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SYNTHESIS OF *GEM*-DIFLUORO-AVERMECTIN DERIVATIVES: POTENT ANTHELMINTIC AND ANTICONVULSANT AGENTS

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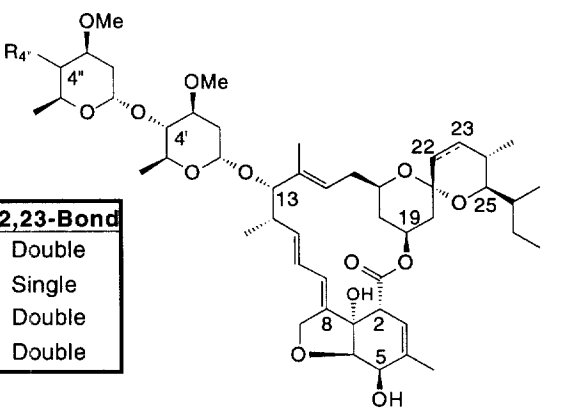
Abstract: A series of *gem*-difluoro-avermectin derivatives was synthesized from the corresponding ketones at positions 4″, 4′, 13, and 23 using diethylaminosulfur trifluoride (DAST). These fluorinated avermectins exhibit potent antiparasitic activity in a new *Haemonchus contortus* larval assay and are equipotent to ivermectin. In addition, 23-*gem*-difluoro-ivermectin displays useful anticonvulsant activity in mouse models.

The discovery of avermectin B_{1a} (**1a**, AVM) and its semi-synthetic derivative, ivermectin (**1b**, IVM), by researchers at Merck[1a,b] profoundly altered the treatment of parasites on plants, animals and humans.[1c] The avermectins, by dint of their remarkable biological activity and complex molecular architecture, stimulated significant interest in the scientific community, leading to the discovery of new derivatives with enhanced biological efficacy such as emamectin[2,3] (**1c**, controls agricultural pests) and eprinomectin[2,4] (**1d**, controls cattle parasites, including those on lactating dairy cattle). Avermectin's useful biological effects are not limited to antiparasitic activity; recent reports[5] demonstrate that certain AVM derivatives possess anticonvulsant and anxiolytic activity at levels comparable to diazepam while lacking valium's undesirable sedative effects and interactions with alcohol.



Entry	Compound	$R_{4"}$	22,23-Bond
1a:	Avermectin B_{1a}	α-OH	Double
1b:	Ivermectin	α-OH	Single
1c:	Emamectin	β-MeNH	Double
1d:	Eprinomectin	β-AcNH	Double

As part of our program to identify new AVM derivatives with improved biological activity profiles, the synthesis of *gem*-difluoro-avermectin derivatives was undertaken, leading to a series of compounds substituted at

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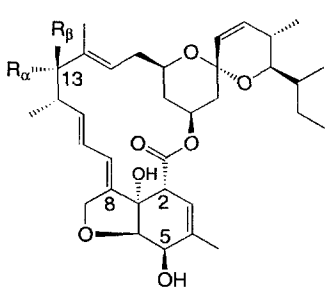
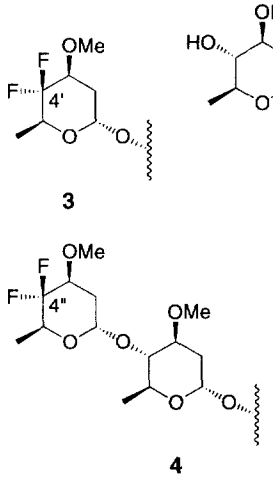
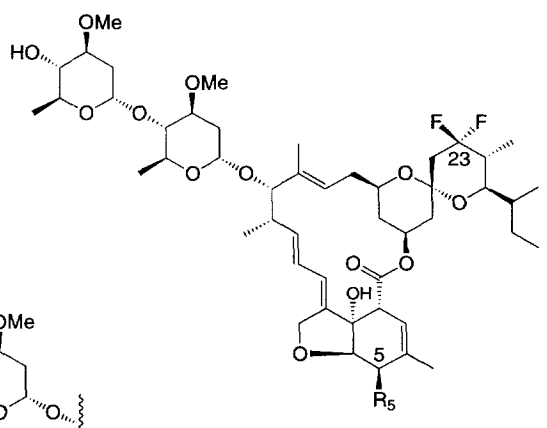
positions 4′, 4″, 13, and 23 with two fluorine atoms. It has long been recognized that fluorination of organic molecules may confer dramatic shifts in their biological activity profile.[6] Fluorine substitution for hydrogen is not merely an isosteric replacement: the larger, more lipophilic fluorine (van der Waals radius: 1.35 Å for F vs 1.20 Å for H)[7] has a stronger C-F bond, increasing metabolic stability and its high electronegativity allows it to function as a H-bond acceptor. The difluoro derivatives described here exhibited potent anthelmintic efficacy in vitro and 23-*gem*-difluoro-IVM (**5**) displayed significant activity in mouse epilepsy models.

