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A Structure–Activity Study of Spermicidal and Anti-HIV Properties of Hydroxylated Cationic Surfactants

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Abstract—The syntheses of 2-hydroxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethylhexadecan-1-aminium chloride [**1**(16)Cl] and iodide [**1**(16)I], 2-hydroxy-*N*,*N*,*N*-trimethylhexadecan-1-aminium chloride (**6**), *N*-(2-hydroxyethyl)-*N*,*N*-dimethylhexadecan-1-aminium chloride (**8**), *N*,*N*-bis(2-hydroxyethyl)-*N*-methylhexadecan-1-aminium chloride (**11**), and 2-hydroxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-4-oxahexadecan-1-aminium chloride (**14**) are reported along with the critical micelle concentrations (cmcs), as measured by conductivity at 25 °C, of **1**(16)Cl, **1**(16)I, **6**, **8**, **11**, and *N*,*N*,*N*-trimethylhexadecan-1-aminium chloride (**12**). All compounds display spermicidal and virucidal activity. A plot of minimum effective concentration (MEC) in the Sander–Cramer spermicidal assay and cmc shows that **1**(16)Cl and **6** have the best spermicidal activity and highest cmcs. Compounds **8**, **11**, and **1**(16)Cl are the most active at 0.05 mg mL^{-1} against cell-free and cell-associated virus. In conclusion, **1**(16)Cl shows the best combination of dual activity against sperm and HIV; it is a promising candidate for further preclinical studies as a topical, contraceptive microbicide.

Introduction

The worldwide increase in human-immunodeficiency-virus (HIV) infections among women[1] has made developing user-controlled, topical vaginal microbicides that provide protection against sexually transmitted pathogens an urgent global priority.[2] Most heterosexual women want to reduce the risk of acquiring a sexually transmitted disease;[3] many want to control their fertility. New dual-active agents (spermicide and microbicide) that do not have the disadvantages of nonoxynol-9 (N-9), currently the most widely used spermicide in the United States, occupy the efforts of several research groups.[4–11] These new agents must be safe, effective, acceptable, and affordable.[12]

N-9, a detergent that disrupts cell walls by solubilizing membranes, has several disadvantages. N-9 increases the risk of urinary tract infections,[13,14] vulvovaginal candidiasis,[15] and genital ulcers.[16] As human trials show, N-9 may increase the risk of HIV transmission,[17] perhaps by initiating interleukin-1-mediated NF-kB activation, which leads to cytokine-induced recruitment of HIV-1 host cells and increased HIV-1 replication.[18] N-9, a mixture of oligomers,[19–23] may violate future federal regulations; pure compounds or mixtures whose individual components have met safety standards will become the norm. Furthermore, the breakdown products of N-9 pose serious health and environmental risks.[24]

Quaternary ammonium salts, well-known microbicides,[25] can act as spermicides (e.g., European and Canadian spermicidal products contain benzalkonium chloride, a popular microbicide). Although comparatively less spermicidal than N-9,[26] benzalkonium chloride has potential as an anti-HIV, topical agent.[27] Alkyl quaternary ammonium salts have potent microbicidal activity, but they have modest solubility in water and can irritate sensitive tissue. We aim to find or synthesize quaternary ammonium salts that minimize these shortcomings,

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especially active compounds that (1) have high critical micelle concentrations (cmcs) and (2) that can be made relatively inexpensively.

Our recent report[11] shows that the water-soluble quaternary ammonium bromides, **1**(p)Br and **2**(p)Br, act as spermicides and anti-HIV agents. The best compounds — **1**(17)Br and **1**(18)Br — have spermicidal activities at minimum effective concentrations (MECs) slightly lower than that of N-9. Compounds **1**(16)Br and **2**(17)Br show the best anti-HIV activity in in-vitro, cell-free and cell-associated virus inactivation assays. Compound **1**(16)Br shows the best combination of dual activity against sperm and HIV.

In this paper, we report a limited structure–activity study, using **1**(16)Br as the lead compound. We use spermicidal and anti-HIV assays to probe structure–activity as a function of different halide counterions, the amount of hydroxylation, and oxygen replacement in the hexadecyl chain. For the most active compounds, we compare the minimum effective concentration (MEC) in the spermicidal assay to the cmc.

