 14 methylenes), the potency increased markely for bis forms but not for mono forms. Ligands with carbon chains longer than 14 methylene groups (e.g. 20 Å) had the optimal distance to allow simultaneous interaction with the two target binding sites. They could also be folded so as to enable better hydrophobic interactions and greater rigidity than a monoammonium whose carbon chain is barely long enough to span the distance between the two presumed anionic domains.

The highest potency ratio between bis and mono analogues was obtained for 21 methylenes, reaching ca. 10^5−10^6 times that of the monovalent analogue (see **G19**, 3 pM/monoammonium analogue, whose IC_{50} can be extrapolated from Figure 1 as ≈1 μM). Hence, assuming that the IC_{50} reflects the affinity for the target, the bivalent **G19** affinity is roughly equal to the square of the monovalent ligand affinity, i.e. the maximal expected value (see above). This suggests that there was almost optimal docking of the free pharmacophore at this spacer length. It also indicates that this spacer (containing 21 methylene groups) permits bridging of vicinal sites without imposing any translational constraints upon the pharmacophore.[17]

Consequently, the relationship between antimalarial potency improvement and the spacer length of bivalent ligands noted for *P. falciparum*-infected erythrocytes might provide a rough indication of the distance between proximal sites in the bound state. These results are consistent with the bridging of proximal sites when the spanner has a linear length of 28 Å (equivalent to roughly 21 methylenes). The linear spacer length is probably greater than the intersite distance because, after proceeding to state (ii), sufficient translational mobility of the free pharmacophore might be required for docking the proximal recognition site.

3. Lipophilic Domain. The nature and length of the hydrophobic/lipophilic domain adjacent to the anionic/electron-rich active site(s) of the target could also be determined. The **TA1** compound including three quaternary ammoniums separated by six methylenes was not very active (IC_{50} 800 μM). In contrast, a bisammonium analogue with a similar total length, but without any cationic charge between the two nitrogen atoms (**G14**, 0.045 μM), showed very potent antimalarial activity. This suggests that the lipophilic region of the target probably does not bear a negative charge, thus enhancing the ability to bind this domain. Moreover, **E70** which possesses a phenyl ring at the end of an octyl chain had the same activity as its saturated dodecyl analogue (**E6**). This electron-rich phenyl ring therefore does not form additional specific bonds. This suggests that there is no positive charge in the long domain adjacent to the anionic (or electron-rich) pocket. This area is probably neutral and purely hydrophobic.

The length of this domain between two active sites is probably higher than 20 Å, i.e. the length of an alkyl chain containing 14 methylene groups (see above). Figure 4 summarizes the main topographic features of the target in malaria-infected red blood cells, regarding the size of the active sites and the length of the lipophilic domain bridging two active sites.

4. Specificity of Quaternary Ammonium to *P. falciparum*-Infected Erythrocytes. We have already discussed the specificity of structural requirements for inhibiting choline entry into infected erythrocytes relative to other cholinergic systems [notably the high-affinity choline transport (HACT) in synaptosomes].[6] Briefly, discrepancies with other systems concern the steric fit of analogues at the active site, the absence of stereospecificity of choline stereoisomers, and the absence of dramatic antimalarial activity of various well-known cholinergic compounds (e.g. hemicholinium, which is a potent HACT inhibitor).

The present study extends these results. Indeed, the absence of a plateau for antimalarial activity inhibition as a function of the polymethylene chain length of bisammonium (up to 21) (see above and Figure 3) strongly contrasts with the patterns noted for nicotinic receptors or HACT in synaptosomes, for which inhibition was maximal for 8−12 and 16−18 methylenes,

respectively, followed by a decrease.[18,19] This indicates that in both cases the intersite distance is much shorter than in infected erythrocytes.

