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New, Potent Anthelmintic and Acaricidal Agents: 4"-Deoxy-4"-Thio-Substituted Avermectin Derivatives

Peter T. Meinke*, Stephen P. O'Connor, D. A. Ostlind, W. L. Shoop, Michael H. Fisher and Helmut Mrozik
Merck Research Laboratories, Department of Basic Medicinal Chemistry,
PO Box 2000, Rahway, NJ 07065-0900

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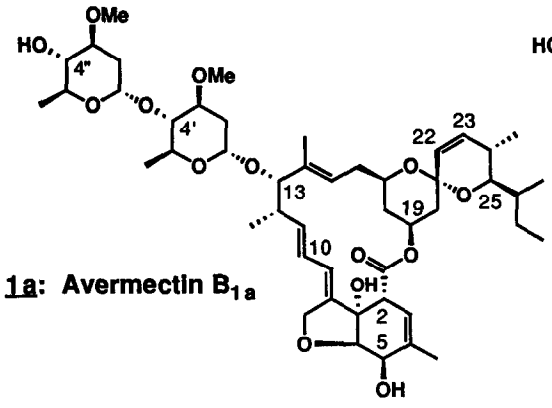
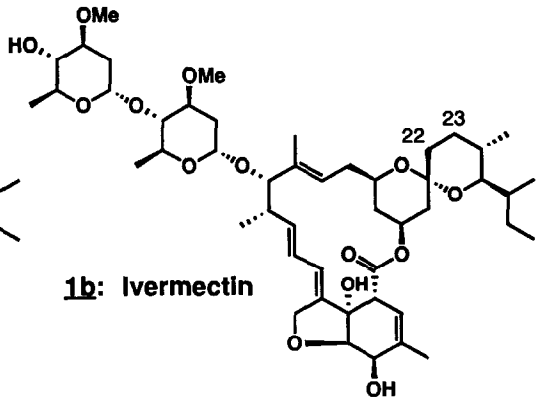
Abstract: *Sulfonylation of the 4"-α(or β)-hydroxyl of 5-OTBDMS-avermectin B_{1a} with trifluoromethanesulfonic anhydride yielded triflates which were displaced stereospecifically with diverse sulfur nucleophiles. This sulfonylation/substitution protocol also was performed on the 4'-α(or β)-hydroxyl of the corresponding avermectin monosaccharide. The sulfides, sulfoxides and sulfones thus obtained exhibited potent, broad spectrum anthelmintic and acaricidal activity.*

The discovery in 1979 of avermectins, a novel class of macrolides with unprecedented activity against endo- and ectoparasites,[1] initiated a new era in the treatment of animal parasitic disease. Although avermectin B_{1a} (**1a**, AVM) and Ivermectin (**1b**, IVM) are primarily employed as animal health agents, IVM additionally has important human clinical uses, specifically for the treatment of individuals afflicted with onchocerciasis[2] (River Blindness). Abamectin, a mixture of AVM isomers (~85:15 sec-butyl:isopropyl at C25), has been developed for horticultural and agricultural applications due to its broad spectrum of activity and residual efficacy against economically significant arthropod pests.[3] The pronounced biological activity exhibited by these unusual macrocycles and their intriguing molecular architecture triggered intense interest in the scientific community, resulting in major advances in AVM synthesis[4] and biology[5a] in addition to mode of action[5b] and receptor identification[5c,d] studies. Efforts to identify interesting, new analogs with enhanced or altered activities also have been reported,[6] demonstrating that synthetic modifications of the terminal sugar are possible with retention of potent antiparasitic activity.



