Synthesis and Biological Activity of 4a,4″-Disubstituted Avermectins

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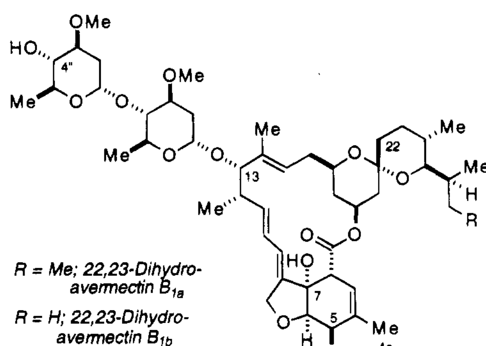
Allylic oxidation of avermectin B_1 and $C_{4''}$-substituted avermectin B_1 analogues at C_{4a} followed by derivatization of the C_{4a} oxygen provides compounds that have an improved safety profile, as judged by mouse LD_{50} data, and are substantially more potent than avermectin B_1 against flies.

Keywords: *Avermectin; anthelmintic; pesticide*

INTRODUCTION

The avermectins are naturally occurring macrocyclic lactones with important anthelmintic and pesticidal activity (Fisher and Mrozik, 1984; Davies and Green, 1986; Pulliam et al., 1989; Lankas and Gordon, 1989; Davies and Green, 1991a,b). Avermectin B_1 (abamectin) (Dybas, 1989) and 22,23-dihydroavermectin B_1 (ivermectin) (Chabala et al., 1980) are widely used anthelmintic agents in animal (Benz et al., 1989) and human health (Greene et al., 1989). The structural novelty of avermectins and their economic importance have attracted the attention of a number of synthetic organic chemistry groups concerned with chemical modification and total synthesis (Blizzard et al., 1990). In this vein, several publications and patents have appeared describing modification of avermectin B_1 at C_{4a} (Mrozik, 1984a,b) and $C_{4''}$ (Mrozik et al., 1989; Mrozik, 1984a,b; Linn and Mrozik, 1991) in an attempt to improve these important antiparasitic drugs.



Avermectin B_1 is isolated as a two-component mixture. The major component (B_{1a}, ≥80%) contains a *sec*-butyl group at C_{25}; the minor component (B_{1b}, ≤20%) contains an isopropyl group at C_{25}. These components are separable by HPLC; however, since they have essentially the same biological activities, they are not separated in practice. All compounds in this paper are mixtures of B_{1a} and B_{1b} components, represented as B_1 and drawn as B_{1a} for clarity.

We report in this paper our observations on the synthesis and biological activity of C_{4a}-substituted avermectin analogues, with emphasis on their activity against flies. We have found that oxidative substitution at C_{4a} increases potency against flies and increases the LD_{50} in mice of these avermectin analogues.

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EXPERIMENTAL PROCEDURES

General. General procedures and instrumentation employed in this work have been reported previously (Jones et al., 1992) except for the following additions. NMR assignments were made with the aid of APT data and literature data (Diez-Martin et al., 1990; Neszmelyi et al., 1989). Standard abbreviations are used for multiplicities with the addition of om for obscured multiplet. Low-resolution FAB mass spectra were acquired on Finnigan MAT TSQ 70B and VG Masslab VG20-250 quadrupole mass spectrometers using as matrix 5:1 dithiothreitol/dithioerythritol doped with lithium acetate or undoped. High-resolution LSIMS data were obtained on a JEOL JMS-HX110 double-focusing (EB) mass spectrometer at 10 kV using Ultramark 1600F as the internal standard and 3-nitrobenzyl alcohol as matrix.

