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Synthesis and Repellent Efficacy of a New Chiral Piperidine Analog: Comparison with Deet and Bayrepel Activity in Human-Volunteer Laboratory Assays Against *Aedes aegypti* and *Anopheles stephensi*

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ABSTRACT Optically active (1*S*, 2′*S*)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide (SS220) is a new synthetic arthropod repellent. A three-step synthesis based on a chiral Diels-Alder reaction and diastereomeric resolution of 2-methylpiperidine was developed to prepare the compound. Quantitative laboratory assays using human volunteers compared the effectiveness of SS220 with the commonly used repellents Deet and Bayrepel against *Aedes aegypti* (Linnaeus) and *Anopheles stephensi* Liston mosquitoes. In two experiments using *Aedes aegypti*, one using a single identical dose and one with varying doses used to develop a dose–response curve, SS220 was as effective as Deet and both compounds were more effective than Bayrepel. The three compounds were equally effective against *An. stephensi.* Based on the ease of its synthetic preparation and its repellent efficacy, we surmise that SS220 is a candidate to serve as a new and effective alternate repellent for protection against arthropod disease vectors.

KEY WORDS (1*S*, 2′*S*)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide, 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester, *N*,*N*-diethyl-3-methylbenzamide, *Aedes aegypti*, *Anopheles stephensi*

THE PURPOSES OF THIS study were to develop an efficient organic chemical synthetic method for preparation of the new chiral piperidine analog candidate repellent and to evaluate the compound's performance as a repellent compared with the benchmark-repellent compounds, Deet and Bayrepel, against two species of mosquitoes that are important vectors of yellow fever, dengue, and malaria. This work is part of a broader objective to develop a new, effective, and safe repellent product for human use against arthropods that are disease vectors.

The study involved three repellents:

1. Deet, (*N*,*N*-diethyl-3-methylbenzamide), a widely used repellent that is registered with the U.S. Environmental Protection Agency (EPA). It is marketed throughout the world by a number of companies, and an extended-duration polymer formulation of 33% Deet is the current standard insect/arthropod repellent of the U.S. military. Despite the compound's extensive use and effectiveness it has some drawbacks including possible health risks (CDC 1989, Qiu et al. 1998) at high dermal doses and damage to certain plastics coming in contact with Deet.
2. Bayrepel, [2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester], is a comparatively new repellent product that is marketed by the Bayer Corporation in many countries and has been recently registered by the EPA (*http://www.autan.com/bayrepel/scientific_en.html*). Formulations of Bayrepel (also known as KBR 3023, Picaridin and Hepidanin) and Deet were found to be equally effective repellents in field studies against *Aedes albopictus* (Skuse), *Culex quinqeuefasciatus* Say and several other mosquito species (Yap et al. 1998, Yap et al. 2000).
3. Racemic, 2-methylpiperidinyl-3-cyclohexene-1-carboxamide, was first identified as an insect repellent by McGovern et al. (1978) and the U.S. Department of Agriculture (USDA) assigned the compound the code number AI3–37220. AI3–37220 (220) contains two asymmetric centers and achiral synthesis yields a racemic mixture of *1S,2′R*, *1R,2′S*, *1R,2′R*, and *1S,2′S* stereoisomers. The racemic mixture proved to be an effective repellent against a variety of blood-feeding arthropods (Robert et al. 1992, Coleman et al. 1993, Walker et al. 1996, Frances et al. 1996, Frances et al. 1998, Debboun et

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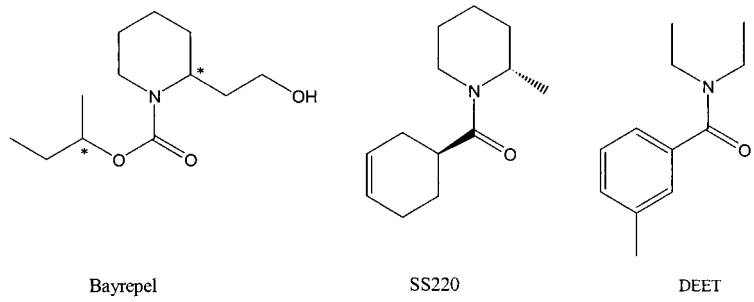

Fig. 1. Chemical structure of three insect repellents.