On fragile, complex natural products such as AVM, the replacement of a hydroxyl with fluorine can be relatively straightforward, proceeding readily at low temperatures. However, the conversion of ketones to the corresponding difluorides requires more forcing conditions, typically heating at 50 °C for protracted times (up to 12 h) and necessitating a significant excess of fluorinating agent (50% by reaction volume). The reagent of choice for this reaction was DAST[8] although morpholinosulfur trifluoride,[8] a somewhat milder reagent, could also be employed. Increasing temperature, reaction times or quantities of fluorinating agent led to sharply decreased yields. DAST was reacted with ketones at positions 4″, 4′, 13, or 23 on suitably protected AVMs (*O*-trimethylsilyl at C7, *O*-*tert*-butyldimethylsilyl at C5 and C4″). In addition, the fluorination reaction at position 13 to prepare **2a**[9] was particularly challenging, as this carbonyl is both sterically congested and electronically deactivated by conjugation with the adjacent 14,15-olefin. In this instance, treatment of 13-oxo-5-OTBDMS-7-OTMS-AVM aglycone with DAST yielded a ~1:1 mixture of the desired 13-difluoride in modest yield (19%) in conjunction with comparable amounts of the corresponding fluorinated 2,7-dehydro product. This elimination product was not observed when introducing fluorine at carbons 4″, 4′, or 23, resulting in somewhat superior yields (28–56%) to generate, after deprotection, **2b**, **2c**, and **5**, respectively. Methylation of the C5-hydroxyl of **5** with MeI or Me_3OBF_4 yielded **6**, its avermectin A_{1a} homolog.



Entry	R_α	R_β
2a:	F	F
2b:	3	H
2c:	4	H





5: R_5 = OH
6: R_5 = OMe

Antiparasitic efficacy of the new difluoro derivatives thus prepared is shown below (Table 1) using two assays: the *Artemia salina* (brine shrimp) immobilization assay[10] (a model for antiparasitic activity) and the *Haemonchus contortus* larval assay[11] (a new model for anthelmintic efficacy). Difluoride **2a** was the most potent

compound evaluated in the brine shrimp assay while fluorination at 4″ and 23 led to decreased efficacy in this model (e.g., **2c** and **5**). Interestingly, fluorine incorporation had little effect on the intrinsic in vitro *H. contortus* anthelmintic potency: each of the five new AVMs was equipotent to IVM (**1b**).

Table 1. Antiparasitic Efficacy of Avermectin Derivatives[12]

Compound	Brine Shrimp (IC_{100}, ng/mL)	*H. contortus* (IC_{100}, ng/mL)
1a	430	----
1b	430	10
2a	325	10
2b	----	10
2c	5,200	10
5	13,009	10
6	----	10

Recent studies[5] also have demonstrated that certain AVM derivatives, particularly those substituted at position 23,[5a] exhibit pronounced efficacy in mouse models of anticonvulsant and anxiolytic activity yet lack undesirable sedative effects or alcohol interactions. Consequently, 23-*gem*-difluoro-IVM (**5**) was evaluated in the pentamethylenetetrazole (PTZ) mouse epilepsy model[5] (Table 2). In this assay, **5** showed a threefold improvement in anticonvulsant efficacy relative to the nonfluorinated homolog IVM (**1b**) in its ability to protect against PTZ-induced seizures. This result is particularly striking given that this same substitution led to a marked decrease in anthelmintic potency. While less potent than diazepam (valium), **5** clearly lacks diazapam's undesirable sedative effects, as determined by the mouse's ability to balance on a 16 rpm rotarod, showing no negative effects at the highest level studied.