***Stomoxys* Assay.** The antifly activity was estimated *in vitro* by evaluating the response of adult *Stomoxys calcitrans* allowed to feed on 2.3 cm diameter Whatman Grade 3 filter paper disks containing bovine blood and test avermectins. Each compound was presented at five or more dosage levels in 0.1 mL of the experimental vehicle (aqueous vehicle containing 0.02% Triton X-100, 5% acetone, and up to 0.2% dimethylformamide) applied to the filter paper disk on top of 7-dram plastic vials. Two hours after application of test compound, 0.4 mL of citrated bovine blood was added to the filter paper disk. Each treatment level consisted of 10 replicate vials, each vial containing four adult flies. The experimental design is such that all groups are completely randomized within the whole, and the test vials are read in numerical order without knowledge of treatment history. Ivermectin is run as a positive control on every assay. The estimated LD_{90} is calculated from the motility of flies 24 h after exposure to test substances. The LD_{90} calculation is based on double arcsine transformed data fitted to straight lines with log-dose response curves. Data are reported in Table 1.

Sheep Assay. Anthelmintic efficacy of the compounds was determined in sheep raised helminth free and experimentally infected with the parasites listed in Table 2. When the infections were patent, the sheep were randomly assigned to a treatment or control group. Each compound was tested in one sheep while two sheep served as controls. The compounds were administered in a single oral dosage of 0.1 mg/kg. Control animals were given only the vehicle. Seven days after dosing, the animals were necropsied and the residual worm burdens were determined. The efficacies of the test compounds were recorded as described in footnote *a* of Table 2.

Estimated Mouse LD_{50} Assay. Acute toxicity of the compounds was evaluated by calculating the LD_{50} for male

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Table 1. Avermectin Analogue Activities against Brine Shrimp (*A. salina*) and *S. calcitrans* Adults

compd[a]	X	R	brine shrimp IC_{100}[b] (ng/mL)	*Stomoxys* LD_{90} (ppm)
1a	OH		356[c]	40.9
1aD	OH		430[c]	29.5
1b	*epi*-AcNH		540[c]	5.3
2a	OH		760[c]	33.0
2b	*epi*-AcNH		870	18.8
4c	*epi*-AcNH	Bz	870	2.1
4d	*epi*-AcNH	THP	1500[c]	2.7
4e	*epi*-AcNH	Me	650	1.3
4f	*epi*-AcNH	CH_2OBn	870	1.7
4g	*epi*-AcNH	MEM	650	1.4
4gD	*epi*-AcNH	MEM	870	0.9
4h	OH	MEM	325	3.6
4hD	OH	MEM	430	2.1
1i	OMEM		1300	75.6
2i	OMEM		1730	8.7
4j	OMEM	MEM	1730	7.1
1k	*epi*-AcNMe		650	13.1
2k	*epi*-AcNMe		650	9.8
4l	*epi*-AcNMe	MEM	870	1.8
1m	OMe		2600	31.7
4mD	OMe	Me	870	22.4

[a] All derivatives are of avermectin unless noted as 22,23-dihydro by the addition of a **D** to the compound number. [b] Brine shrimp data as previously described (Blizzard et al., 1989), average of two assays unless otherwise noted. [c] Multiple assays.

Table 2. Anthelmintic Efficacy of Avermectin Analogues in Sheep[a]

compd	*H.c.*[b]	*Os.c.*	*T.a.*	*T.c.*	*C.c.*	*C.onc.*	*Oe.c.*
1a	3	3	3	3	3	3	3
1aD	3	3	3	3	3	3	3
1b	3	3	3	3	3	3	3
2a	3	2	3	3	0	3	3
2b	0	0	0	0	0	0	0
4c	1	0	0	0	0	0	0
4g	3	3	3	3	3	3	3
1i	3	0	1	0	0	0	3
2i	3	2	3	2	3	1	3
4j	3	0	3	3	3		3

[a] Sheep data obtained as described under Experimental Procedures, all compounds tested at 0.1 mg/kg, efficacy as percent reduction from control: 0 = <50%, 1 = 51−75%, 2 = 76−95%, 3 = >95%. [b] *H.c.*, *Haemonchus contortus*; *Os.c.*, *Ostertagia circumcincta*; *T.a.*, *Trichostronglyus axei*; *T.c.*, *Trichostrongylus colubriformis*; *C.c.*, *Cooperia curticei*; *C.onc.*, *Cooperia oncophora*; *Oe.c.*, *Oesophagostomum columbianum*.