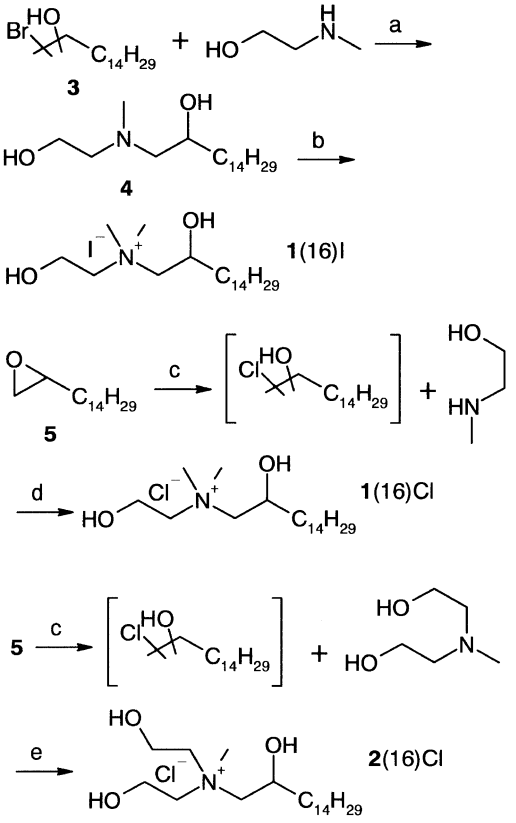
Results

Chemistry

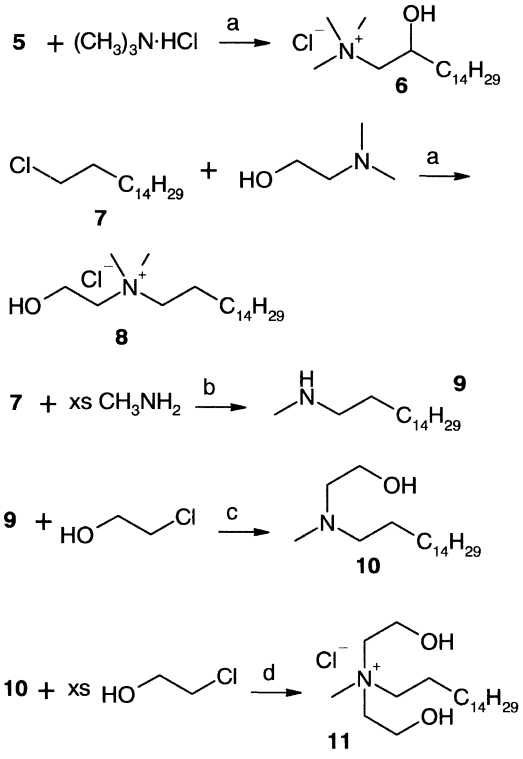
To explore the effect of counterions, we synthesized **1**(16)Cl, **1**(16)I, and **2**(16)Cl (Scheme 1). We had **1**(16)Br from our initial study.[11] Reaction of 2-[(*N*-methyl)amino]ethanol with a mixture of bromohydrins (**3**) gave the desired long-chain alkyl tertiary aminodiol (**4**),[28] which reacted with methyl iodide to give **1**(16)I.[29] Reaction of tetradecyloxirane (**5**) with hydrogen chloride followed by addition of 2-(dimethylamino)ethanol[30] gave **1**(16)Cl. Compound **1**(16)Cl was the most active of the three halides in initial spermicidal assays. (data not shown) We then made **2**(16)Cl[31] from the reaction of **5** with hydrogen chloride followed by addition of 2-{(2-hydroxyethyl)methylamino}ethanol. As **1**(16)Br is more potent spermicide than **2**(16)Br,[11] we needed to verify that **1**(16)Cl is more potent than **2**(16)Cl.

To probe the structure–function of hydroxylation, we synthesized **6**, **8**, and **11** (Scheme 2), which structurally resemble **1**(16)Cl and **2**(16)Cl, but lack one or two hydroxyl groups.

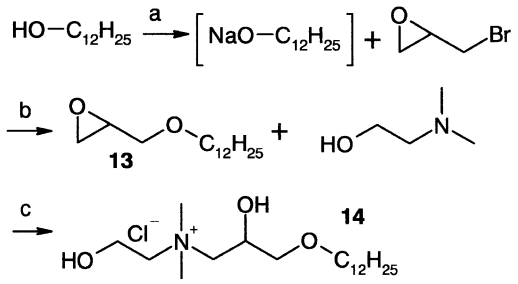
Reaction of **5** with trimethylamine hydrochloride gave **6**.[32] Reaction of 1-chlorohexadecane (**7**) with 2-(dimethylamino)ethanol gave **8**.[33,34] The three-step synthesis of **11** began with the synthesis of *N*-methyl-1-hexadecanamine (**9**) from **7** and excess methylamine.[35] Reaction of **9** with 2-chloroethanol gave **10**,[36,37] which we isolated by chromatography. Treatment of **10** in a sealed tube with excess 2-chloroethanol gave **11**.[38] We recrystallized a commercial sample of *N*,*N*,*N*-trimethyl-1-hexadecanaminium chloride (**12**), which served as the reference, non-hydroxylated alkyl quaternary ammonium salt.



Scheme 1. (a) Δ, 16 h, MeOH; (b) CH_3I, Et_2O, 16 h; (c) aq HCl, Δ, 3 h; (d) Δ, 3 h; (e) Δ, 16 h.



Scheme 2. (a) Δ, 16 h, MeOH; (b) K_2CO_3, KI, Δ, 3 days, EtOH; (c) K_2CO_3, Δ, 1 day, MeOH; (d) Δ, 5 days, MeOH.



Scheme 3. (a) NaH, THF, 15 °C→rt, 4 h; (b) rt, 16 h; (c) $HOCH_2CH_2NMe_2 \cdot HCl$, Δ, 16 h, MeOH.

To test how hydrophobicity affected activity, we synthesized **14** (Scheme 3), which structurally resembles **1**(16)Cl but has one oxygen in place of the methylene at carbon 4 of the hexadecyl group. Reaction of (bromomethyl)oxirane with the in-situ generated sodium dodecan-1-oxide[39] gave oxirane **13**. Reaction of **13** with 2-(dimethylamino)ethanol in the presence of an equivalent of 2-(dimethylamino)ethanol hydrochloride yielded **14**.

The cmcs determined from conductivities of dilute solutions of **1**(16)X (X = Cl, Br, I), **2**(16)Cl, **6**, **8**, **11**, and **12** are given in Table 1. Two reported[40,41] values for the cmc (1.36 and 1.41×10^{-3} mol dm^{-3}) of **12** in water agree with the value determined by our procedure. As we anticipated from others,[42,43] the cmcs and alpha for **1**(16)X (X = Cl, Br, I) decrease as halide counterion increases in size. As we anticipated from previous data on bromide[44] and chloride[38] salts, hydroxylation has small but significant effects on the cmc.

Replacing a hydrogen at carbon 2 of a hexadecyl group with a hydroxyl group increases the cmc; replacing a methyl with a 2-hydroxyethyl group decreases the cmc. Comparison of three pairs of compounds **6**/**12**, **1**/**8**, and **2**/**11** shows that replacing a hydrogen at carbon 2 of a hexadecyl group with a hydroxyl group increases the cmc by 41, 51, and 58%, respectively. Comparison of two pairs of compounds **8**/**12** and **1**/**6** shows that replacing one methyl with one 2-hydroxyethyl group decreases the cmc by 15 and 8%, respectively. Comparison of two pairs of compounds **11**/**12** and **2**/**6** shows that replacing two methyls with two 2-hydroxyethyl groups decreases the cmc by 22 and 12%, respectively. In summary, hydroxylation at carbon 2 on the hexadecyl group increases the cmc by a greater amount than replacement of a methyl with a 2-hydroxyethyl group decreases the cmc.