Assuming that, in *Plasmodium*, the IC_{50} is directly related to the affinity constant *K* for the target[6] (likely the choline carrier of infected erythrocytes[7]), the increment in affinity can be estimated in association with the addition of a CH_2 group to the molecule and compared with other cholinergic models. For the striatal synaptosome HACT, the affinity increased by 1 order of magnitude when the polymethylene chain of the bisammonium salt was increased from 10 to 18 units,[19] as compared to more than 3 orders of magnitude for the activity against *Plasmodium*-infected erythrocytes (see Figure 3). Regarding cholinesterase activity, both absolute values of affinity constants *K* for mono- and bisquaternary ammonium were 2-fold lower[20] than in *Plasmodium*, also indicating a lower affinity for choline esterase than for the malarial choline carrier.

Finally, regarding the steric fit at the active site, bistrimethyl analogues were less active in comparison to other more hindered compounds (bistriethyl, etc.) against *Plasmodium*-infected erythrocytes than against nicotinic receptor.[18]

Tubocurarine, a cholinergic cyclic bisquaternary ammonium salt, was also shown to be the most potent compound possessing nicotinic curare-like activity relative to other bivalent ligands (such as decamethonium).[18] In contrast, tubocurarine exhibited very low antimalarial activity ($IC_{50} = 100\ \mu M$; data not shown, i.e. 100-fold higher than decamethonium).

The marked differences in response, in terms of pattern and relative affinity as a function of polymethylene chain length and of steric fit at the active site, relative to the nitrogen substitution bulk between antimalarial effect and other cholinergic models (e.g. HACT, nicotinic or muscarinic cholinergic receptor, cholinesterase, etc.) reflect considerable differences in these different targets (see above).

In summary, with these compounds, a suitable very high level of activity has now been reached in vitro against *P. falciparum* (eight molecules with an IC_{50} lower than 10 nM). When tested (e.g. **G25**), they were equally potent against various resistant isolates. The compounds also exerted potent antimalarial activity in vivo both against murine malaria in the rodent model and against human malaria in monkeys (manuscript in preparation). The potent in vitro antimalarial activity noted in the present study and the probable specificity of the choline carrier of infected erythrocytes (compared to other cholinergic systems such as HACT in synaptosomes) suggest in vivo specificity. This is supported by the high in vitro therapeutic index against various eukaryotic cells of other analogues in these series.[6] This also concerns some bis analogues of the present study. We have shown that they were much less toxic in vitro against mammalian cells (macrophages, lymphoblastoid cells) than against *P. falciparum* with a differential of activity ranging between 300 (**G12**) and 1.2×10^6 (**G19**) (data not shown). Altogether this indicates a promising future for the development of this new pharmacological model against malaria.

This study provides a rough topographic model of the ligand binding site. The highest activity (IC_{50} lower than 1 nM) was obtained for bisquaternary ammonium salts containing more than 14 methylene groups in the internitrogen spacer. Moreover the longer the hydrophobic alkyl interchain, the better the antimalarial activity (up to 21 methylene groups, **G19**, IC_{50} 3×10^{-12} M). *N*-Methylpyrrolidinium was the polar head that probably fits best into the active site of the target molecule. These results suggest the presence of two anionic sites (with a radius of the globular pocket of 4 ± 0.4 Å) in the target (likely the choline carrier). Between these sites, there is a long hydrophobic domain corresponding to a length of at least 14 methylene groups (higher than 20 Å).

Experimental Section

Chemistry. General Procedure for the Synthesis of Ammonium Quaternary Salts. Most of the quaternary ammonium salts indicated in Table 1 were synthesized by alkylation of a tertiary amine with an alkyl halide in a polar solvent or without solvent. In this way, using the appropriate alkyl bromide, trimethylamine gave **E2a** and **E70**, *N*-methylpyrrolidine led to **E24**−**E27**, triethylamine gave **E60**, **E11,** and **E12**, 3-dimethylaminopropyne provided **E36**, 1-dimethylamino-2-propanol gave **F30** and **F32,** and dimethylaminoethanol gave **F2a** and **F25**. 1-Dimethylaminododecane led to **E35** by action of allyl bromide and to **E34** by reaction with 4-tosylbutyne. **E37** and **E38** were produced by reaction, under basic conditions, of *N*-methyldodecylamine with an excess of 3-bromopropene and 3-bromopropyne, respectively.