al. 2000). Klun et al. (2001) showed that the *1S,2'S* stereoisomer (SS220) was the most effective isomer of the four in reducing bites by *Ae. aegypti*, and they surmised that enhanced repellent effects could be realized through specific formulation of the most active stereoisomer. Klun and Schmidt (2001) have a U.S. patent pending that covers this concept, and SS220 is as a candidate for use as a new standard repellent for the military. SS220 was cleared for use on humans (Snodgrass and Harvey 1998) and the compound has since undergone additional standardized toxicology tests at the U.S. Army Center for Health Promotion and Preventive Medicine in Aberdeen Proving Ground, MD. Tests with SS220 included acute dermal toxicity in guinea pigs (Snodgrass and Houpt 2002), acute oral toxicity in rats (Snodgrass 2002a), eye irritation in rabbits (Snodgrass 2002b), skin sensitization to SS220 in guinea pigs (Houpt 2002), primary skin irritation in rabbits (Snodgrass 2002c), and mutagenic potential studies (Covance Laboratories, Inc., Vienna, VA). All of these tests indicated that SS220 is biologically pacific and amenable to dermal application to humans as protectant against blood-feeding arthropods.

The chemical structures of Bayrepel, SS220, and Deet are shown in Fig. 1, and it is apparent that the three compounds have some structural resemblance. They also have similar molecular weights, of 229.3, 207.1, and 191.3, respectively. Bayrepel and SS220 are piperidine analogs and each contains two asymmetric centers. The absolute configurations of the stereogenic centers of SS220 are depicted in Fig. 1 and asymmetric centers in Bayrepel are noted by asterisks. Bayrepel is marketed as a mixture of four stereoisomers (racemate) and the compound used in this study was racemate. To our knowledge, the four isomers of Bayrepel have not been prepared individually and evaluated for differential repellent effects (Klun et al. 2001). As was discovered with SS220, it is plausible that one of the stereoisomers comprising Bayrepel might possess greater repellent effects than racemate or the other stereochemical configurations.

Materials and Methods

Chemical Analytical Methods. Gas chromatography (GC) analyses were carried out in a split mode on a Shimadzu GC-17A with FID detector fitted with columns: (1) DB-5, 15 m × 0.25 mm, film thickness 0.25 μm (J&W Scientific) for chemical purity, and (2) Chiraldex B-DM (β- cyclodextrin, dimethyl) 30 m × 0.25 mm, film thickness 0.25 μm, (J&W Scientific) for stereoisomeric purity determination. (*S*)-2-Methylpiperidine and (*S*)-3-cyclohexen-1-carboxylic acid was derivatized using standard acetylation (CH_3COCl/Py) and methylation (CH_2N_2) procedures, respectively, before analyses on the chiral column. Liquid chromatographic analysis of SS220 diastereoisomer composition was performed in an isocratic solvent mode (hexane/iso- propanol, 95:5) on a Beckman System Gold instrument fitted with a Chiralpak AS column (Chiral Technologies, Inc., Exton, PA) 25 cm × 0.46 cm using UV detection at 232 nm. ^{1}H NMR spectra were recorded with TMS as an internal standard in $CDCl_3$ on a Bruker QE-300 spectrometer. IR spectra were obtained in KBr tablets on a Perkin-Elmer 1320 spectrophotometer. Optical rotations were measured in chloroform at 22°C on a Perkin-Elmer model 241 polarimeter. The reagents were purchased from Aldrich (Milwaukee, WI) unless otherwise specified.

Commercial Repellents. Deet was obtained from Morflex, Inc. (Greensboro, NC) and Bayrepel from Bayer Corporation (Bayer Consumer Care, Morristown, NJ). The compounds were at least 98% pure chemically according to GC analyses.

(-)-Bornane-2,10-sultam (or (-)-2,10-camphorsultam). Commercial (*S*)-(+)-10- camphorsulfonic acid was chlorinated to (*S*)-(+)-10-camphorsulfonyl chloride according to Bartlett and Knox (1973). The acyl chloride was converted to the amide, and the latter was cyclized to (-)-(camphorsulfonyl)imine as described by Towson et al. (1990). Following the procedure by Weismiller et al. (1990) the imine was reduced with lithium aluminum hydride to provide crystalline (-)-bornane-2,10-sultam.

