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SYNTHESIS OF AVERMECTIN B_1-4",4"a-OXIDE: A PRECURSOR OF POTENT ANTHELMINTIC AGENTS

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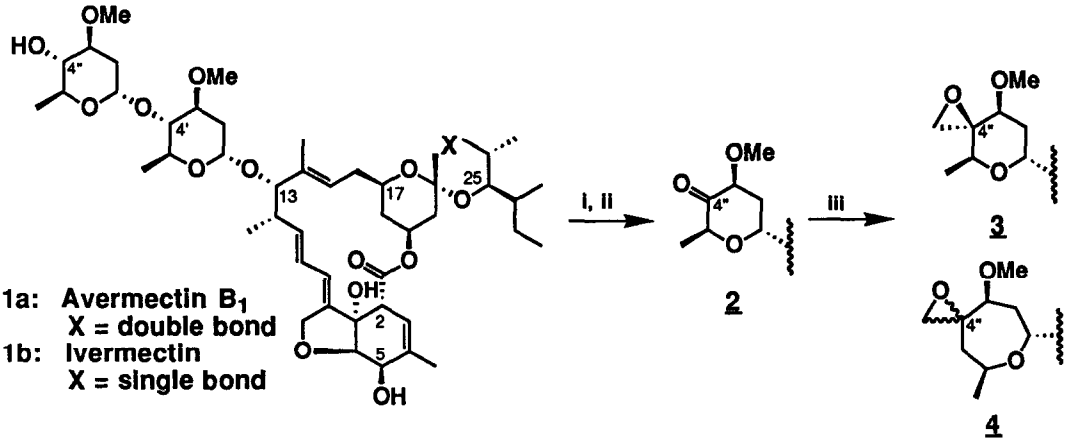
Abstract: *Treatment of 4"-oxo-5-OTBDMS-Avermectin B_1 (2) with trimethylsilyldiazomethane or diazomethane resulted in the stereoselective formation of the 4",4"a-oxide (3) in addition to the ring-expanded oxepinyl epoxide (4). The resultant epoxides were opened regiospecifically with sulfur, amine and halogen nucleophiles. The new 4"-substituted avermectins thus formed exhibited potent, broad-spectrum anthelmintic activity.*

The avermectins (e.g. **1a** and **1b**) are a family of macrocyclic lactones that exhibit potent parasiticidal activity[1] and whose complex molecular structure has elicited considerable interest among synthetic chemists.[2] We envisaged that structural modifications of the terminal oleandrosyl unit would result in unnatural avermectins displaying enhanced or shifted biological activity. Synthetic modifications of the terminal sugar are possible with retention of potent antiparasitic activity and have led to interesting shifts of the insecticidal spectrum in the case of the 4"-amino analogs.[3,4] Our interest in these complex natural products has led to the synthesis of the 4",4"a-oxide as a convenient intermediate for the preparation under mild conditions of diverse analogs bearing heteroatoms at the 4"a-position. These new avermectin derivatives were evaluated for use as broad-spectrum drugs for the treatment of a variety of animal metazoan parasitic infections.

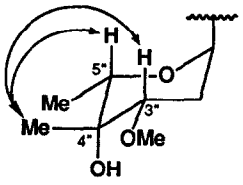


I. TBDMSCl, imidazole; II. $PhOP(O)Cl_2$, DMSO, Et_3N, -20 °C - RT; III. $TMSCHN_2$

Avermectin B_1, the primary fermentation product of *Streptomyces avermitilis*, was protected selectively at the 5-hydroxyl position[3] (85%). Subsequent Pfitzner-Moffatt oxidation of the 4"-hydroxyl using $PhOP(O)Cl_2$/DMSO[5] produced the requisite ketone **2**[4] (85-95% on multi-gram scale). Treatment of **2** with 1.3

equivalents of trimethylsilyldiazomethane[6] resulted in the formation of epoxide **3** (31%) and oxepinyl epoxide[7] **4** (37%). Oxirane **3** was produced as a 9:1 mixture of isomers with the major diastereomer as shown. No selectivity was observed in the formation of oxide **4** which was generated as a 1:1 mixture of diastereomers. Alternatively, the epoxidation could be run with a substantial excess of diazomethane although the yields were poorer. Interestingly, the diazomethane-mediated epoxidation, unlike the $TMSCHN_2$ reaction, exhibited a strong solvent dependence. Generation of **3** was favored in Et_2O, whereas in MeOH, the ring-expanded analog **4** predominated.