Similarly, most of the bisquaternary ammonium salts (Table 2) were obtained by alkylation of a tertiary amine with an alkyl dihalide. Action of the suitable α,ω-dihaloalkane in ethanol with an excess of (a) trimethylamine yielded **G5**, (b) triethylamine led to **G12**, **G14**, **G15,** and **G19**, (c) *N*-methylpyrrolidine gave **G23**−**G25**, (d) 3-dimethylaminopropyne led to **G94**, (e) 2-diethylaminoethanol gave **H5**, and (f) 1-dimethylaminododecane yielded **G40**, **G41**, **G44,** and **G45**. Finally, **G74** was synthesized by reaction of *N*,*N*,*N*′,*N*′-tetramethyldiaminododecane with an excess of 4-tosylbutyne, and **G84** was obtained by alkylation of *N*,*N*,*N*′,*N*′-tetraethyldiaminododecane with allyl bromide.

Pentamethylenebis(1-methylpyrrolidinium) oxalate (**G20**) and *N*-dodecyl-*N*-methylephedrinium bromide (**F50**) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). 1,-14-Tetradecamethylenebis(*N*-methylpyrrolidinium) dibromide (**G22**) was synthesized and provided by Virbac laboratories.

Melting points were determined in capillary tubes on a Thomas-Hoover apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Brucker AC 250 (250 MHz). Chemical shifts (δ) are expressed in ppm downfield from the internal standard tetramethylsilane (TMS). Elemental analyses were within ±0.4 of calculated values. Yields, melting points, and ^{1}H NMR data of products are reported in Table 3.

***N*,*N*,*N*-Trimethyl-1-octanaminium Bromide (E2a), *N*,*N*,*N*-Trimethyl-1-(8-phenyl)octanaminium Chloride (E70), and *N*,*N*,*N*,*N*′,*N*′,*N*′-Hexamethyl-1,16-hexadecanediaminium Dibromide (G5).** 10 mmol of halogenated derivative (1-bromooctane for **E2a**, 1-choro-8-phenyloctane for **E70**) or dihalogenated derivative (1,16-dibromohexadecane for **G5**) was added to 25 mL solution of methylamine in ethanol (33%). The mixture was heated under reflux for 12 h. The solvent was removed and the salt was recrystallized from a mixture of acetone and diethyl ether. **E2a**: Anal. ($C_{11}H_{26}NBr$) C, H, N. **E70**: Anal. ($C_{17}H_{30}NCl$) C, H, N. **G5**: Anal. ($C_{22}H_{50}N_2Br_2$) C, H, N.

***N*-Methyl-*N*-hexylpyrrolidinium Bromide (E25), *N*-Methyl-*N*-dodecylpyrrolidinium Bromide (E24), *N*-Methyl-*N*-tetradecylpyrrolidinium Bromide (E27), *N*-Methyl-*N*-hexadecylpyrrolidinium Bromide (E26), 1,12-Dodecamethylenebis(*N*-methylpyrrolidinium) Dibromide (G24), and 1,16-Hexadecamethylenebis(*N*-methylpyrrolidinium) Dibromide (G25).** 5 g (60 mmol) of *N*-