Table 1. Critical micelle concentrations (cmcs) and alpha (ratio of slopes before and after the cmc) for **1**(16)X, **2**(16)Cl, **6**, **8**, **11**, and **12** at 25 °C as measured by conductance in distilled water

Compd	10^3 cmc (mol dm^{-3})	Alpha
1(16)Cl	1.77 ± 0.01	0.290 ± 0.002
	1.791 ± 0.009	0.299 ± 0.002
	1.75 ± 0.01	0.305 ± 0.002
1(16)Br	1.36 ± 0.02	0.213 ± 0.003
	1.34 ± 0.02	0.264 ± 0.006
1(16)I	0.88 ± 0.01	0.181 ± 0.004
	0.833 ± 0.006	0.160 ± 0.002
2(16)Cl	1.70 ± 0.03	0.299 ± 0.003
	1.68 ± 0.02	0.346 ± 0.004
6	1.87 ± 0.03	0.350 ± 0.005
	1.97 ± 0.01	0.33 ± 0.01
	1.94 ± 0.02	0.303 ± 0.008
8	1.176 ± 0.007	0.387 ± 0.004
	1.162 ± 0.009	0.374 ± 0.001
11	1.055 ± 0.01	0.3626 ± 0.0007
	1.091 ± 0.009	0.375 ± 0.003
12	1.37 ± 0.03	0.371 ± 0.002

Biology

Table 2 presents MECs for spermicidal activity in a modified Sander–Cramer assay[45] for **1**(16)X (X = Cl, Br, I), **2**(16)Cl and N-9. MECs represent a measure of potency — the lower the MEC, the more potent the compound. The Sander–Cramer assay determines the minimum concentration required to stop all sperm motility in 20 s. The MEC decreases; that is, the potency increases, as the size of the halide counterion decreases to **1**(16)Cl. The MEC of **1**(16)Cl, which is the most potent compound in this series, is nearly half the MEC of N-9. As with the bromide salts,[11] **1**(16)Cl is more potent than **2**(16)Cl. Compounds **1**(16)Br and **2**(16)Cl are as effective spermicides as N-9.

Table 3 presents the MECs for spermicidal activity in a modified Sander–Cramer assay for **6**, **8**, **11**, **12**, **14**, and

Table 2. Spermicidal activity of **1**(16)X (X = Cl, Br, I) and **2**(16)Cl in the Sander–Cramer assay

Compd	Highest spermicidal dilution (HSD) (1/*X*)	MEC (mg mL^{-1})
1(16)Cl	362.7 ± 44.8	0.072 ± 0.012
1(16)Br	250.7 ± 46.8	0.120 ± 0.022
1(16)I[a]	245.3 ± 58.2	0.221 ± 0.061
2(16)Cl[b]	93.3 ± 16.6	0.140 ± 0.019
N-9	213.3 ± 40.9	0.124 ± 0.014

Sperm samples ($n = 12$) were mixed with 2-fold serial dilutions of the compounds in 0.9% NaCl (initial concentration of compound in distilled water, 20 mg mL^{-1}) and observed under the microscope for 20 s. Those dilutions that completely immobilized all screened spermatozoa were further diluted in excess buffer and incubated for 1 h to verify lack of motility recovery. The highest dilution that successfully passed both assessments was considered the HSD and was used to calculate the MEC.
[a]Requires warm water (40 °C) to remain in solution at 20 mg mL^{-1}.
[b]Initial concentration, 10 mg mL^{-1}.

Table 3. Spermicidal activity of **6**, **8**, **11**, **12**, **14**, and N-9 in the Sander–Cramer assay

Compd	HSD (1/*X*)	MEC (mg mL^{-1})
6	256.0 ± 0.0	0.078 ± 0.000
8	320.0 ± 32.0	0.068 ± 0.005
11	170.7 ± 17.4	0.130 ± 0.011
12	320.0 ± 32.0	0.068 ± 0.005
14	61.3 ± 2.6	0.339 ± 0.025
N-9[a]	181.3 ± 25.9	0.081 ± 0.016

Sperm samples ($n = 12$) were mixed with 2-fold serial dilutions of the compounds in 0.9% NaCl (initial concentration of compound in distilled water, 20 mg mL^{-1}) and observed under the microscope for 20 s. Those dilutions that completely immobilized all screened spermatozoa were further diluted in excess buffer and incubated for 1 h to verify lack of motility recovery. The highest dilution that successfully passed both assessments was considered the HSD and was used to calculate the MEC.
[a]Initial concentration, 10 mg mL^{-1}.

N-9. The MECs for **6**, **8**, and **12** are better than the MEC for N-9. In this particular assay, N-9 has a lower MEC than is typically observed (i.e., 0.12 mg mL^{-1}). This batch of sperm is apparently more sensitive to spermicidal agents. Regardless, the trends are clear. Compound **14** is much less effective than the others. Compound **11** is less active than **6**, **8**, **12**, and N-9.

Hydroxylation can either not change or decrease the MEC compared to the reference compound, **12**. In comparing **6** and **12**, we find that replacing a hydrogen at carbon 2 of a hexadecyl group with a hydroxyl group does not change the potency. In comparing **8** and **12**, we find that replacing one methyl with one 2-hydroxyethyl group does not change the potency. In comparing **11** and **8**, we find that replacing a second methyl with a 2-hydroxyethyl groups decreases potency (i.e., increases the MEC). A similar result is seen (Table 2) when comparing **1**(16)Cl and **2**(16)Cl; a second 2-hydroxyethyl group decreases potency. We conclude that **1**(16)Cl, **6**, **8**, and **12** are similarly potent spermicides and that hydroxylation at carbon 2 of a hexadecyl group and replacing one methyl with one 2-hydroxyethyl group do not affect the potency of this series of alkyl quaternary ammonium chlorides.

Figure 1 presents the data for anti-HIV activity of **1**(16)X (X = Cl, Br, I), **2**(16)Cl and N-9 in two in-vitro assays:[46] cell-free and cell-associated virus inactivation. All five compounds are effective, that is, ≥ 3 log units of infectious titer reduction at 1.0 and 0.5 mg mL^{-1} in both assays. (data not shown) At 0.05 mg mL^{-1}, **1**(16)Br and **1**(16)I are more potent than **1**(16)Cl against both cell-free and cell-associated HIV. Compound **1**(16)Br is more potent than N-9 against cell-free HIV at 0.05 mg mL^{-1}.

Figure 2 presents the data for anti-HIV activity of **6**, **8**, **11**, **12**, **14**, **1**(16)Cl, **1**(16)Br, and N-9 in two in-vitro assays: cell-free and cell-associated virus inactivation. All compounds are effective, that is ≥ 4 log units of infectious titer reduction at 1.0 and 0.5 mg mL^{-1} in both assays. At 0.05 mg mL^{-1}, **6** and **1**(16)Cl are more potent than N-9 against cell-free HIV; **11**, **1**(16)Cl, **8**, and **1**(16)Br are more potent than N-9 against cell-associated HIV. In these two assays, **1**(16)Cl is the only compound that is consistently more potent than N-9.

