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ACYLCARNITINE ANALOGUES AS TOPICAL, MICROBICIDAL SPERMICIDES.

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Abstract: Acylcarnitine analogues, (+)-6-Carboxylatomethyl-2-alkyl-4,4-dimethylmorpholinium (**Z-n**, where n = the number of carbons in the alkyl chain), synthesized in multi-gram quantities show in vitro activities as spermicides, anti-HIV agents, and inhibitors of the growth of *Candida albicans*. Activity improves with increasing chain length. Compound **Z-15** is a candidate for further study as a topical, microbicidal spermicide.

Many women want to control their fertility and reduce their risk of becoming infected with a sexually transmitted disease (STD).[3] The HIV epidemic intensifies the need[4] for female-controlled methods that provide effective protection against both pregnancy and STDs. No currently available agent simultaneously protects against pregnancy and infection. There is a need to develop safe prophylactic agents that are spermicidal, anti-HIV, and anti-STD pathogens.

Nonoxynol-9 (**N-9**), a nonionic surfactant, is the most widely used spermicide in the U.S. It does not appear to reduce the risk of HIV infection.[5,6] It also increases the risk of urinary tract infections,[7] vulvovaginal candidiasis,[8] and genital ulcers.[9] Furthermore, **N-9** is a mixture of oligomers,[10] which may not meet future regulations as the health-care industry moves toward using pure compounds or mixtures whose individual components have met safety standards. For reasons of environmental toxicity,[11] several European nations have banned or restricted the use of **N-9** and related compounds, sparking a debate[12] about the health risks of **N-9**.

Two recent leads for improving the properties of **N-9** are an analogue[13] of zidovudine, a clinically proven reverse transcriptase inhibitor, and C31G,[14] a mixture of long-chain alkyldimethylamine oxide and long-chain *N*-alkyl-*N*,*N*-dimethylglycine. The zidovudine analogue, a non-detergent compound, has excellent anti-HIV properties.[13] The surfactant mixture, C31G, shows promising spermicidal activity and a broad spectrum of microbicidal activities;[15] it appears safe and nonirritating.[14]

Acylcarnitines have structural features similar to *N*-alkyl-*N*,*N*-dimethylglycine, a component of C31G. Our interest in making acylcarnitine analogues as inhibitors of carnitine acyltransferases[16] leads us to study these compounds as spermicides and microbicides. Furthermore, carnitine and acylcarnitines abound in semen[17] and carnitine acetyltransferase promotes growth of *Candida albicans*.[18] Sperm and *Candida albicans* likely contain proteins that recognize carnitine and acylcarnitines. Consequently, acylcarnitine analogues are excellent candidates for topical, microbicidal spermicides because, at low concentrations, they might inhibit the functions

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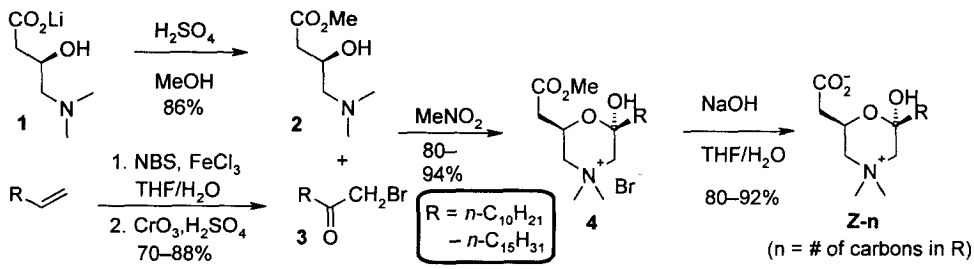
of these sperm and yeast. The lower the concentration that is required for efficacy, the less likely will be unwanted side effects (e.g., irritation).

In this letter, we report an improved synthesis of acylcarnitine analogues and assays of their in vitro activities as spermicides, growth inhibitors of *Candida albicans*, and anti-HIV agents.

Synthesis (Scheme 1). Demethylation of (*R*)-carnitine by thiophenol in 2-(dimethylamino)ethanol,[19] followed

Scheme 1.



by precipitation with LiOH gave **1** in 89% yield. We modified the esterification[16] to improve the yield of **2**. Bromohydroxylation of a series of 1-alkenes, followed by in situ oxidation of the resulting secondary alcohol to a ketone by Jones' reagent, gave **3** in good yields. Our previous synthesis,[20] a minor modification of Zav'yalov et al.'s procedure,[21] used acetone as a solvent and gave yields of **3** in the range of 30–55%. Bromoacetone, a by-product and a strong lachrymator, made the workup tedious. The procedure with THF eliminated this difficulty. Initially, the reaction was irreproducible. After systematic variation of solvent and alkene, the reaction proceeded reliably in THF/H_2O/alkene [(12:12:1)(v/v/w)].[22]

Condensation[16] of **3** with **2** gave **4**. The key to isolating "clean" **4** was triturating the oily residue with EtOAc, then stirring until precipitation occurred. The hydrolysis of **4** with 0.05-*N* NaOH furnished the desired zwitterions **Z-10**–**Z-15**. Multi-gram quantities of **Z-10**–**Z-15** were separated from NaBr with reverse-phase (RP-8) column chromatography instead of HPLC[16] and, then, recrystallized to yield analytically pure samples.