Table 3. Physicochemical and Spectral Data for Synthesized Compounds

compd	yield (%)	mp (°C)	^{1}H NMR (δ in ppm)
E2a	48	201	0.90 (t,3H); 1.05 (m,10H); 1.5 (m,2H); 3.2 (s,9H); 3.3 (m,2H)[a]
E70	86	215−220	1.2 (m,8H); 1.5 (m,2H); 1.6 (m,2H); 2.5 (t,2H); 3.4 (s,9H); 3.5 (m,2H); 7.1 (m,3H); 7.2 (m,2H)[a]
E25	70	74	0.70 (t,3H); 1.1 (m,6H); 1.5 (m,2H); 2.0 (m,4H); 3.1 (s,3H); 3.35 (m,2H); 3.5 (m,4H)[a]
E24	52	186	0.80 (t,3H); 1.2 (m,18H); 1.65 (m,2H); 2.2 (m,4H); 3.2 (s,3H); 3.6 (m,2H); 3.7 (m,4H)[a]
E27	61	204	0.65 (t,3H); 1.05 (m,22H); 1.55 (m,2H); 2.05 (m,4H); 3.05 (s,3H); 3.45 (m,2H); 3.6 (m,4H)[a]
E26	72	209	0.78 (t,3H); 1.15 (m,26H); 1.7 (m,2H); 2.23 (m,4H); 3.21 (s,3H); 3.55 (m,2H); 3.75 (m,4H)[a]
E60	51	104	0.8 (t,3H); 1.4 (m,15H); 1.6 (m,2H); 3.3 (m,8H)[a]
E11	61	155	0.8 (t,3H); 1.4 (m,35H); 1.7 (m,2H); 3.3 (m,8H)[a]
E12	38	160	0.8 (t,3H); 1.3 (m,39H); 1.6 (m,2H); 3.2 (m,8H)[a]
E35	62	63	0.9 (t,3H); 1.3 (m,18H); 1.8 (m,2H); 3.4 (s,6H); 3.5 (m,2H); 4.4 (d,2H); 5.8 (m,2H); 6,0 (m,1H)[a]
E36	65	70	0.8 (t,3H); 1.2 (m,18H); 1.7 (m,2H); 3.0 (t,1H);3.35 (s,6H); 4.0 (t,2H); 4.7 (d,2H)[a]
E34	61	105	0.8 (t,3H); 1.15 (m,18H); 1.55 (m,2H); 2.15 (t,1H); 2.3 (s,3H); 2.7 (td,2H); 3.2 (s,6H); 3.3 (m,2H); 3.5 (t,2H); 7.1 (d,2H); 7.7 (d,2H)[a]
E37	65	20	0.8 (t,3H); 1.25 (m,18H); 1.7 (m,2H); 3.2 (s,3H); 3.3 (m,2H); 4.25 (m,4H); 5.7 (m,4H); 5.9 (m,2H)[a]
E38	70	49−51	0.9 (t,3H); 1.3 (m,18H); 1.9 (m,2H); 3.0 (t,2H); 3.5 (s,3H); 3.7 (m,2H); 4.7 (m,4H)[a]
F2a	70	116−118	0.80 (t,3H); 1.2 (m,10H); 1.6 (m,2H); 3.2 (s,6H); 3.4 (m,2H); 3.55 (m,2H); 3.9 (m,2H); 4.85 (t,1H)[a]
F25	68	203−204	0.90 (t,3H); 1.32 (m,26H); 1.75 (m,2H); 3.41 (s,6H); 3.60 (m,2H); 3.80 (m,2H); 4.15 (m,2H); 5.10 (m,1H)[c]
F32	43	hygroscopic	0.8 (t,3H); 1.2 (d,3H); 1.3 (m,4H); 1.7 (m,2H); 3.3 (s,6H); 3.4 (m,2H); 3.8 (m,2H); 4.4 (m,1H); 4.9 (m,1H)[a]
F30	73	60	0.8 (t,3H); 1.2 (d,3H); 1.3 (m,18H); 1.7 (m,2H); 3.3 (s,6H) 3.4 (m,2H);3.8 (m,2H); 4.4 (m,1H); 5.0 (m,1H)[a]
G5	61	227−230	1.4 (m,24H); 1.8 (m,4H); 3.2 (s,18H); 3.3 (t,4H)[b]
G12	88	130 (dec)	1.4 (m,18H); 1.5 (m,8H); 1.85 (m,4H); 3.7 (m,16H)[b]
G14	52	162	1.3 (m,16H); 1.4 (t,18H); 1.7 (m,4H); 3.5 (m,16H)[b]
G15	70	198	1.25 (m,18H); 1.38 (m,24H); 1.82 (m,4H); 3.32 (m,16H)[b]
G19	60	205	1.2 (s,34H); 1.35 (t,18H); 1.5 (m,4H); 3.4 (m,16H)[a]
G24	67	204	1.40 (m,16H); 1.85 (m,4H); 2.2 (m,8H); 3.15 (s,6H); 3.55 (m,12H)[b]
G25	63	180	1.45 (m,24H); 1.93 (m,4H); 2.38 (m,8H); 3.20 (s,6H); 3.63 (m,12H)[b]
G94	50	145	1.3 (m,16H); 1.7 (m,4H); 3.15 (s,12H); 3.40 (m,4H); 4.1 (t,2H); 4.5 (d,4H)[a]
G74	10	170	1.3 (m,16H); 1.65 (m,4H); 2.3 (s,6H); 2.75 (td,4H); 3.1 (t,2H); 3.25 (m,4H); 3.45 (t,4H); 7.1 (d,2H); 7.5 (d,2H)[a]
G84	75	pasty	1.2 (t,12H); 1.3 (m,16H); 1.6 (m,4H); 3.15 (m,4H); 3.25 (q,8H); 4.0 (d,4H); 5.6 (m,4H); 6.0 (m,2H)[a]
H5	60	120	1.45 (m,36H); 1.85 (m,4H); 3.5 (m,16H); 4.15 (t,4H)[b]
G40	70	195	0.8 (t,6H); 1.25 (m,36H); 1.75 (m,4H); 2.75 (m,2H); 3.5 (s,12H); 3.9 (m,8H)[a]
G41	80	235	0.9 (t,6H); 1.3 (m,40H); 1.65 (m,4H); 1.75 (m,4H); 3.5 (s,12H); 3.7 (m,8H)[a]
G44	80	235	0.8 (t,6H); 1.3 (m,52H); 1.7 (m,4H); 1.8 (m,4H); 3.5 (s,12H); 3.7 (m,8H)[a]
G45	76	128	0.9 (t,6H); 1.3 (m,60H); 1.65 (m,4H); 1.75 (m,4H); 3.6 (s,12H); 3.7 (m,8H)[a]
TA1	45	262	1.5 (m,40H); 3.3 (m,24H)[b]