(-)-*N*-Propenoylbornane-2,10-sultam. A solution of (-)-bornane-2,10-sultam (10.0 g, 46.5 mmol), acryloyl chloride (15.2 ml, 187.1 mmol) and anhydrous $CuCl_2$ (0.625 g, 4.4 mmol) in anhydrous benzene (65 ml) was

refluxed under N_2 for 4 h. The mixture was filtered while still warm, the reaction vessel was washed with dichloromethane (30 ml), and the combined filtrate was concentrated in vacuo to give a solid product. Crystallization from anhydrous ethyl alcohol yielded (92.4%) 11.56 g pure sultam as white needles, m. p. 203–205°C. IR (KBr tablet): υ = 2920 s, 1678 m, 1620 m, 1320 s, 1225 s, 1135 s, 1065 s, 980 s, 880 w, 805 m, 765 m cm^{-1}. ^1H NMR: δ = 6.83 (1H, dd, J = 10.0, 16.5Hz), 6.48 (1H, dd, J = 1.5, 16.5Hz), 5.83 (1H, dd, J = 1.5, 10.0Hz), 3.93 (1H, dd, J = 5.0, 7.0Hz), 3.49 (1H, d, J = 14.0Hz), 3.46 (1H, d, J = 14.0Hz), 2.20–2.02 (2H, m), 1.98–1.80 (^3H, m), 1.47–1.30 (2H, m), 1.11 (^3H, s), 0.91 (^3H, s). $[\alpha]_D$ −102.3 (c 1.7, $CHCl_3$)

(*S*)-3-Cyclohexene-1-carboxylic acid. The chiral Diels-Alder reaction of (-)-*N*- propenoylbornane-2,10-sultam with butadiene was conducted in a 0.2 molar scale as described by Thom et al. (1993). The yield of the acid was 80–82% with >99% optical and chemical purity.

(S)-2-Methylpiperidine. To a 0°C solution of (*R*)-(-)-mandelic acid (24.0 g, 157.8 mmol) in anhydrous methanol (63 ml), a solution of racemic 2-methylpiperidine (19.4 ml, 165.3 mmol) in anhydrous ethyl ether (450 ml) was added. The reaction mixture was kept at 0°C overnight and filtered. The crystals were washed with cold anhydrous ether and dried under vacuum to give (*S*)- 2-methylpiperidinium mandelate (12.28 g, 31%, m.p. 118–120°C) of >97% stereochemical purity determined by converting to a free amine). Ethyl ether (60 ml) was added to the mother liquor and the mixture was kept at (−12°C) for 3 h to furnish an additional portion of the salt (3.17 g) with m.p. 116–119°C. Overall yield was 39% and stereoisomeric purity >96%. (-)-2- Methylpiperidinium mandelate from several runs (36.00 g, 143.4 mmol) was dissolved in water (50 ml) and treated with dry powdered K_2CO_3 until the layers separated. The organic phase was separated, the aqueous phase was extracted with ether (5 × 50 ml), and combined organic solution was dried ($MgSO_4$). Distillation under atmospheric pressure gave pure (*S*)-2-methylpiperidine (12.49 g, 88%) as a colorless liquid with b.p. 118–120°C, and *S*/*R* ratio 97/3.

(1*S*, 2′*S*)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide. To a stirred solution of (*S*)-3-cyclohexene-1-carboxylic acid (7.56 g, 60.0 mmol) in anhydrous dichloromethane (52 ml), *N*,*N*-dimethylformamide (8.0 μl) was added followed by oxalyl chloride (10.5 ml, 120.3 mmol). The reaction mixture was stirred for two hours at room temperature and evacuated. The remainder was cooled in an ice bath, and a mixture of (*S*)-2-methylpiperidine (7.3 ml, 62.0 mmol) and pyridine (5.3 ml, 62.0 mmol) in dichloromethane (25 ml) was added. The resulting mixture was stirred overnight at room temperature. After addition of water (15 ml), the organic layer was separated, washed with saturated aqueous $NaHCO_3$ (20 ml), brine (20 ml), 2% HCl (20 ml), again with brine (20 ml), and dried ($MgSO_4$). Concentration of the extract in vacuo and distillation under reduced pressure gave a colorless oil (10.59 g, 86%); b.p. 95–96°C (0.025 torr). The chemical purity was >99% and the stereochemical purity >94%. The compound was identical by GC and HPLC with an authentic sample (Klun et al. 2000).