CD-1 mice. Each compound was evaluated at several doses in treatment groups of five mice per dose allocated at random from a pool of mice. The compounds were administered orally in a vehicle via a calibrated syringe with a blunt-tipped needle. Seven days after dosing, the number of deaths was determined and the estimated LD_{50} values were determined by the method of dose-pair responses. This method effectively emulates linear regression while minimizing the number of animals required.

General Procedure A: SeO_2 Oxidation. A 250-mL round-bottom flask fitted with a magnetic stirring bar, septum, and nitrogen inlet was charged with the desired avermectin analogue in CH_2Cl_2 (0.15 M). To the resulting clear solution was added 0.50 equiv of SeO_2 followed by 4 equiv of 90% *t*-BuOOH at room temperature. The resulting solution was stirred at room temperature for ~24 h. The reaction mixture was then concentrated by rotary evaporation and chromatographed with a CH_2Cl_2/MeOH solvent mixture on SiO_2 to provide the 4a-hydroxyavermectin analogue as a foam.

Data for 4a-Hydroxyavermectin B_1 (2a) (Mrozik, 1984a,b): 2.04 g (50%); R_f = 0.17 (94:6 CH_2Cl_2/MeOH). MS and combustion data are in the supplementary material.

Data for 4″-*epi*-(Acetylamino)-4″-deoxy-4a-hydroxyavermectin B_1 (2b): 6.10 g (50%); R_f = 0.27 (93:7 CH_2Cl_2/MeOH); ^1H NMR δ 5.86 (m, H_9), 5.78−5.63 (om, H_3, H_{10}, H_{11}, H_{23}, NH), 5.55 (dd, J = 9.9, 2.5 Hz, H_{22}), 5.38−5.25 (om, H_{19}, $H_{1''}$), 4.95 (m, H_{15}), 4.74 (d, J = 3.2 Hz, $H_{1'}$), 4.65 (m, H_{8a}), 4.55 (br d, J = 5.0 Hz, H_5), 4.38, (dd, J = 10.0, 3.2 Hz, $H_{4''}$), 4.22 (br s, H_{4a}), 4.03 (m, $H_{5''}$), 3.97 (d, J = 6.3 Hz, H_6), 3.90 (br s, H_{13}), 3.90−3.72 (om, H_{17}, $H_{5'}$), 3.71−3.50 (om, $H_{3'}$, $H_{3''}$), 3.45 (d, J = 10 Hz, H_{25}), 3.42 (s, OCH_3), 3.35 (s, OCH_3), 3.32 (m, H_2), 3.18 (t, J = 9.0 Hz, $H_{4'}$), 2.70 (br s, 2 × OH), 2.50 (m, H_{12}), 2.32−2.15 (om, 2 × H_{16}, H_{24}, $H_{2'eq}$), 2.07 (s, CH_3CO), 2.05−1.95 (om, H_{20eq}, $H_{2''eq}$), 1.75 (m, H_{18eq}), 1.65−1.40 (om, H_{20}, H_{26}, 2 × H_{27}, $H_{2'}$, $H_{2''}$), 1.48 (s, 3 × H_{14a}), 1.21 (d, J = 6.2 Hz, 3 × $H_{6'}$), 1.15 (d, J = 6.9 Hz, 3 × H_{12a}), 1.10 (d, J = 6.6 Hz, 3 × $H_{6''}$), 0.95−0.85 (om, 3 × H_{24a}, 3 × H_{26a}, 3 × H_{28}, H_{18ax}); ^{13}C NMR δ 173.2 (C_1), 170.9 [(C=O)NH], 140.2, 139.3 (C_{14}, C_8), 138.1 (C_{11}), 136.3 (C_{23}), 135.1 (C_4), 127.7 (C_{22}), 124.7 (C_{10}), 120.7, 119.9, 118.3 (C_3, C_9, C_{15}), 98.6 ($C_{1''}$), 95.8 (C_{21}), 94.9 ($C_{1'}$), 81.9 (C_{13}), 81.0 ($C_{4'}$), 80.6 (C_7), 79.3 ($C_{3'}$), 79.2 (C_6), 74.9 (C_{25}), 73.3 ($C_{3''}$), 68.6, 68.3 (C_{17}, C_{19}), 68.4 (C_{8a}), 67.1, 65.5 (C_5, $C_{5'}$, $C_{5''}$), 64.4 (C_{4a}), 56.6, 56.1 (2 × OCH_3), 48.5 ($C_{4''}$), 45.6 (C_2), 40.5 (C_{20}), 39.8 (C_{12}), 36.5 (C_{18}), 35.2 (C_{26}), 34.5 ($C_{2'}$), 34.2 (C_{16}), 31.8 ($C_{2''}$), 30.6 (C_{24}), 27.5 (C_{27}), 23.4 (CH_3C=O), 20.2 (C_{12a}), 18.3 ($C_{6'}$), 17.0 ($C_{6''}$), 16.4 (C_{24a}), 15.1 (C_{14a}), 13.0 (C_{26a}), 12.0 (C_{28}); MS (FAB) 952 (M + Na, 4), 330 (26), 305 (22), 300 (20), 221 (20), 186 (100), 154 (82), 112 (34). Anal. Calcd for $C_{50}H_{75}NO_{15}$: C, 64.57; H, 7.95; N, 1.51. Found: C, 64.53; H, 7.95; N, 1.52.