[a] In $CDCl_3$. [b] In D_2O. [c] In DMSO-d_6.

methylpyrrolidine was added to 1.1 equiv of halogenated derivative (1-bromohexane for **E25**, 1-bromododecane for **E24**, 1-bromotetradecane for **E27**, 1-bromohexadecane for **E26**) or to 0.55 equiv of dihalogenated derivative (1,12-dibromododecane for **G24,** 1,16-dibromohexadecane for **G25**) in 100 mL of ethanol. The solution was heated under reflux for 24 h. The solvent was removed and the salt was recrystallized from a mixture of methanol and diethyl ether. **E25**: Anal. ($C_{11}H_{24}$NBr) C, H, N. **E24**: Anal. ($C_{17}H_{36}$NBr) C, H, N. **E27**: Anal. ($C_{19}H_{40}$NBr) C, H, N. **E26**: Anal. ($C_{21}H_{44}$NBr) C, H, N. **G24**: Anal. ($C_{22}H_{46}N_2Br_2$) C, H, N. **G25**: Anal. ($C_{26}H_{54}N_2Br_2$) C, H, N.

***N,N,N*-Triethyl-1-hexanaminium Bromide (E60), *N,N,N*-Triethyl-1-hexadecanaminium Bromide (E11), and *N,N,N*-Triethyl-1-octadecanaminium Bromide (E12).** 5 g (50 mmol) of triethylamine was added to 1.1 equiv of halogenated derivative (1-bromohexane for **E60**, 1-bromohexadecane for **E11**, 1-bromooctadecane for **E12**) in 100 mL of ethanol. The solution was heated under reflux for 18 h. The solvent was removed and the salt was recrystallized from a mixture of methanol and diethyl ether or ethanol and diethyl ether. **E60**: Anal. ($C_{12}H_{28}$NBr) C, H, N. **E11**: Anal. ($C_{22}H_{48}$NBr) C, H, N. **E12**: Anal. ($C_{24}H_{52}$NBr) C, H, N.