Table 2. Comparison of Anticonvulsant and Sedative Effects

Compound	Mouse PTZ Model (ED_{50}, mg/kg)	Mouse Rotarod (MED, mg/kg)
diazepam	0.26	3
1b	28.2	>300
5	9.0	>40

In summary, the first preparation of a series of *gem*-difluoro-avermectin derivatives is reported. These new AVMs exhibit anthelmintic activity comparable to ivermectin and **5** displays useful anticonvulsant activity.

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9. **13-Desoxy-13-*gem*-difluoro-avermectin B_{1a} aglycone (2a)**. To 13-oxo-5-OTBDMS-7-OTMS-AVM aglycone (360 mg, 0.468 mmol) in toluene (2 mL) at 25 °C was added DAST (2 mL) and the solution was heated to 50 °C for 4 h. The solution was then cooled to rt, poured into Et_2O (30 mL), quenched by careful dropwise addition of saturated $NaHCO_3$ (aq) and extracted with Et_2O. The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Pure 13-desoxy-13-*gem*-difluoro-5-OTBDMS-7-OTMS-AVM aglycone (68 mg, 19%, TLC: 85/15 hexanes/acetone, R_f = 0.45) was obtained following flash chromatography on silica gel using 85/15 hexanes:acetone as eluant. To 13-*gem*-difluoro-13-desoxy-5-OTBDMS-7-OTMS-AVM aglycone (68 mg, 86 μmol) in THF (3 mL) at 25 °C was added HF•pyridine solution[13] (1 mL, prepared using 25 g HF•pyridine, 10 mL pyridine, 25 mL THF). The solution was stirred for 72 h, then cooled to 0 °C. Pyridine (3 mL) was added and the solution then poured into 1/1 H_2O/Et_2O (50 mL). The layers were separated and neutralized separately with saturated $NaHCO_3$(aq). The combined aqueous layers were extracted with Et_2O and then the combined organic layers were washed with saturated NaCl (aq) and dried ($MgSO_4$). The solution was filtered and concentrated under reduced pressure. Pure **2a** (31 mg, 51%) was obtained following preparative RP-HPLC on a Waters C18-ODS 4.6 × 150-mm column using 4/1 MeOH/H_2O as eluant. TLC: 85/15 hexanes/acetone, R_f = 0.23; RP-HPLC: Zorbax RX-8 4.6 × 250-mm column, 85/15 MeOH/H_2O, 2 mL/min, t_R = 4.07 min; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.81–5.88 (m, 3H), 5.61 (br d, J = 11.7 Hz, 1H), 5.74 (dd, J = 1.5, 9.9 Hz, 1H), 5.44 (t, J = 11.1 Hz, 1H), 5.38 (s, 1H), 5.33 (m, 1H), 4.66 (AB, J_{AB} = 14.4 Hz, 2H), 4.27 (br d, J = 5.8 Hz, 1H), 3.90 (m, 1H), 3.94 (d, J = 6.2 Hz, 1H), 3.85 (br t, J = 10.9 Hz, 1H), 3.42 (d, J = 9.9 Hz, 1H), 3.24 (br s, 1H), 2.66–2.80 (m, 1H), 2.17–2.40 (m, 4H), 1.99 (d, J = 4.8 Hz, 1H), 1.84 (s, 3H), 1.62 (s, 3H), 1.58 (m, 2H), 1.45 (m, 3H), 1.16 (d, J = 6.68 Hz, 3H), 0.94 (t, J = 7.3 Hz, 3H), 0.89 (d, J = 6.5, 6H), 0.87 (m, 1H); ^{19}F NMR (376 MHz, $CDCl_3$): δ −101.4 (d, J = 233.1 Hz), −122.5 (dd, J = 26.1, 233.9 Hz); MS: 604.7 calcd for $C_{34}H_{46}F_2O_7$, found 611.7 (M + Li).
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11. Fresh *H. contortus* eggs, each containing a single embryo, were collected from sheep feces and placed in a 48-well microtiter plate (50 eggs/well). Tap water (0.5 mL) was added to each well followed by AVMs dissolved in DMSO (2.5 μL). The eggs hatched within 24 hours and newly hatched L1 larvae molt to L2 larvae and then to L3 larvae during the 5 day test period. At day 5, readout occurs by visually comparing larvae from treated wells to those in untreated and vehicle-treated wells. Thus, this is a developmental test in which the embryo-containing egg and three different larval stages are exposed to drug.
12. All new compounds were characterized by ^{1}H NMR, ^{19}F NMR, and mass spectral data.
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