Insects. *Ae. aegypti* (red eye Liverpool strain) and *An. stephensi* used in the study were from colonies maintained at the Walter Reed Army Institute of Research. The insects were reared (Gerberg et al. 1994) by feeding larvae ground tropical fish flakes (Tetramin Tropical Fish Flakes, Tetra Sales, Blacksburg, VA, www.tetra-fish.com). Adults were maintained in a photoperiod of 12:12 (L:D) h at 27°C and 80% RH with cotton pad moistened with 10% aqueous sucrose solution.

Bioassay Methods. In conducting this research, we adhered to the guidelines established by the National Institutes of Health for tests involving human subjects, and protocols were approved by the Human-Use Review Board of the Walter Reed Army Institute of Research. As stated earlier, SS220, Deet and Bayrepel have abundant safety databases that permitted applications to human volunteers. Experiment 1 measured the biting frequency of *Ae. aegypti* females in response to 0.0, 0.3, 0.6, 1.2, 2.4 and 4.8 × 10^{-2} μmol/cm^2 skin doses of SS220, Bayrepel and Deet applied to human volunteers using ethanol solutions. In experiments 2 and 3, the three compounds were tested at 2.4 × 10^{-2} μmol/cm^2 skin against *Ae. aegypti* and *An. stephensi*, respectively.

Experiments were conducted by using K & D modules and methods described by Klun and Debboun (2000). A human volunteer wearing short pants was seated. Using a skin-marking template and a washable-ink marker, skin areas representing 3 cm × 4 cm floor openings of the K & D module were outlined on the outer, top, and inner thigh positions of each leg. Six areas to be treated with doses of compound (experiment 1) or four areas treated with stoichiometrically equivalent amounts of each compound at a fixed dose and control (experiments 2 and 3) were assigned randomly.

Experiment 1 dose–response testing of each compound against *Ae. aegypti* used three volunteers. Each volunteer represented an incomplete block with total numbers of mosquitoes per dose and treatments assigned as follows. Volunteer one was assigned 30 mosquitoes per dose for Deet and SS220, and 60 mosquitoes per dose for Bayrepel; volunteer two was assigned 90 mosquitoes per dose for Deet and 60 for SS220; and volunteer three was assigned 60 mosquitoes per dose for SS220 and 30 for Bayrepel. Thus, considering each volunteer as a block, this experiment was conducted as an incomplete block design (one volunteer received all three treatments). The sample sizes used for each dose-compound combination were sufficient to establish a well-defined dose–response curve for each compound, indicated by the small standard errors of the parameter estimates referred to in the Results and Discussion section.

Fixed-dose tests of the three compounds against *Ae. aegypti* and *An. stephensi* used 27 replicates over four volunteers and 42 replicates over six volunteers,

respectively. Five mosquitoes were used for each treatment in each replicate.

All treatments were pipetted onto a 4 cm × 5 cm rectangular area, 0.5 cm outside of the template marks, of the subjects' skin in 55 μl ethanol/treatment. Treating outside template marks assured that areas beneath each K & D module cell contained no untreated skin. Skin treated with ethanol alone served as control. In all tests, adjacent cells of the K & D modules were provided with five female mosquitoes randomly selected from cages containing ≈200 adults. Mated nulliparous females (5–15 d old) had access only to water 24 h before testing. The K & D module was positioned over the treated skin areas, trap doors above the areas opened, and the number of females biting (proboscis inserted into skin and/or observed blood-engorged) within each of the cells in a 2 min skin exposure was recorded, then trap doors were closed. Individual mosquitoes were recorded as either having fed or not fed during a trial. The experiments were done in a walk-in incubator (27°C and 80% RH) in ambient fluorescent light from 0730 hours to 1030 hours over 4–6 d. Mosquitoes were used once in a test and then frozen.

Statistical Methods. The range of doses selected for experiment 1 was based on previous empirical tests with *Ae. aegypti.* We used Proc NLMixed (SAS Institute, Inc., 1999) to analyze the data sets, fitting a generalized linear mixed model with a logit link (McCullagh and Nelder, 1989). In this model, estimates for the dependent variable, logit (p) = log (p/[1 − p]), where p is the (true) proportion of nonbiting mosquitoes, depends on both fixed (compounds, and in experiment 1, doses) and random (volunteer, where each volunteer acts as a block) effects.