Hydroxylation increases or decreases the antiviral activity. In comparing **6** and **12**, we find that replacing a hydrogen at carbon 2 of a hexadecyl group with a

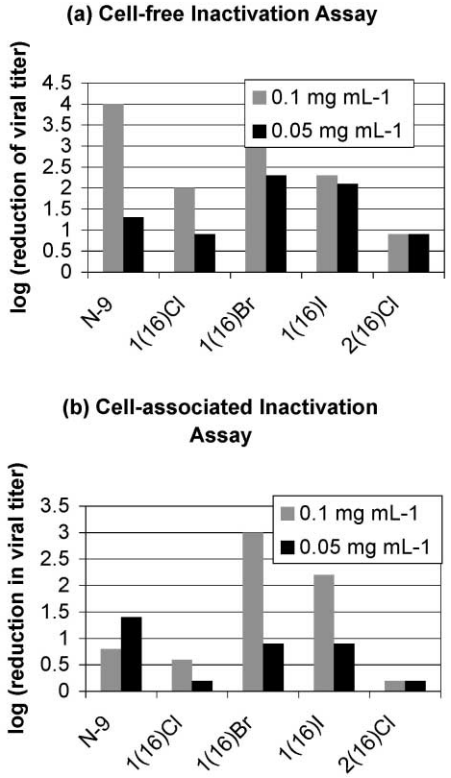


Figure 1. In vitro cell-free (a) and cell-associated (b) inactivation of HIV assays for **1**(16)X (X = Cl, Br, I), 2(16)Cl, and N-9. Cell-free or cell-associated HIV-1 was incubated with multiple concentrations of the test agents for 2 min at 37 °C. Compound effect was then terminated by 10-fold serial dilutions, and the treated virus was further incubated with infection-susceptible MT-2 cells for 6 days. Virus-induced cytopathic effects (formation of cell syncytium) were recorded under microscopic observation. The values presented in the body of the table represent the reduction in the infectious titer of the untreated virus (in logs) effected by each compound concentration.

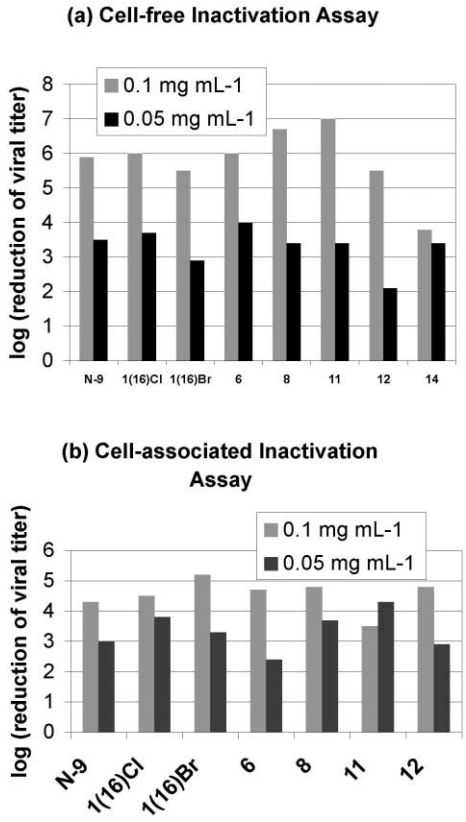


Figure 2. In vitro cell-free and cell-associated inactivation of HIV assays for **6**, **8**, **11**, **12**, **14**, **1**(16)Cl, **1**(16)Br, and N-9. Cell-free or cell-associated HIV-1 was incubated with multiple concentrations of the test agents for 2 min at 37 °C. Compound effect was then terminated by 10-fold serial dilutions, and the treated virus was further incubated with infection-susceptible MT-2 cells for 6 days. Virus-induced cytopathic effects (formation of cell syncytium) were recorded under microscopic observation. The values presented in the body of the table represent the reduction in the infectious titer of the untreated virus (in logs) effected by each compound concentration.

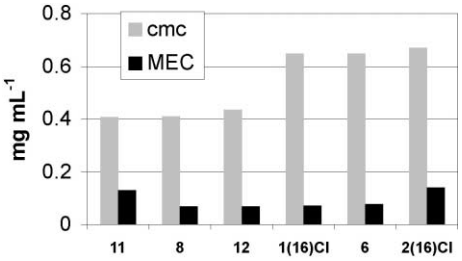


Figure 3. Comparison of MECs and cmcs of **1**(16)Cl, **2**(16)Cl, **6**, **8**, **11**, and **12**.

hydroxyl group improves potency against cell-free HIV but not against cell-associated HIV. In comparing **8** and **12**, we find that replacing one methyl with one 2-hydroxyethyl group improves potency against both cell-free and cell-associated HIV. In comparing **11** and **8**, we find that replacing a second methyl with a 2-hydroxyethyl groups increases potency against cell-associated HIV. In contrast, when comparing **1**(16)Cl and **2**(16)Cl (Fig. 1), a second 2-hydroxyethyl group decreases potency. We conclude that **1**(16)Cl, **8**, and **11** are similarly potent antiviral agents. Furthermore, hydroxylation at carbon 2 of a hexadecyl group increases potency against cell-free HIV, but decreases potency against cell-associated HIV. Replacing a methyl with a 2-hydroxyethyl group increases potency against both cell-free and cell-associated HIV. Hydroxylation at carbon 2 of a hexadecyl group in combination with replacing methyl with a 2-hydroxyethyl group produces the best result.

Discussion

Spermicidal activity and micellar concentration

Figure 1 presents the MECs for spermicidal activity and cmcs for **1**(16)Cl, **2**(16)Cl, **6**, **8**, **11**, and **12**. We show the cmcs in mg mL^{-1} to facilitate comparison with the bioassays. The cmcs, which were determined in distilled water, represent an upper limit of the cmc under the conditions of the Sander–Cramer assay. That is, $\sim$1% bovine serum albumin (BSA), 0.9% saline, and Baker's (phosphate and glucose) solution might decrease the cmc. Studies[47,48] on the binding of **12** to BSA and human serum albumin reveal $\sim$100 binding sites on each protein. This surfactant–protein binding should increase the total amount of surfactant needed to form a micelle. Salt significantly decreases the cmc of hydroxy-functionalized alkyl quaternary ammonium chlorides.[49] In the presence of salt, the cmcs would probably be lower.