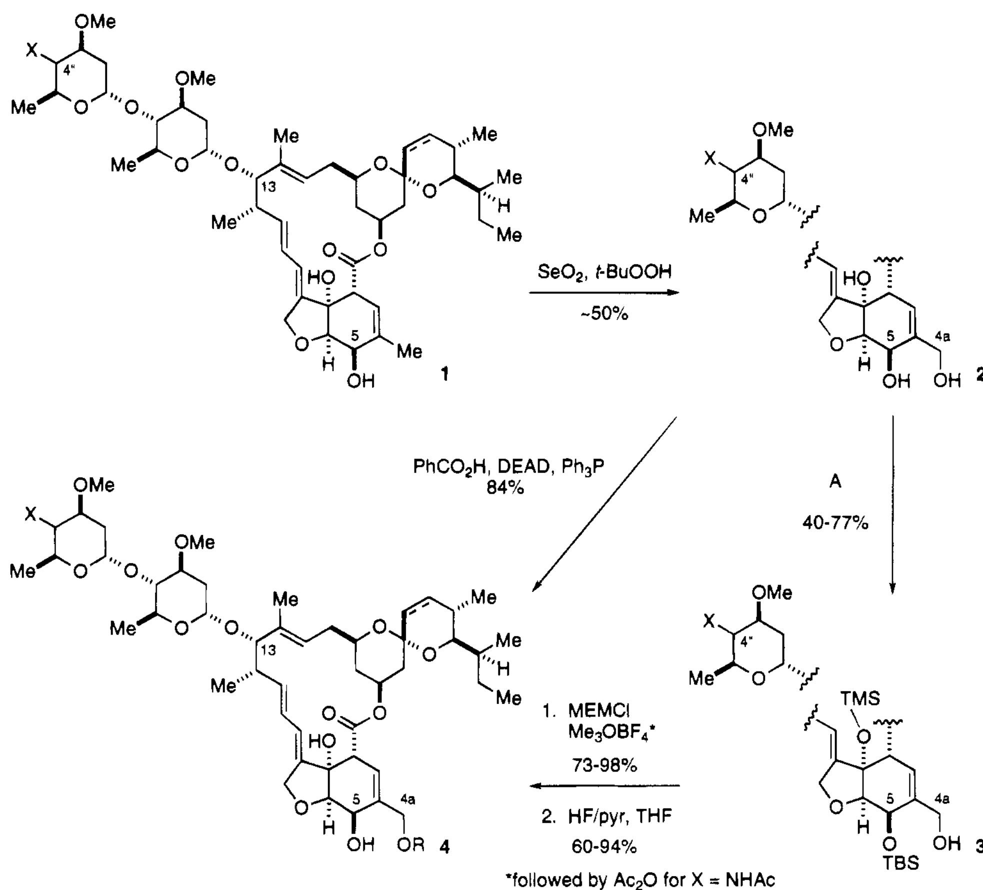
Data for 4″-*epi*-*N*-Acetyl-4″-deoxy-*N*-(methylamino)-4a-hydroxyavermectin B_1 (2k): 1.35 g (46%); R_f = 0.19 (94:6 CH_2Cl_2/MeOH); ^1H NMR (selected data) δ 5.86 (m, H_9), 5.78−5.63 (om, H_3, H_{10}, H_{11}, H_{23}), 5.55 (dd, J = 9.9, 2.5 Hz, H_{22}), 5.45 (d, J = 3.0 Hz, $H_{1''}$), 5.40−5.30 (m, H_{19}), 4.95 (m, H_{15}), 4.74 (d, J = 3.2 Hz, $H_{1'}$), 4.65 (m, H_{8a}), 4.55 (t, J = 5.0 Hz, H_5), 4.22 (br s, H_{4a}), 4.15 (m, $H_{5''}$), 3.95 (d, J = 6.3 Hz, H_6), 3.90 (br s, H_{13}), 3.42 (s, OCH_3), 3.35 (s, OCH_3), 3.32 (m, H_2), 3.18 (s, NCH_3), 2.85 (d, J = 5 Hz, OH); MS (FAB) 966 (M + Na, 80), 344 (90), 312 (100), 221 (95). Anal. Calcd for $C_{51}H_{77}NO_{15}$: C, 64.88; H, 8.22; N, 1.48. Found: C, 64.62; H, 8.33; N, 1.41.