***N,N,N,N′,N′,N′*-Hexaethyl-1,8-octanediaminium Dibromide (G12), *N,N,N,N′,N′,N′*-Hexaethyl-1,12-dodecanediaminium Dibromide (G14), *N,N,N,N′,N′,N′*-Hexaethyl-1,16-hexadecanediaminium Dibromide (G15), and *N,N,N,N′,N′,N′*-Hexaethyl-1,21-heneicosanediaminium Dibromide (G19).** 10 g (0.1 mol) of triethylamine was added to a solution of 25 mmol of dihalogenated derivative (1,8-dibromooctadecane for **G12**, 1,12-dibromododecane for **G14**, 1,16-dibromohexadecane for **G15,** 1,21-dibromoheneicosane[21] for **G19**) in 100 mL of ethanol. The solution was heated under reflux for 18 h. The solvent was removed and the salt recrystallized from a mixture of methanol and diethyl ether or ethanol and diethyl ether or acetone and diethyl ether. **G12**: Anal. ($C_{20}H_{46}N_2Br_2$) C, H, N. **G14**: Anal. ($C_{24}H_{54}N_2Br_2$) C, H, N. **G15**: Anal. ($C_{28}H_{62}N_2Br_2$) C, H, N. **G19**: Anal. ($C_{32}H_{72}N_2Br_2$) C, H, N.

***N,N*-Dimethyl-*N*-(2-propynyl)-1-dodecanaminium Iodide (E36).** 20 mmol of 1-iodododecane was added to a solution of 15 mmol of 3-dimethylaminopropyne in 60 mL of anhydrous acetone. The solution was heated under reflux for 24 h; the solvent was removed and the salt recrystallized from acetone. Anal. ($C_{17}H_{34}$NI) C, H, N.

***N,N*-Dimethyl-*N*-(2-hydroxypropyl)-1-pentanaminium Bromide (F32) and *N,N*-Dimethyl-*N*-(2-hydroxypropyl)-1-dodecanaminium Bromide (F30).** 20 mmol of halogenated derivative (1-bromopentane for **F32**, 1-bromododecane for **F30**) was added to a solution of 15 mmol of 1-dimethylamino-2-propanol in 50 mL of ethanol. The solution was heated under reflux for 12 h; the solvent was removed and the salt recrystallized from ethanol−diethyl ether. **F32**: Anal. ($C_{10}H_{24}$NOBr) C, H, N. **F30:** Anal. ($C_{17}H_{38}$NOBr) C, H, N.

***N*-(2-Hydroxyethyl)-*N,N*-dimethyl-1-octanaminium Bromide (F2a) and *N*-(2-Hydroxyethyl)-*N,N*-dimethyl-1-hexadecanaminium Bromide (F25).** 0.12 mol of 2-(dimethylamino)ethanol and 0.11 mol of the corresponding brominated derivative (1-bromooctane for **F2a**, 1-bromohexadecane for **F25**) were added to 100 mL of methanol and heated under reflux conditions for 20 h. The solvent was removed and the crude product recrystallized from a mixture of methanol and diethyl ether. **F2a**: Anal. ($C_{12}H_{28}$NOBr) C, H, N. **F25**: Anal. ($C_{16}H_{36}$NOBr) C, H, N.

***N,N*-Dimethyl-*N*-(2-propenyl)-1-dodecanaminium Bromide (E35).** 10 mmol of 3-bromopropene was added to a

solution of 15 mmol of dimethyldodecylamine in 50 mL of ethanol. The solution was heated under reflux for 24 h; the solvent was removed and the salt recrystallized from acetone−diethyl ether. Anal. ($C_{17}H_{36}NBr$) C, H, N.