Visually inspecting graphs of the data and experience from previous analyses suggested that a square-root transformation on dose would create a linear relationship between dose and logit (p) for the range of doses used for Deet, Bayrepel and SS220 in experiment 1. However, with Bayrepel, mosquitoes did not appear to respond differently to controls and to the lowest dose used. We accommodated for this fact by slightly altering the dose–response equation for Bayrepel, stated below and illustrated in Fig. 2. Slope estimates for repellents were allowed to differ but intercepts were not, since the only factor that should affect responses at a zero dose is volunteer-to-volunteer variability in attractiveness to mosquitoes.

We jointly estimated models for the three compounds as:

$$\text{Deet:} \quad \text{logit}(p) = b_0 + b_D \times \sqrt{(\text{dose}_i)} + u_j,$$

$$\text{SS220:} \quad \text{logit}(p) = b_0 + b_S \times \sqrt{(\text{dose}_i)} + u_j,$$

$$\text{Bayrepel:} \quad \text{logit}(p) = b_0 + u_j, \text{ if } \sqrt{(\text{dose}_i)} < 0.5,$$

$$= b_0 + b_B \times (\sqrt{(\text{dose}_i)} - 0.5) + u_j, \text{ otherwise,}$$

where p is the proportion of nonbiting mosquitoes, i indexes the different doses, b_0, b_D, b_S, b_B are estimated parameters, and u_j is the random effect of the jth subject, assumed to be a draw from a normal distribution with mean zero and variance estimated from the model fitting procedure. While there was a volunteer effect in both this and other studies using similar methodology, in experiment 1 the magnitude (a variance component) of the effect was poorly estimated because it involved only three volunteers. Nevertheless, allowing for volunteer to volunteer variability in the model provided an estimate of the relationship between dose and compound efficacy free of volunteer effects.

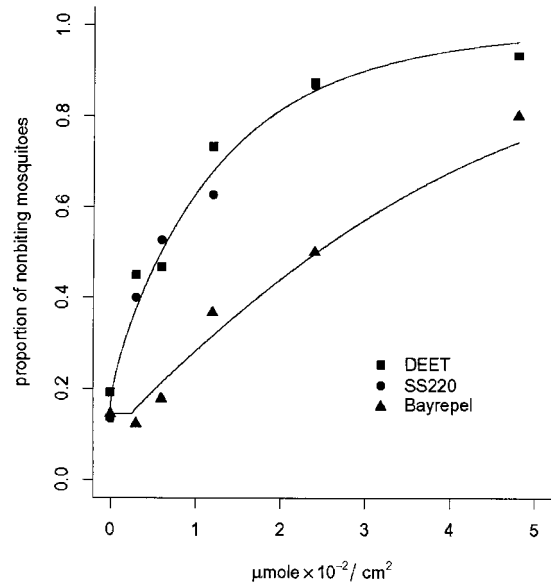

Fig. 2. Estimated dose–response curves for Deet and SS220 (upper line) and Bayrepel (lower line) for *Ae. aegypti* based on a generalized linear mixed model for the logit of the proportion of nonbiting mosquitoes. Empirical proportions of nonbiting mosquitoes calculated from the same data are also plotted with the estimated curves.

In experiment 2 with *Ae. aegypti* and experiment 3 with *An. stephensi,* we used a similar analysis approach to determine if mosquitoes were differentially repelled by the three compounds at a fixed dose of 2.4×10^{-2} μmol/cm^2 skin. We estimated the following model: logit $(p) = \tau + u_j$, where p is the proportion of nonbiting mosquitoes, i indexes the control, Deet, SS220, or Bayrepel treatment (τ), and $_j$ indexes the volunteers (u), as in experiment 1.

Results and Discussion

Synthesis of SS220. A three-step method was employed to synthesize SS220; (1) Enantioselective synthesis of (*S*)-3-cyclohexen-1-carboxylic acid, (2) Resolution of racemic 2-methylpiperidine, and (3) Acylation of (*S*)-2-methylpiperidine with (*S*)-3-cyclohexen-1- carboxylic acid.