RESULTS AND DISCUSSION

Chemistry. We initially focused on one $C_{4''}$ substituent (*epi*-acetylamino) (Mrozik et al., 1989; Mrozik, 1984a,b; Linn and Mrozik, 1991) and varied the groups at C_{4a}. Once the structure activity relationship was established, we examined the most desirable C_{4a} substituents with other $C_{4''}$ groups. In general, these compounds were prepared by first oxidizing at C_{4a} with SeO_2 as previously reported (Fisher and Mrozik, 1984; Mrozik, 1984a,b; Fraser-Reid et al., 1988), to provide alcohols **2a**, **2b**, **2i**, and **2k**. A series of protection and deprotection reactions selectively revealed $C_{4a}OH$ in **3** for derivatization. Finally, complete deprotection provided the desired compounds (**4**) for testing (Scheme 1).

The oxidation reproducibly provided 4a-hydroxyavermectins in a yield of ~50%; however, only very low yields were obtained with C_5OH protected with either silyl or ester groups. Esters at $C_{4a}OH$ were directly introduced through a Mitsunobu reaction (Mrozik, 1984a,b; Mitsunobu, 1981; Hughes et al., 1988) without the aforementioned protection/deprotection sequence to provide compounds like **4c**. Ethers, however, were prepared by silylating with *t*-$BuSiMe_2Cl$ (TBSCl) at C_5-OH and $C_{4a}OH$, desilylating $C_{4a}OTBS$, and trimethylsilylating C_7OH and $C_{4a}OH$ with bis(trimethylsilyl)trifluoroacetamide (BSTFA) followed by hydrolysis of the primary silyl ether with aqueous HOAc to provide **3** from **2**. The final silylation with BSTFA was necessary since the tertiary alcohol at C_7 was competitively alkylated by Me_3OBF_4, (2-methoxyethoxy)methyl chloride (MEMCl), and benzyloxymethyl chloride (BOMCl). Alkylation at $C_{4a}OH$ followed by deprotection with pyridine/HF (Trost et al., 1983) provided the desired compounds (**4**) for testing. The methyl ethers were prepared by alkylation with Me_3OBF_4. This reagent competitively alkylates the $C_{4''}$ acetylamino group, so

Scheme 1



A: a) TBSCl, imidazole, DMF, rt; b) HF/pyr, THF, rt; c) BSTFA, DMF, rt; d) HOAc, H_2O

the reaction was run to alkylate both the amide and alcohol functional groups. Upon complete alkylation, the reaction mixture was quenched with water to provide a $C_{4''}$ amino group that was reacylated with Ac_2O.