We group the compounds in Figure 3 by cmc to show the structural similarities that produce higher cmcs. Each compound in the left triad — **1**(16)Cl, **6**, and **2**(16)Cl-has a hydroxyl at carbon 2 of a hexadecyl group; compounds in the right triad — **11**, **8**, and **12**-do not. The two compounds at the ends, **11** and **2**(16)Cl, are 2-fold less potent as spermicides than the four compounds in the middle. Both of these compounds have two 2-hydroxyethyl groups. The four compounds in the middle have similar potency despite different amounts and positions of hydroxylation. Compound **12** has no hydroxyl groups; **11** and **6** have one hydroxyl group each; **1**(16)Cl has two hydroxyl groups. From these data, we conclude that (1) hydroxylation affects the cmc and MEC independently and (2) the position and amount of hydroxylation either increase or decrease spermicidal potency and cmc.

Compounds **14**, which is quite water-soluble, is not a potent spermicidal agent (Table 3) Although we did not measure the cmc of **14**, we can estimate it from the cmc of 2-hydroxy-*N*,*N*,*N*-trimethyl-4-oxahexadecan-1-aminium chloride, which is 5.6×10^{-3} M.[50] Given the slight decrease in cmc expected for replacing a methyl with a 2-hydroxyethyl group, we estimate that the cmc of **14** is $\sim5.1\times10^{-3}$ M, which is 3-fold greater than the cmc for **1**(16)Cl (Table 1). As a spermicide, **1**(16)Cl is nearly 5-fold more potent than **14**. We conclude that replacing a methylene with oxygen at carbon 4 of a hexadecyl chain decreases the spermicidal potency more than it increases the cmc.

Anti-HIV properties

Those compounds — **8**, **11**, and **1**(16)Cl — that are active ($\geq$3 log units of infectious titer reduction) at 0.05 mg mL^{-1} against cell-free and cell-associated virus have at least one 2-hydroxyethyl group (Fig. 2). Compound **14** has one 2-hydroxyethyl group. Although it was dropped from consideration as a dual agent because of relatively low potency in the spermicidal assay, it shows excellent activity against cell-free virus. Compound **6** differs the most in activity between the cell-free and cell-associated virus. At 0.05 mg mL^{-1}, **6** is the most potent against the cell-free virus and the least potent against the cell-associated virus. Compound **11**, which has two 2-hydroxyethyl groups, is the most active against the cell-associated virus. Of the three best compounds in this assay, **1**(16)Cl has the highest cmc (Fig. 1).

Comparison with previous work

The antibacterial activities and applications in shampoo formulations of **1**(16)Cl, **2**(16)Cl, and **6** are known from the patent literature.[30–32] Cognis markets **1**(16)Cl as the active ingredient in Dehyquart® E-CA as wetting agent for hair-care products. The antimicrobial activities of (2-hydroxyethyl)-*N*,*N*-dimethyl-1-alkanaminium halides have been well known since the 1950s.[51–53] Compound **8** has excellent bacteriostatic[53,54] and fungiostatic[53] activities. However, **8** is quite cytotoxic to Chinese hamster lung fibroblasts and a strong irritant in the Draize eye test.[55] Our study extends these previous results to spermicidal and antiviral activities. Furthermore, it compares these compounds in a manner previously unreported.

Conclusion

Hydroxylation and oxygen replacement affect the cmc, spermicidal potency, and antiviral activity of alkyl quaternary ammonium salts. Replacing a hydrogen with a hydroxyl group at carbon 2 of a hexadecyl group increases the cmc, improves spermicidal potency, and

has little effect on antiviral activity. Replacing a methyl with a 2-hydroxyethyl group decreases the cmc, has little effect on spermicidal potency, and improves antiviral activity. Replacing two methyls with two 2-hydroxyethyl groups decreases the cmc, decreases spermicidal potency, and improves activity against cell-associated virus. Replacing one oxygen for a methylene group at carbon 4 in the hexadecyl chain increases the cmc, decreases spermicidal activity, and has little effect on activity against cell-free virus. For this limited set of compounds, hydroxylation and oxygen replacement affect the cmc, spermicidal potency, and antiviral activity independently.

The best candidate for further evaluation as a dual agent is **1**(16)Cl. It has the best combination of spermicidal and antiviral activities, which are below the cmc. Compound **1**(16)Cl is a racemic mixture; it has spermicidal and antiviral potencies that are indistinguishable from the individual enantiomers, which we synthesized and assayed. (data not shown) Further preclinical tests[12] are warranted to see if **1**(16)Cl can be formulated into a safe, effective topical microbicide that will protect against mucosal infections by sexually transmitted pathogens.

Experimental

General

Reagents and HPLC-grade solvents were used as received. Bromohydrins **3** were prepared as described.[11] Tetradecyloxirane (**5**) and 1-chlorohexadecane (**7**) were used as received. Solutions were concentrated by rotary evaporation. Flash chromatography was performed with silica gel, 60 X, 230–400 mesh. TLC was performed with PE SIL G/UV 250-μm plates. Apparent coupling constants, J_{app}, were the measured peak widths. Atlantic MicroLab Inc. performed the elemental analyses.

Syntheses

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*-methyl-1-hexadecanamine, 4. A solution of bromohydrins **3** (2.00 g, 6.22 mmol) and 2-[(*N*-methyl)amino]ethanol (1.12 g, 14.9 mmol) in CH_3OH (40 mL) was heated to reflux overnight. The solvent was removed under reduced pressure to give a colorless oil, which was purified twice with flash column chromatography (1.75-inch diameter×5.5-inch height) by eluting with $CHCl_3/CH_3OH$ (10:1) to give a thick, colorless oil that solidified in vacuo overnight to give a white solid (1.51 g, yield 77%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70–3.61 (comp, 3H), 2.67 (ddd, 1H, J_{app} = 13.0, 6.8, 4.7 Hz), 2.52 (ddd, 1H, J_{app} = 13.0, 5.6, 4.4 Hz), 2.35 (m, 2H), 2.31 (s, 3H), 1.46–1.20 (comp, 26H), 0.87 (t, 3H, J_{app} = 6.9 Hz; ^{13}C NMR (100 MHz, $CDCl_3$) δ 67.6, 64.1, 59.6, 59.3, 42.4, 34.9, 31.9, 29.7, 29.63, 29.61, 29.55, 29.3, 25.6, 22.6, 14.1.