(*S*)-Cyclohex-3-enecarboxylic acid was prepared via an asymmetric Diels-Alder reaction (Thom et al. 1993) of butadiene and a derivative of acrylic acid bearing a chiral auxiliary, (-)-2,10- camphorsultam,

Table 1. Proportions (p) of nonbiting mosquitoes and sample sizes (n) used for creating dose-response functions for three repellents, tested using *Ae. aegypti* (experiment 1)

Compound	Dose (10^{-2} μmol/cm^2 skin)					
	0	0.3	0.6	1.2	2.4	4.8
Deet						
p	0.19	0.45	0.47	0.73	0.88	0.93
n	120	120	120	120	120	120
SS220						
p	0.13	0.40	0.53	0.63	0.87	0.93
n	150	150	150	150	150	150
Bayrepel						
p	0.14	0.12	0.18	0.37	0.50	0.80
n	90	90	90	90	90	90

Table 2. Estimated proportions (p) of nonbiting mosquitoes and sample sizes (n) used for assessing repellency against Deet, SS220, and Bayrepel each at a dose of 2.4×10^{-2} μmol/cm^2 skin, tested using *Ae. aegypti* (experiment 2) and *An. stephensi* (experiment 3).

Species	Control	Deet	SS220	Bayrepel
Ae. aegypti				
p (SE)	0.22 (0.11)	0.85 (0.08)	0.86 (0.08)	0.67 (0.14)
n	135	135	135	135
An. stephensi				
p (SE)	0.26 (0.07)	0.88 (0.04)	0.88 (0.04)	0.83 (0.06)
n	210	210	210	210

Standard errors (SE) of the estimated proportions are in parentheses.

readily available from the commercial (*S*)-(+)-10-camphorsulfonic acid (Bartlett and Knox 1973, Towson et al. 1990, Weismiller et al. 1990). Synthesis of the chiral dienophile was described by Oppolzer et al. (1986) and Binger et al. (1989) via acylation of the sodium derivative of bornane-2,10-sultam with propenoyl chloride. Thom and Kocienski (1992) encountered two problems in this preparation: (1) sodium salt formed a gray sludge that was difficult to manipulate, and (2) a major by-product incorporating two molecules of auxiliary was formed. To overcome these difficulties, Thom and Kocienski (1992) developed a high-yielding two-step method consisting of a silylation of (+)-bornane-2,10-sultam with chlorotrimethylsilane and further acylation of the *N*-trimethylsilyl derivative with propenoyl chloride in the presence of copper (II) chloride. We found that the moisture-sensitive silylation of bornane-2,10-sultam could be eliminated, and *N*-propenoylbornane-2,10-sultam could be easily obtained in a high yield (>90%) by direct acylation of (-)-bornane-2,10-sultam with propenoyl chloride in the presence of copper (II) chloride in refluxing benzene for just 4 h (instead of 16 h reflux in the original procedure).

The diastereoisomeric resolution of 2-methylpiperidine was accomplished by using commercially available (*R*)-(-)-mandelic acid as described by Craig and Pinder (1971) and Rauk et al. (1983). This procedure was supposedly improved by Adamo et al. (1999), but in our hands it failed to reproduce the reported yield. (Precipitation of the salt was not noticeable even after a final portion of ether was added, and after extended exposure of the mixture to 0°C, the yield did not exceed 30%.) We optimized conditions of the resolution and attained (*S*)-2-methylpiperidine of >96% stereochemical purity in 34–40% yield (see Materials and Methods).

In the final step, (*S*)-3-cyclohexen-1-carboxylic acid was converted to acyl chloride using oxalyl chloride, which reacted with (*S*)-2-methylpiperidine in the presence of a base. Importantly, we found that use of rather basic triethylamine gave rise to a partial epimerization and the stereoisomeric purity of SS220 dropped to 91%. However, less basic pyridine and quinoline were significantly more suitable for the acylation affording 94–95% stereoisomeric and >99% chemical purity. The overall yield of the three-step process was 22%.