***N,N*-Dimethyl-*N*-(3-butynyl)-1-dodecanaminium Tosylate (E34).** 16.3 g (85.5 mmol) of 4-methylbenzenesulfonyl chloride was added to a solution of 3 g (43 mmol) of 3-butyn-1-ol in 135 mL of anhydrous pyridine at 0 °C. The reaction mixture was kept at 0 °C for 12 h and poured in 250 mL of cold water. The mixture was extracted with three portions of diethyl ether, washed with diluted HCl then with water, dried over $MgSO_4$, and evaporated under reduced pressure. 5.5 g of 3-butynyltosylate (57%) was obtained as an oil, which was used in the next step. The previously obtained tosylate was added to 1.5 equiv of dimethyldodecylamine in 50 mL of acetone. The solution was heated under reflux for 24 h; the solvent was removed and the salt recrystallized from acetone. Anal. ($C_{25}H_{43}NO_3S$) C, H, N.

***N,N*-Di(2-propenyl)-*N*-methyl-1-dodecanaminium Bromide (E37) and *N,N*-Di(2-propynyl)-*N*-methyl-1-dodecanaminium Bromide (E38).** 30 mmol of appropriate halogenated derivative (3-bromopropene for **E37**, 3-bromopropyne for **E38**) was added to a solution of 10 mmol of methyldodecylamine and excess Na_2CO_3 in 50 mL of acetonitrile. The solution was heated under reflux for 24 h. The reaction mixture was cooled; the precipitate was filtered and the filtrate evaporated. The residue was recrystallized from a mixture of dichloromethane−diethyl ether. **E37**: Anal. ($C_{19}H_{38}NBr$) C, H, N. **E38**: Anal. ($C_{19}H_{34}NBr$) C, H, N.

***N,N,N′,N′*-Tetraethyl-*N,N′*-di(2-propenyl)-1,12-dodecanediaminium Dibromide (G94).** A mixture of 2 g (6 mmol) of 1,12-dibromododecane and 1.5 g (18.3 mmol) of 1-dimethylamino-2-propyne in 15 mL of acetone was heated under reflux for 48 h. The white precipitate was filtered. Anal. ($C_{22}H_{42}N_2Br_2$) C, H, N.

***N,N,N′,N′*-Tetramethyl-*N,N′*-di(3-butynyl)-1,12-dodecanediaminium Ditosylate (G74).** A mixture of 3 g (15 mmol) of 1,12-diaminododecane and 7.5 g (250 mmol) of paraformaldehyde in 250 mL of methanol was heated under reflux for 1 h. After cooling, 3.6 g (95 mmol) of $NaBH_4$ was added, and the mixture was stirred for 1 h at room temperature; then 100 mL of water was added. The excess paraformaldehyde was filtered and the filtrate extracted with three portions of dichloromethane, washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The oily residue was diluted with 50 mL of diethyl ether, in which HCl gas was bubbled. The precipitate of 1,12-bis(dimethylamino)-dodecane dichlorhydrate was filtered and recrystallized from acetone. Yield: 10%. Mp:169 °C.

1,12-Bis(dimethylamino)dodecane was regenerated by treatment in basic conditions, and 0.3 g (1 mmol) of this was refluxed with 0.57 g (2.5 mmol) of 3-butynyl tosylate in 10 mL of acetone for 12 h; the solvent was removed and the salt recrystallized from dichloromethane−diethyl ether. Anal. ($C_{38}H_{60}N_2O_6S_2$) C, H, N.

***N,N,N′,N′*-Tetramethyl-*N,N′*-di(2-propenyl)-1,12-dodecanediaminium Dibromide (G84).** A mixture of 5 g (15 mmol) of 1,12-dibromododecane and 11.1 g (150 mmol) of diethylamine in 75 mL of ethanol was heated under reflux for 12 h. The solvent was removed and the residue diluted with 100 mL of diethyl ether, in which HCl gas was added. The precipitate of 1,12-bis(diethylamino)dodecane dichlorhydrate was filtered and recrystallized from acetone. Yield: 55%. Mp: 170 °C.