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-1-hexadecanaminium iodide, 1(16)I. A solution of **4** (1.00 g, 3.17 mmol) and CH_3I (2.14 g, 15.1 mmol) in ether (10 mL) was stirred at room temperature under N_2 overnight. The reaction mixture was diluted with ether (10 mL) and filtered to collect the white solid. The white solid was recrystallized twice in $EtOAc/CH_2Cl_2$ (10:1, 11 mL) to give colorless crystals (1.20 g, yield 83%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.36 (br m, 1H), 4.16 (br m, 3H), 3.90 (br m, 2H), 3.84 (d, 1H, J_{app} = 6.7 Hz), 3.64 (dd, 1H, J_{app} = 13.5, 10.0 Hz), 3.45 (comp, 7H), 1.58–1.24 (comp, 26H), 0.87 (t, 3H, J_{app} = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.1, 66.7, 65.6, 56.0, 53.9, 53.7, 35.9, 31.9, 29.7, 29.64, 29.59, 29.5, 29.3, 25.1, 22.6, 14.1. Anal. calcd for $C_{20}H_{44}NO_2I$: C, 52.51; H, 9.69; N, 3.06. Found: C, 52.45; H, 9.62; N, 3.09.

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-1-hexadecanaminium chloride, 1(16)Cl. A mixture of **5** (10.55 g of 85% tech, 37.30 mmol) and aq HCl (8 mL, 6 M solution) was heated to reflux for 3 h. 2-(Dimethylamino) ethanol (8.97 g, 100.63 mmol) was added. The resulting mixture was heated to reflux for 3 h. The warm reaction mixture was poured into CH_3CN (350 mL), which was then heated to dissolve the reaction mixture. EtOAc (150 mL) was added while the CH_3CN was still hot. The solution was cooled at room temperature for 1 h; crystals formed slowly. The crystals were collected by vacuum filtration, and washed with $EtOAc/CH_3CN$ (2:1, 300 mL). The filtrate stood at room temperature overnight; a second crop of crystals formed and were collected as before. The combined crystals were dried in vacuo for 3 days to give a white crystalline powder (10.22 g, yield 75%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.49 (br t, 1H, J_{app} = 5.3 Hz), 5.32 (d, 1H, J_{app} = 7.3 Hz), 4.23 (br m, 1H), 4.09 (br m, 2H), 3.83 (m, 2H), 3.61 (dd, 1H, J_{app} = 13.6, 10.1 Hz), 3.41 (s, 6H), 3.32 (d, 1H, J_{app} = 13.0 Hz), 1.58–1.24 (comp, 26H), 0.87 (t, 3H, J_{app} = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.4, 66.8, 65.6, 56.0, 53.5, 53.1, 36.0, 31.9, 29.7, 29.65, 29.59, 29.3, 25.3, 22.6, 14.1. Anal. calcd for $C_{20}H_{44}NO_2Cl$: C, 65.63; H, 12.12; N, 3.83. Found: C, 65.56, H, 12.09, N, 3.90.

2-Hydroxy-*N*,*N*-bis(2-hydroxyethyl)-*N*-methyl-1-hexadecanaminium chloride, 2(16)Cl. A mixture of **5** (2.06 g, 8.57 mmol) in aq HCl (16 mL, ~0.6 M solution) was heated to reflux for 3 h. 2-[(2-Hydroxyethyl)methylamino] ethanol (2.47 g, 20.73 mmol) was added. The heterogeneous reaction mixture was heated to reflux overnight. The reaction mixture turned into a clear, homogeneous, light-yellow solution; turned opaque when it was cooled to room temperature. The reaction mixture was washed with EtOAc (2×25 mL). The aqueous layer was collected, concentrated, and dried in vacuo to give a thick oil. To purify the product, several crystallizations were attempted. The oil was dissolved in boiling $EtOAc/CH_3CN$ (2:1, 105 mL) and cooled at room temperature. A layer of semi-solid, sticky oil was formed on the glass surface. Scraping the beaker with a spatula induced solidification. The solid was collected by vacuum filtration and washed with CH_3CN (2×10 mL) to give a white powder (1.47 g, yield 43%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 (two overlapping triplets, 2H), 5.06 (d, 1H, J_{app} = 7.0 Hz), 4.20 (m, 1H),

4.07 (m, 4H), 3.91–3.81 (comp, 4H), 3.63 (m, 1H), 3.38 (overlapping d and s, 4H and NCH_3), 1.53–1.24 (comp, 26H), 0.87 (t, 3H, J_{app} = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 68.4, 65.6, 65.3, 65.1, 55.9, 55.8, 51.0, 36.1, 31.9, 29.83, 29.80, 29.78, 29.76, 29.69, 29.4, 25.4, 22.7, 14.1. Anal. calcd for $C_{21}H_{46}NO_3Cl$: C, 63.69; H, 11.71; N, 3.54. Found: C, 63.74; H, 11.71; N, 3.52.

2-Hydroxy-*N*,*N*,*N*-trimethyl-1-hexadecanaminium chloride, 6. A mixture of **5** (3.69 g, 15.3 mmol) (not very soluble in CH_3OH at room temperature) and $(CH_3)_3N{\cdot}HCl$ (1.23 g, 12.9 mmol) in CH_3OH (50 mL) was heated to reflux overnight. The reaction mixture was concentrated to give a sticky solid, which was recrystallized in CH_3CN (250 mL). A solid started forming after 1 h at room temperature. The solid was collected by vacuum filtration, and washed with CH_3CN, to give a white solid (2.54 g, yield 59%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (d, 1H, J_{app} = 7.0 Hz), 4.22 (br m, 1H), 3.53 (dd, 1H, J_{app} = 13.2, 10.3 Hz), 3.45 (s, 9H), 3.32 (m, 1H), 1.56–1.21 (comp, 26H), 0.84 (t, 3H, J_{app} = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.5, 65.5, 54.8, 35.8, 31.9, 29.7, 29.62, 29.59, 29.56, 29.3, 25.3, 22.6, 14.1. Anal. calcd for $C_{19}H_{42}NOCl$: C, 67.92; H, 12.60; N, 4.17. Found: C, 67.87; H, 12.60; N, 4.12.