Silylation at $C_{4''}OH$ (and C_7OH) was sufficiently slower than silylation at C_5OH and $C_{4a}OH$ (see structure **2**), so that good yields of either silylated (allowing the reaction to run for extended periods of time) or free alcohol (stopping the reaction when C_5OH was silylated) at $C_{4''}$ could be obtained. Identical groups could therefore be introduced at $C_{4a}OH$ and at $C_{4''}OH$ by selectively silylating $C_{4a}OH$ and C_5OH and then selectively hydrolyzing the primary silyl group to provide a C_5 monosilyl ether—C_{4a}, $C_{4''}$, C_7 triol. Pertrimethylsilylation followed by aqueous hydrolysis gave a C_{4a}, $C_{4''}$ diol ready for alkylation. Deprotection once again provided the desired compounds (**4**) for testing. The analogues with a hydroxyl at $C_{4''}$ were prepared by silylating $C_{4a}OH$, $C_{4''}$-OH, and C_5OH and then selectively hydrolyzing the primary silyl ether at C_{4a}. As described above, the alcohols at C_7 and C_{4a} were then trimethylsilylated and the primary silyl ether was hydrolyzed with aqueous HOAc. Alkylation followed by hydrolysis with pyridine/HF yielded the desired compounds (**4**).

A tetrahydropyran (THP) substituent was attached on $C_{4a}OH$ through a slightly different sequence as shown in Scheme 2. Silylation of $C_{4a}OH$ followed by esterification of C_5OH provided **5**. Desilylation at C_{4a} provided the primary alcohol, **6**, for derivatization with 3,4-dihydro-2*H*-pyran (DHP). The ester at C_5 was then removed by methanolysis to provide **4d** for testing.

Biological Evaluation. The new avermectin analogues were evaluated for activity in two *in vitro* assays against brine shrimp (Blizzard et al., 1989) (*Artemia salina*) and flies (*Stomoxys calcitrans* adults, Table 1). As shown in Table 1, derivatization at C_{4a} usually increased activity against flies relative to the parent $C_{4''}$ substituted avermectins. Nonbranched ethers (**4e**–**g**) were more active than free hydroxyls (**2a**, **2b**, **2i**, **2k**), an ester (**4c**), or branched ether (**4d**). Saturating the C_{22}–C_{23} double bond of the avermectin analogues produced compounds with increased activity against flies (compare **1aD**, **4gD**, and **4hD** to **1a**, **4g**, and **4h**). The more easily installed MEM group appeared to be as good as or better than a simple methyl substituent for increasing potency against flies (compare **4g**, **4mD** to **4e**, **4j**). Both of the acylamino groups at $C_{4''}$ and the groups at C_{4a} served to increase potency against flies relative to **1a**. Combined on the same molecule, they provided even more potent compounds (**4g** and **4l**), with the C_{4a} substituent providing a larger impact on efficacy. No strong correlation existed between the brine shrimp and fly data.