Bioassays. Table 1 presents empirical proportions for nonbiting mosquitoes (mosquitoes not biting divided by total mosquitoes) and total mosquitoes for each dose/compound combination in experiment 1. We found that Deet and SS220 did not differ in effectiveness ($P = 0.65$, $t = 0.46$, df = 20), with parameter estimates (standard error in parenthesis) for both compounds of intercept −1.78 (0.24) and slope 23.0 (1.1). Figure 2 shows the fitted relationship between dose and proportion of nonbiting *Ae. aegypti* on the original scale, along with empirical proportions averaged over volunteers, and overlayed on the modeled curves. Note that, because of the nature of the incomplete block design (each volunteer was not tested using all three compounds), the fit to the data are actually better than it appears, since adjustments in the fitted lines made to each volunteer are not depicted. While this model fits these data well, it should only be used to interpret relative differences among the compounds for reasons discussed below. In our model, Bayrepel produced no effect until $\sqrt{\text{dose}}$ equals $\sqrt{0.5 \times 10^{-2}}$, at which point logit (p) increased with slope 16.8 (1.8). Thus, Bayrepel's effectiveness was everywhere lower than Deet and SS220, and the difference increased (on the logit scale) with increased dose because the slope parameter for Bayrepel was significantly ($P < 0.01$, $t = -4.36$, df = 20) lower than that of the other two compounds.

Table 2 provides estimates and their standard errors for the proportion of nonbiting mosquitoes and sample sizes for experiments 2 and 3. In experiment 2 at a fixed dose of 2.4×10^{-2} μmol/cm^2 skin, significantly fewer *Ae. aegypti* bit subjects treated with Bayrepel than in the control ($P < 0.01$, $t = 6.03$, df = 5), but Bayrepel was significantly less effective than either Deet or SS220 in reducing *Ae. aegypti* bites ($P < 0.02$, $t = 3.45$, df = 5). SS220 and Deet had a similar repellency ability and did not differ significantly from each other ($P > 0.75$, $t = 0.33$, df = 5). These results independently confirmed those obtained in experiment 1. The estimated proportions of nonbiting mosquitoes obtained in experiment 2 fell close to the values seen in experiment 1 at the 2.4×10^{-2} μmol/cm^2 dose (Table 1).

Our experience (and previous results with this and other mosquito species) suggests that the overall proportion of nonbiting mosquitoes can vary from one experiment to another. Mosquitoes' tendency to bite or not bite appears to depend on many factors other than the amount of repellent used. Some factors, such as differential host attractiveness to mosquitoes, can be easily incorporated into statistical models. Others, however, are less well understood, and may depend on complex interactions between host factors, physiological factors, genetics, conditions of larval development, and environmental factors. What seems to be constant is the relative difference in repellent effectiveness from one compound to another. In other words, whether the mosquitoes are tending to bite more or less frequently, Deet and SS220 seem to provide similar levels of protection. Because Bayrepel was less effective than Deet and SS220 in experiments 1 and 2, our results indicate that racemic Bayrepel is significantly less effective than the other two compounds in preventing bites by *Ae. aegypti*. We surmise that the relative effectiveness of the three compounds against *Ae. aegypti* will be confirmed in the field, and it may well be that the performance of Bayrepel against this species could be improved by increasing the amount of compound applied to the skin or by using an optimized stereochemical formulation containing the most active stereoisomer of Bayrepel rather than racemate.

Unlike tests with *Ae. aegypti*, experiment 3 showed that at 2.4×10^{-2} μmol/cm^2 skin all three compounds were equally effective against *An. stephensi* (Table 2). The compounds were significantly more effective than the control ($P < 0.01$, $t = 10.19$, df = 5).

Discovering that *An. stephensi* possessed equal sensitivity to the three repellents while *Ae. aegypti* showed tolerance to Bayrepel provides evidence that the repellent receptor systems of the species are physically different. This is not surprising and one should logically expect that different species of arthropods, strains within species, and individuals within strains can vary in their susceptibility to repellent compounds. This premise is supported by Rutledge et al. (1978), who observed that 18 mosquito species and strains displayed significantly different levels of susceptibility to the repellent effects of Deet.

SS220 is derived from a parent compound, AI3-37220 racemate, that has performed well as repellent in laboratory and field trials against species of ticks, mites, blackflies, sand flies, and mosquitoes (see aforementioned citations.). It is known that SS220 has enhanced repellent effects that exceed the parent racemic compound (Klun et al. 2001). In this study we have demonstrated that SS220 can be prepared synthetically with ease, and that it can be as effective or more effective than the most widely used repellents. Based upon these facts, indications are that SS220 could eventually serve as a new practical and effective third-generation repellent against arthropods carrying disease.

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