***N*-(2-Hydroxyethyl)-*N*,*N*-dimethyl-1-hexadecanaminium chloride, 8.** A mixture of **7** (3.01 g, 11.5 mmol) (insoluble in CH_3OH) and 2-(dimethylamino)ethanol (1.64 g, 18.4 mmol) in CH_3OH (35 mL) was heated to reflux overnight. The solvent was removed under reduced pressure to give a white gummy solid. EtOAc (400 mL) was added to triturate the product. The EtOAc solution was heated to the bp for 30 min, but some solid remained. The EtOAc solution was cooled at room temperature overnight. Crystals were collected by vacuum filtration, and washed with EtOAc (100 mL) and then hexanes (50 mL) to give colorless crystals (2.12 g, yield 52%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (t, 1H, J_{app} = 5.6 Hz), 4.08 (br m, 2H), 3.70 (m, 2H), 3.51 (m, 2H), 3.34 (s, 6H), 1.72 (m, 2H), 1.32–1.23 (comp, 26H), 0.85 (t, 3H, J_{app} = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.0, 65.7, 55.9, 51.9, 31.9, 29.7, 29.62, 29.57, 29.5, 29.4, 29.3, 29.2, 26.3, 22.8, 22.6, 14.1. Anal. calcd for $C_{20}H_{44}NOCl$: C, 68.63; H, 12.67; N, 4.00. Found: C, 68.54; H, 12.65; N, 3.93.

***N*-Methyl-1-hexadecanamine, 9.** A mixture of **7** (2.29 g, 8.78 mmol), CH_3NH_2 (30 mL of 8.03 M solution in abs EtOH, 241 mmol), K_2CO_3 (1.21 g, 8.75 mmol) and KI (1.70 g, 10.21 mmol) was heated to reflux for 3 days. The reaction mixture was diluted with ether (150 mL), and washed with brine (100 mL). The ethereal solution was dried over Na_2SO_4, filtered, and concentrated to give a pale-yellow liquid. The crude product was purified with flash column chromatography (1.75-inch diameter × 5.5-inch height) by eluting with $CHCl_3/CH_3OH$ (10:1, 5:1, and 3:1, 1000 mL each) to give dihexadecylmethylamine (0.81 g) as a side product, which was identified by ^{1}H NMR, and the desired product as an off-white solid (1.00 g, yield 45%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.55 (t, 2H, J_{app} = 7.2 Hz), 2.42 (s, 3H), 1.49–1.43 (comp, 2H), 1.28–1.25 (comp, 26H), 0.88 (t, 3H, J_{app} = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.2, 36.6, 31.9, 30.0 29.7, 29.60, 29.58, 29.3, 27.3, 22.7, 14.1.

***N*-(2-Hydroxyethyl)-*N*-methyl-1-hexadecanamine, 10.** A mixture of **9** (1.00 g, 3.91 mmol), 2-chloroethanol (711 mg, 8.83 mmol) and K_2CO_3 (677 mg, 4.90 mmol) in CH_3OH (10 mL) was heated to reflux for 24 h. The solution was concentrated, and ether (200 mL), EtOAc (100 mL), and water (100 mL) were added. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated to give a mixture of oil and crystals. This crude product was purified with flash column chromatography (1.75-inch diameter × 4.5-inch height) by eluting with $CHCl_3/CH_3OH$ (10:1, 1000 mL) to give an oily crystalline solid (630 mg, yield 54%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.59 (t, 2H, J_{app} = 5.0 Hz), 2.54 (t, 2H, J_{app} = 5.0 Hz), 2.42 (t, 2H, J_{app} = 7.2 Hz), 2.26 (s, 3H), 1.47 (m, 2H), 1.28–1.25 (comp, 26H), 0.88 (t, 3H, J_{app} = 6.5 Hz); (lit.[36,37] 60 MHz ^{1}H NMR) ^{13}C NMR (100 MHz, $CDCl_3$) δ 58.7, 58.2, 57.7, 41.5, 31.9, 29.7, 29.64, 29.60, 29.5, 29.3, 27.3, 27.1, 22.7, 14.1.

***N*,*N*-Bis(2-hydroxyethyl)-*N*-methyl-1-hexadecanaminium chloride, 11.** A solution of **10** (630 mg, 2.10 mmol) and 2-chloroethanol (687 mg, 8.53 mmol) in MeOH (12 mL) was heated to boiling in a heavy-walled sealed tube for 5 days. The solvent was removed under reduced pressure to give a colorless oil, which was triturated in EtOAc. Fine crystals were collected by vacuum filtration to give a powder. ^{1}H NMR showed that the powder was a mixture of the desired product and the hydrochloride salt of amine **10**. The powder was dissolved in $CHCl_3$ (10 mL) and treated with deactivated basic alumina (160 mg). The mixture was allowed to stand at room temperature for 4 days with occasional swirling. The basic alumina particles were removed by filtration through a small cotton wool in a 6-inch pipet. The filtrate was concentrated to give a white, oily solid, which was recrystallized in EtOAc (50 mL) to give white crystals (399 mg, yield 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 (t, 1H, J_{app} = 5.4 Hz), 4.07 (br m, 4H), 3.69 (m, 4H), 3.51 (m, 2H), 3.30 (s, 3H), 1.72 (br m, 2H), 1.32–1.24 (comp, 26H), 0.86 (t, 3H, J_{app} = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 64.2, 64.0, 55.8, 50.3, 31.9, 29.7, 29.6, 29.53, 29.48, 29.34, 29.25, 26.4, 22.7, 22.6, 14.1. Anal. calcd for $C_{21}H_{46}NO_2Cl$: C, 66.37; H, 12.20; N, 3.69. Found: C, 66.43; H, 12.18; N, 3.73.

Recrystallization of *N*,*N*,*N*-Trimethyl-1-hexadecanaminium chloride, 12. Compound **12** (1.02 g, Eastman Kodak) was added to acetone/EtOAc/EtOH (80:80:1, 80.5 mL), which was heated to boiling. Not all the solid dissolved. The hot solution was decanted and filtered through a filter paper; some crystals formed on the filter paper and some crystals formed on cooling the filtrate. These crystals were combined and recrystallized with acetone/EtOAc (1:1, 80 mL) then washed with EtOAc (20 mL) and hexanes (20 mL) to give colorless crystals (210 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (m, 2H), 3.45 (s, 9H), 1.73 (m, 2H), 1.34–1.19 (comp, 26H), 0.87 (t, 3H, J_{app} = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ

67.0, 53.2, 31.9, 29.65, 29.63, 29.60, 29.5, 29.4, 29.3, 29.2, 26.2, 23.2, 22.6, 14.1. Anal. calcd for $C_{19}H_{42}NCl$: C, 71.31; H, 13.23; N, 4.38. Found: C, 71.32; H, 13.18; N, 4.41.