The stereochemistry of epoxide **3** was determined by addition of MeMgBr to ketone **2**, producing diastereomeric alcohols **5** and **6**. These alcohols were desilylated (HF·pyridine),[8] separated by reversed-phase HPLC and examined using 2D NOE techniques. As anticipated, alcohol **5** exhibited crosspeaks between the 4"-methyl and the 3"- and 5"-methines. No corresponding crosspeaks were observed for the other diastereomer. Treatment of **3** with DIBAH generated a single alcohol which was correlated to **5** after deprotection.



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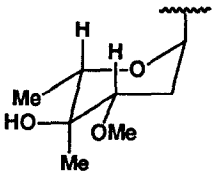

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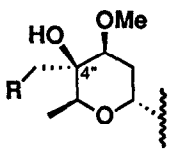
Oxiranes **3** and **4** were opened regioselectively with diverse sulfur, amine or halogen nucleophiles yielding **7** and **8**, respectively. Representative examples are shown in **Table I**. For instance, nitriles **7a** and **8a** were formed by the reaction of the requisite epoxide with 10 eq. Et_2AlCN[9a] in toluene. In a similar manner, amines **7b-e** and **8b** were produced using 5 eq. of 1:1 Et_2AlCl:H_2NR'[9b] in CH_2Cl_2.



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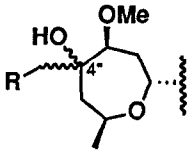

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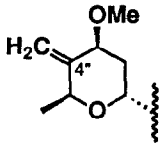

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Sulfur nucleophiles required considerably less stringent conditions to effect oxirane opening. Typically, reaction of a suitable thiol in Et_2O using neutral alumina[9c] as activating agent (**7g-j**, **8c**) or simply AcSK (**7f**, **8d**) in MeOH yielded the desired sulfides.

Functionalization of epoxides **3** and **4** was not restricted exclusively to sulfur and nitrogen nucleophiles. Bromohydrins **7k** and **8e** were generated by the reaction of **3** with 10 eq Li_2NiBr_4[9d] in MeCN. Alternatively, deoxygenation of **3** was effected with 5 eq. SmI_2[9e] in THF containing 3 eq. t-BuOH to yield the 4"-exomethylene **9** (45%) in addition to iodohydrin **7l** (36%). Removal of the 5-OTBDMS protecting groups of the avermectin derivatives in **Table I** proceeded smoothly using HF·pyridine (THF, RT, overnight).[8,10]

The biological activities of these new 4"-substituted avermectins were evaluated using an *Artemia salina* (brine shrimp) immobilization assay,[11a] a *Caenorhabditis elegans* motility screen[11b] and an *in vivo Trichostrongylus colubriformis* assay using gerbils.[11c] The data presented in Table I demonstrate that significant modification of the 4"-position is possible while retaining high biological activity. Inspection of

these data clearly reveals that nitrile **7a** and 2-imidazoylthio analog **7i** are two of the most potent derivatives in this series of compounds. Consequently, **7a** and **7i** were evaluated for oral efficacy against six species of adult gastrointestinal helminths in experimentally infected sheep. Employing a dosage level of 0.1 mg/kg, **7a** and **7i** exhibited greater than 90% efficacy against *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *Cooperia curticei* and *Oesphagostomum columbianum*. These results are identical to that observed for ivermectin at the same dosage.[11d] Only against *Trichostrongylus axei* did analog **7i** exhibit intermediate potency (50 - 75% efficacy), whereas **7a** remained fully active.

TABLE I: 4"-SUBSTITUTED AVERMECTINS: YIELDS & BIOACTIVITIES[10]