1,12-Bis(diethylamino)dodecane was regenerated by treatment under basic conditions, and 1.4 g (4.4 mmol) of this was refluxed with 1.62 g (13.3 mmol) of 3-bromopropene in 15 mL of acetone for 48 h; the solvent was removed and the pasty residue could not be recrystallized. Anal. ($C_{26}H_{54}N_2Br_2$) C, H, N.

***N,N,N′,N′*-Tetraethyl-*N,N′*-di(2-hydroxyethyl)-1,16-hexadecanediaminium Dibromide (H5).** 1 g (2.6 mmol) of 1,16-dibromohexadecane was refluxed with 2 mL of 2-diethylaminoethanol for 3 h; the excess amine was evaporated under reduced pressure and the salt recrystallized from ethanol−diethyl ether. Anal. ($C_{28}H_{62}N_2O_2Br_2$) C, H, N.

***N,N,N′,N′*-Tetramethyl-*N,N′*-didodecyl-1,3-propanediaminium Dibromide (G40), *N,N,N′,N′*-Tetramethyl-*N,N′*-didodecyl-1,6-hexanediaminium Dibromide (G41), *N,N,N′,N′*-Tetramethyl-*N,N′*-didodecyl-1,12-dodecanediaminium Dibromide (G44), and *N,N,N′,N′*-Tetramethyl-*N,N′*-didodecyl-1,16-hexadecanediaminium Diiodide (G45).** 1 equiv of the appropriate dihalogenated derivative (1,3-dibromopropane for **G40**, 1,6-dibromohexane for **G41**, 1,12-dibromododecane for **G44**, 1,16-diiodohexadecane for **G45**) was refluxed for 48 h with 3 equiv of dimethyldodecylamine in ethanol. The solvent was evaporated and the salt recrystallized from dichloromethane−diethyl ether. **G40**: Anal. ($C_{31}H_{68}N_2Br_2$) C, H, N. **G41**: Anal. ($C_{34}H_{74}N_2Br_2$) C, H, N. **G44**: Anal. ($C_{40}H_{86}N_2Br_2$) C, H, N. **G45**: Anal. ($C_{44}H_{94}N_2I_2$) C, H, N.

***N,N,N,N′,N′,N″,N″,N″*-Octaethylbis(hexamethylene)-triaminium Tribromide (TA1).** 5 g (23.3 mmol) of bis-(hexamethylene)triamine, 25 mL (340 mmol) of diisopropylethylamine, and 15.3 g (140 mmol) of bromoethane were refluxed for 72 h in 25 mL of ethanol. The solvent and the excess bromoethane were evaporated, and the salt was recrystallized from dichloromethane−diethyl ether. Anal. ($C_{28}H_{64}N_3Br_3$) C, H, N.

Biological Activity. Human blood or AB human serum came from the local blood bank. The Nigerian strain of *P. falciparum*[22] was maintained at 37 °C by serial passages in human erythrocytes suspended in Hepes-buffered RPMI 1640 (Gibco, France) supplemented with 10% AB human serum using the Petri dish candle jar method.[23]

The antimalarial activity of the compound was measured in microtiter plates according to the method of Desjardins et al.[24] The final volume of the incubation medium in each well was 200 μL and the hematocrit of the *P. falciparum*-infected erythrocyte suspension was 1−2%, with an initial parasitemia (i.e. the percentage of infected erythrocytes) of 0.3−0.5%. In some cases, the drugs were dissolved in DMSO and then further diluted in culture medium so that the final DMSO concentration never exceeded 0.25%. After contact of the drug with the parasite for one parasite cycle (48 h), [^{3}H]hypoxanthine (Amersham, France) was added for 18 h to assess parasite viability. The results are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth and are means of at least two independent experiments performed in triplicate using different drug stock solutions. Inside each experiment, standard deviation was always lower than 20% of the mean (intraexperiment) and when comparing the two mean values for each experiment (interexperiment) they did not differ by more than 50% (mean values of IC_{50} do not differ significantly using Student's *t* test). In the few cases for which the two means differed by more than 50%, a third experiment was performed to ascertain the value.

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