Selected compounds were further evaluated for *in vivo* activity against several parasites in sheep (Table 2) (Chabala et al., 1980). As shown in Table 2, a free hydroxyl or ester group at C_{4a} generally reduced antiparasitic activity in sheep (compare **2a**, **2b**, and **4c** to **1a**, **1b**, and **1b**). However, substitution with the chemically less reactive (and less polar) MEM group restored the activity to levels at or above the parent avermectin's levels (compare **4g** and **4j** to **1b** and **1i**). Finally, analysis of these avermectin analogues in a mouse LD_{50} assay showed that C_{4a} substitution generally provided compounds that were potentially safer in mammals than

Scheme 2

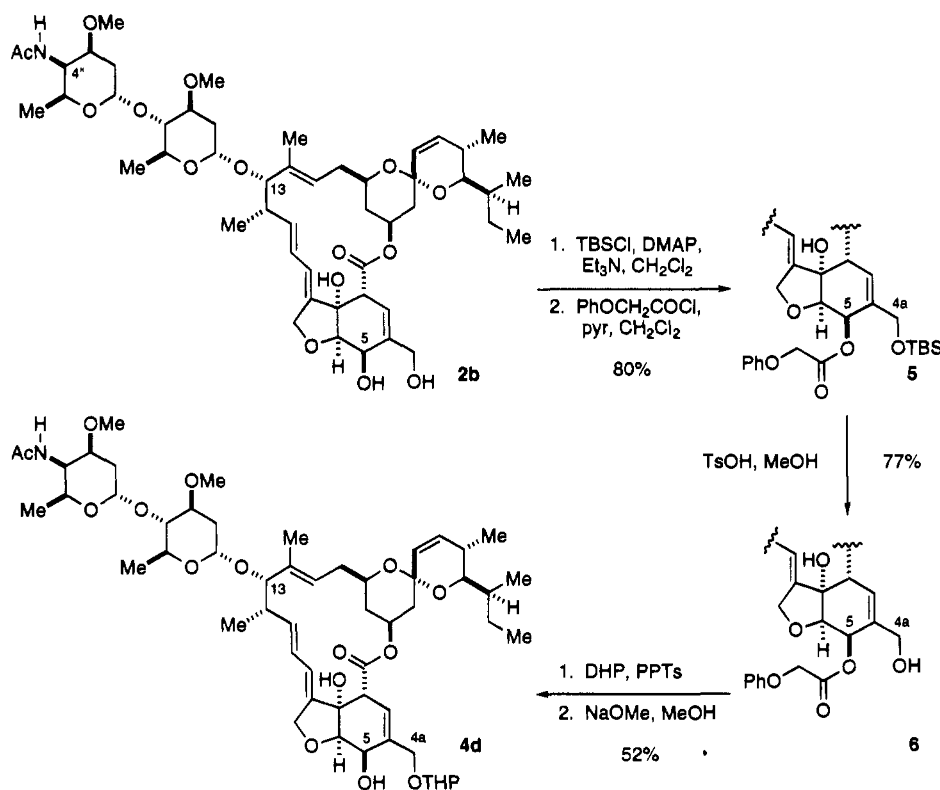


Table 3. Acute Toxicity of Avermectin Analogues in Mice[a]

compd	LD_{50} (mg/kg)	compd	LD_{50} (mg/kg)
1a	19	**4c**	≫560
1aD	27	**4e**	175
1b	24	**4g**	>560
2a	205	**4gD**	≫320
		4h	≫100
		4hD	310

[a] Estimated mouse LD_{50} as described under Experimental Procedures.

the parent derivatives, with the MEM ether (**4g**) superior to a methyl ether (**4e**) (Table 3).

Conclusion. Oxidation of avermectin analogues at C_{4a} followed by derivatization of the added oxygen provides safer compounds, as judged by mouse LD_{50} data, with substantial improvement in activity against flies. The antiparasitic activity of avermectin can be fully retained (see **4g**) by forming a MEM ether with the added C_{4a} oxygen while increasing activity against flies. The improved safety profile and efficacy against flies may potentially expand the use of avermectins.

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Supplementary Material Available: A procedure for **4c**, general procedures B–F, selected mass spectral, combustion analytical, and transcribed and assigned NMR data for **2a**, **4c**, **4e**, **4f**, **4g**, **4gD**, **4h**, **4hD**, **2i**, **4j**, **4l**, and **4mD**; procedures and data for 4a-O-TBS-**2b**, **5**, **6**, and **4d** (14 pages). Ordering information is given on any current masthead page.

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