Table 1. Spermicidal Activity of **Z-10**–**Z-15** and **N-9** in the Sander–Cramer Test.

compound	Highest Spermicidal Dilution (1/X)[a]	MEC (mg/mL)[b]
Z-10	4.8 ± 0.5	2.3 ± 0.2
Z-11	7.6 ± 0.4	1.4 ± 0.1
Z-12	27.2 ± 2.3	0.407 ± 0.7
Z-13	48.0 ± 5.1	0.235 ± 0.025
Z-14	102.4 ± 13.5	0.121 ± 0.014
Z-15	134.4 ± 14.2	0.109 ± 0.012
N-9	83.2 ± 18.2	0.144 ± 0.011

[a]Solvent = distilled H_2O, initial concentration = 10 mg/mL, Number of replicates/samples = 12. Values are expressed as means ± SEM
[b]Minimum Effective Concentration

Spermicidal Activity.[23] Table 1 presents the in vitro assay results for **Z-10**–**Z-15** and **N-9**. For **Z-10**–**Z-15**, the potency increases (i.e., MEC decreases) as the length of the alkyl chain increases until **Z-14**, the tetradecyl analog. Within experimental errors, **Z-14** and **Z-15** have similar MECs; both are slightly lower than that of **N-9**.

Inhibition of *Candida albicans*.[25] In vitro assays for growth inhibition of *Candida albicans* also show that the minimum inhibitory concentration (MIC) decreases as the chain length increases. The MICs (in mg/mL) are **Z-10** (>0.1), **Z-11** (>0.1), **Z-12** (0.08–0.1), **Z-13** (0.04), **Z-14** (0.01), and **Z-15** (0.002). Compound **Z-15** is the best;

Z-14 has an acceptable activity. By comparison, C31G has an MIC of 0.04 mg/mL,[27] **N-9** does not inhibit growth at concentrations up to 10 mg/mL.[28]

Anti-HIV Activity.[29] Table 2 presents the results of **Z-10** – **Z-15** and **N-9** in-vitro assays for inhibition of cell-free HIV-1 (RF strain). As in the other assays, the effective concentration of antiviral agent decreases as the length of the alkyl chain increases. Within experimental errors, **Z-13**, **Z-14** and **Z-15** have similar activities; **N-9** is slightly more active than these compounds at 0.032 and 0.01 mg/mL. In a different assay,[31] C31G inactivates HIV-1 infectivity at 0.0125 mg/mL.

Table 2. In Vitro Assay for Inhibition of HIV: Cell-free Inactivation Assay. The effect of the concentration on the reduction of virus infectivity.

	Concentration (mg/mL)					
Compound	10	3.2	1	0.32	0.1	0.032
Z-10	≥4.0[a]	2.8	1.0	0.8	n.a.[b]	n.a.
Z-11	4.7	2.7	2.0	0.8	n.a.	n.a.
Z-12	≥4.0	≥3.5	2.8	2.0	0.3	0.0
Z-13	3.5	3.5	3.3	2.7	1.5	0.5
Z-14	≥4.0	≥4.0	3.5	2.8	1.5	0.5
Z-15	≥4.0	3.8	3.2	2.7	1.5	0.3
N-9	n.a.	n.a.	≥4.0	3.5	2.2	0.5

[a]In log units, values indicate reduction of infective viral titer [b]Not assayed

Summary. With excellent spermicidal activity, *Candida albicans* inhibition, and HIV inhibition, **Z-15** shows promise for development as a topical, microbicidal spermicide. The mechanism of action for spermicidal and HIV activities may be due to the surfactant properties of **Z-15**; further studies are needed. The striking activity against *Candida albicans* distinguishes **Z-15** from **C31G** and **N-9**. This high potency occurs at a concentration 50-fold lower than that needed for spermicidal and anti-HIV activities. This low concentration suggests a specific interaction between **Z-15** and this yeast. Whether this activity is due to inhibition of acylcarnitine-dependent enzymes awaits further study. In addition to use as a topical microbicide, the anti-Candida activity suggests the potential to develop **Z-15** as a lead compound for systemic treatment of this pathogen.

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22. **Synthesis of 1-bromo-2-alkanones, 3.** To a solution of 1-alkene (20 mmol) in THF:H_2O (1:1, 24 mL/g of alkene), *N*-bromosuccinimide (NBS) (24 mmol, 1.2 equiv) and $FeCl_3 \cdot 6H_2O$ (50 mg) were added. The resulting orange solution was stirred until all NBS dissolved (ca. 4–5 h). To this solution, Jones' reagent [CrO_3 (6.1 g, 61 mmol) and conc H_2SO_4 (6 mL)] was added while cooling the reaction flask. The dark green reaction-mixture was stirred overnight and then diluted with water (50 mL). The reaction mixture was extracted with Et_2O (3 × 30 mL). The ethereal extract was washed with satd $NaHCO_3$ (2 × 30 mL), brine (1 × 20 mL), and dried. Concentration of the extract gave a wax, which was chromatographed on silica. Eluting with hexanes followed by 10% Et_2O-hexanes gave a colorless waxy solid (yield 70–88%). ^1H NMR (400 MHz) δ 0.75 (3H, t, J_{app}=7.1 Hz), 1.44–1.75 ((n-7)H, br m), 2.65 (2H, t, J_{app}=7.1 Hz), 3.89 (2H, s); IR (film) ν_{max} 1719.
23. Semen samples were collected from healthy volunteers; only specimens with > 60 × 10^6 motile sperm/mL and 50% motility were used. Two-fold serial dilutions of the compounds were prepared in 0.9% saline. Positive dilutions (i.e., all observed sperm were immotile) were further incubated at 37 °C for 1 h with 2 vol of buffer, and re-examined for sperm motility. If no motile sperm were seen, the positive score was maintained. MEC for the compound was calculated using the highest sperm immobilizing dilution and its initial concentration.
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