#	R	Yield[a] (%)	*A. salina*[b] IC_{100} (ng/mL)	*C. elegans* Binding[b] IC_{50} (ng/mL)	*T. colubriformis*[b] ED_{85} (mg/kg)
1b	Ivermectin	---	430	0.20	0.063
7a	NC	74	650	0.10	0.031
7b	furfuryl amine	69	10415	0.10	0.063
7c	c-$C_6H_{11}CH_2NH$	64	55500	0.13	0.250
7d	$(EtO)_2CHCH_2NH$	59	13900	0.15	0.125
7e	n-PrNH	42	6930	0.17	0.125
7f	AcS	96	1730	0.18	0.125
7g	MeO_2CCH_2S	88	870	1.28	0.250
7h	c-$C_6H_{11}S$	66	6930	1.28	>0.250
7i	2-imidazoylthio	95	430	0.13	0.031
7j	4-pyridylthio	69	6930	0.11	0.125
7k	Br	81	------	-----	-------
7l	I	36	------	-----	-------
8a	NC	88	870	0.70	0.031
8b	n-PrNH	33	27800	1.39	0.125
8c	MeO_2CCH_2S	41	1300	0.88	0.063
8d	AcS	57	1300	1.11	0.031
8e	Br	85	------	-----	-------

(a) Yields refer to the oxirane-opening reaction.
(b) Bioactivities were determined for deprotected avermectin derivatives.

In summary, the facile epoxidation and subsequent ring-opening reactions with diverse nucleophiles represents an attractive and efficient method for the synthesis of new heteroatom-substituted avermectin derivatives under mild conditions. Avermectin derivatives thus modified at the 4"-position exhibit potent, broad-spectrum anthelmintic activity.

Experimental: 3.26 g 4"-Oxo-5-OTBDMS-avermectin B_1 (**2**) in 10 mL MeOH at RT was treated with 7 mL 10% w/w $TMSCHN_2$ in hexanes for 2 hrs. The reaction was quenched with 1 mL glacial HOAc at 0°C and poured into 10 mL sat. $NaHCO_3$. The aqueous layer was extracted with EtOAc, dried ($MgSO_4$), filtered and concentrated in vacuo. The crude material was purified by flash chromatography on silica gel with EtOAc:hexanes (1:4) as eluant to yield 1.02 g **3**[12] (31%) and 1.21 g **4** (37%).

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(12) Data for **3**: Partial **^{1}H NMR** (300 MHz, $CDCl_3$, δ): 5.68-5.79 (m, 4H, C9, C10, C11, C22), 5.53 (dd, J_1 = 2.5 Hz, J_2 = 9.8 Hz, 1H, C23), 5.42 (d, J = 2.5 Hz, 1H, C1"), 5.37 (m, 1H, C19), 5.32 (s, 1H, C3), 4.97 (br. s, 1H, C15), 4.75 (d, J = 3.2 Hz, 1H, C1'), 4.67 (AB, J_{AB} = 14.5 Hz, 2H, C8a), 4.40 (br. s, 1H, C5), 4.31 (q, J = 6.4 Hz, 1H, C5"), 4.10 (s, 1H, 7-OH), 3.75-3.95 (m, 5H, C6, C13, C17, C3", C5'), 3.63 (m, 1H, C3'), 3.43 (s, 3H, OMe), 3.40 (s, 3H, OMe), 3.40-3.50 (m, 2H, C2, C25), 3.25 (t, J = 9.1 Hz, 1H, C4'), 3.01 (d, J = 5.0 Hz, 1H, C4"a), 2.82 (d, J = 5.0 Hz, 1H, C4"a), 2.50 (m, 1H, C12), 2.24-2.40 (m, 5H), 2.00 (dd, J_1 = 3.5 Hz, J_2 = 12.2 Hz, 1H, C18), 1.77 (s, 3H, C4a), 1.48 (s, 3H, C14a), 1.25 (d, J = 6.2 Hz, 3H, C5'a), 1.15 (d, J = 7.1 Hz, 3H, C12a), 1.00 (d, J = 6.5 Hz, 3H, C5"a), 0.91 (s, 9H, Si(tBu)), 0.11 (s, 6H, $SiMe_2$). **^{13}C NMR** (75 MHz, $CDCl_3$, δ): 173.9, 140.2, 137.5, 137.4, 136.1 135.1, 127.7, 124.8, 119.2, 118.2, 117.1, 98.4, 95.7, 94.9, 82.0, 80.5, 80.1, 80.0, 79.3, 74.7, 72.4, 69.4, 68.3, 68.2, 67.8, 67.1, 66.0, 61.8, 58.9, 56.5, 45.7, 44.4, 40.4, 39.6, 37.4, 36.5, 35.1, 34.5, 34.2, 30.5, 27.4, 25.8, 20.2, 20.0, 18.3, 18.2, 16.3, 15.1, 13.2, 12.9, 12.0, -4.7, -5.0.