2-Oxatetradecyloxirane, 13. To a suspension of NaH (500 mg, 20.8 mmol) in THF (10 mL) at 15 °C was added a solution of 1-dodecanol (1.972 g, 10.58 mmol) in THF (20 mL) dropwise over 10 min. After stirring at room temperature for 4 h, (bromomethyl)oxirane (2.479 g, 18.10 mmol) was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was added to brine (50 mL) in a 500-mL separatory funnel. The aqueous solution was extracted with ether (2×100 mL). The combined ether extracts were washed with water (50 mL), dried over Na_2SO_4, filtered and concentrated to give an oil. The crude product was purified with flash column chromatography (1.75-inch diameter×5.5-inch height) by eluting with hexanes/EtOAc (5:1) to give a clear colorless oil (2.18 g, yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 (dd, 1H, J_{app} = 11.5, 3.1 Hz), 3.48 (m, 2H), 3.38 (dd, 1H, J_{app} = 11.5, 5.8 Hz), 3.14 (m, 1H), 2.79 (dd, 1H, J_{app} = 5.2, 4.3 Hz), 2.60 (dd, 1H, J_{app} = 5.2, 2.7 Hz), 1.57 (m, 2H), 1.35–1.25 (comp, 18H), 0.87 (t, 3H, J_{app} = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.7, 71.4, 50.9, 44.3, 31.9, 29.7, 29.63, 29.60, 29.57, 29.56, 29.4, 29.3, 26.0, 22.7, 14.1. (lit.[56] ^{1}H and ^{13}C NMR, MHz unspecified).

2-Hydroxy-*N*-(2-hydroxyethyl)-*N*,*N*-dimethyl-4-oxahexadecan-1-aminium chloride, 14. To a solution of **13** (600 mg, 2.47 mmol) and 2-(dimethylamino)ethanol (206 mg, 2.31 mmol) in CH_3OH (8 mL) was added a solution of 2-(dimethylamino)ethanol hydrochloride in CH_3OH (2.5 mL of 0.92 M). The pH of the resulting solution was about 8. This reaction mixture was heated to reflux overnight and then concentrated to give a colorless thick oil, which was dissolved in boiling $CHCl_3$ (10 mL). The solution was boiled for 10–15 min to concentrate to about 5 mL. After the solution had been cooled to room temperature and EtOAc (90 mL) added, the solution turned white. Scratching the beaker with spatula induced the formation of a solid. The white solid was collected by vacuum filtration, washed with EtOAc/$CHCl_3$ (9:1, 50 mL), and then ether (20 mL). The white solid was dissolved in $CHCl_3$ (1 mL) and filtered through a small amount of flash silica gel (1-cm height×0.7-cm diameter) in a 6-inch pipet, and rinsed with $CHCl_3$ (2×1 mL). EtOAc (170 mL) was added; the solution was allowed to stand at room temperature overnight. The fine crystals were collected by vacuum filtration, washed with EtOAc (100 mL), and then hexanes (50 mL), to give white crystals (0.453 g, Yield 53%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46 (d, 1H, J_{app} = 6.4 Hz), 5.40 (t, 1H, J_{app} = 5.3 Hz), 4.39 (m, 1H), 4.09 (br m, 2H), 3.79 (m, 2H), 3.61 (dd, 1H, J_{app} = 13.8, 9.4 Hz), 3.45–3.48 (comp, 2H), 3.41 (t, 2H, J_{app} = 6.9 Hz), 3.38 (comp, 7H), 1.56–1.48 (comp, 2H), 1.30–1.24 (comp, 18H), 0.86 (t, 3H, J_{app} = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 72.4, 71.8, 68.7, 66.8, 64.5, 56.0, 53.6, 53.2, 31.9, 29.7, 29.6, 29.54, 29.50, 29.3, 26.0, 22.7, 14.1. Anal. calcd for $C_{19}H_{42}NO_3Cl$: C, 62.01; H, 11.50; N, 3.81. Found: C, 61.92; H, 11.48; N, 3.79.

Determination of critical micelle concentrations by conductivity

As described previously in detail,[11] conductivity measurements were made with a conductance meter connected to a conductance cell, containing platinum-iridium electrodes coated with platinum black and gold soldered to platinum lead wires, a dip-type cell made from ABS plastic with a cgs cell constant of 1.0 cm^{-1}. The meter gave readings in siemens/cm (S/cm). A temperature probe connected directly to the meter measured the temperature of the sample solution before and after each set of data was collected. A temperature of 25 °C was maintained with a water bath. Typically, 20 data points were collected for each set; data points up to but not exceeding 5 times the cmc were collected.

Semen samples

Semen samples were collected by healthy volunteers by masturbation and allowed to liquefy for 30 min at room temperature. If completely liquefied, they were evaluated for sperm concentration and motions parameters with a computer-assisted semen analyzer (Hamilton Thorne Research, Beverly, MA, USA). Only specimens with $>60\times10^6$ motile sperm/mL and 50% motility were used.

In vitro spermicidal activity

Spermicidal activities of the compounds were evaluated at once using a modified version to the protocol originally described by Sander and Cramer.[45] Semen samples were adjusted with a buffered glucose solution (Baker's buffer) supplemented with 0.5–1.0% BSA in order to contain 60×10^6 motile sperm/mL; 2-fold serial dilutions of the compounds were prepared in 0.9% NaCl (saline). Fifty microliters of semen were pipetted into 250 μL of compound/saline dilutions and gently vortexed for 3 s. A drop of this mixture was placed on a pre-warmed glass slide, coverslipped, and analyzed under a dark-field microscope with a 10× objective during 30 s. If any motile spermatozoon was seen, the dilution was considered as negative. Positive dilutions (i.e., all observed sperm immotile) were further incubated at 37 °C for 1 h with two volumes of Baker's buffer, and then re-examined for sperm motility. If no motile sperm were seen, the positive score was maintained. The MEC of a compound was calculated using the highest sperm-immobilizing dilutions and the initial concentration of the compound. Several different donor semen samples were assayed to ensure consistency of results. N-9 was used as positive control in all assays.

Statistical analysis

Comparisons between pairs of spermicidal results were done with the non-parametric Mann–Whitney test.

In vitro anti-HIV activity

Following the method of Resnick et al.,[46] cell-free (RF strain) and cell-associated (RF-infected H9 cells) HIV-1

stocks, displaying titers around 6 $-\log_{10}$ $TCID_{50}$, were incubated with half-log serial dilutions of the test agents (starting at 0.1%) for 2 min. Compound effect was terminated by 10-fold serial dilutions, which were then incubated with plated MT-2 cells for 4–6 days (in quadruplicates). Endpoint: syncytium formation and compound cytotoxicity (agents + MT-2 cells without virus). Results were calculated by the Reed–Muench method.[57] N-9 was used as positive control.

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