1a: Avermectin B_{1a}

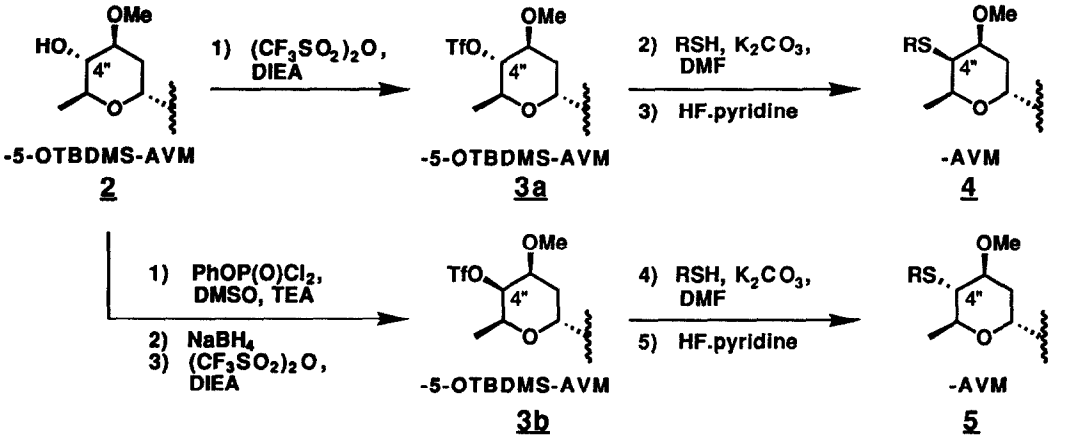


1b: Ivermectin

Our interest in these complex natural products has led to the preparation of a new class of AVM derivatives in which the 4"(or 4')-hydroxyl of the terminal oleandrosyl unit has been replaced with an alkyl thio moiety. Sulfur derivatives possessing either the natural equatorial (α) or the epimeric, axial (β) stereochemistry at the 4"- or 4'-position were synthesized in good yields under mild conditions via nucleophilic displacement of

an appropriate triflate precursor with thiolates. Similar reaction conditions have been employed previously[7] to introduce sulfur with inversion of stereochemistry, although on carbohydrates which were significantly less complex structurally. The sulfides, sulfoxides and sulfones thus obtained were evaluated for use as potent, broad-spectrum anthelmintic and acaricidal agents.

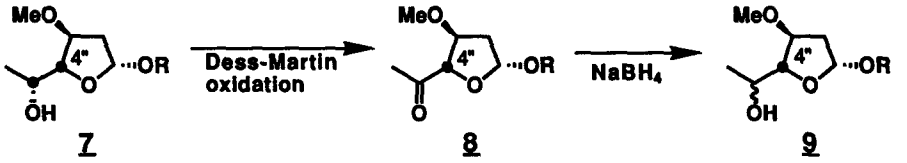
The preparation of the 4"-α-triflate **3a** proceeded as follows: 5-OTBDMS-AVM (**2**) was sulfonylated using $(CF_3SO_2)_2O$ in excellent yield (83%). Although the 4"-α-trifluoromethanesulfonate (**3a**) thus formed was highly sensitive to moisture, after lyophilization it was stable for >six months under a nitrogen atmosphere at -16°C, permitting the preparation of the multigram quantities necessary for the subsequent displacement reactions. Similarly, the 4"-β-triflate **3b** was synthesized from the corresponding 4"-epi alcohol, which was available via a simple two-step oxidation/reduction protocol from **2**. The synthesis of the corresponding 4'-α- or 4'-β-triflates in the AVM monosaccharide series proceeded with equal facility.



Nucleophilic displacement of the 4"-α-triflate **3a** with thiolates proceeded smoothly to yield 4"-epi-sulfides (**4**). Analogously, utilizing 4"-β-triflate **3b** provided the 4"-α-sulfides (**5**). These substitution reactions were stereospecific: no 4"-epimers were detected during the displacements. The β-triflate **3b** reacted appreciably faster than the corresponding α-triflate **3a**, resulting in fewer byproducts and superior chemical yields. High yielding, selective oxidation of these new sulfides to form the corresponding sulfoxides (two diastereomers produced, 1 eq $NaIO_4$, 1:1 $MeOH/H_2O$, RT) or sulfones (2 eq MCPBA, CH_2Cl_2, RT) was readily accomplished. Representative mono- (**6a-d**) and disaccharide (**4a-n, 5a-j**) analogs are shown in **Table I**.

Anhydrous DMF proved to be the best solvent for these sulfur substitution reactions. Use of other solvents or prolonged reaction times produced significant quantities of 2-epi analogs, Δ-2,3-olefin isomers and material wherein aromatization of the hexahydrobenzofuran ring occurred. When the substitution reactions of α-triflate **3a** were not run under anhydrous conditions, the ring-contracted product **7** was generated.[8] Although **7** was present in small quantities in many of the reactions examined, its formation was only prevalent when the solvent was insufficiently dry or when using recalcitrant sulfur nucleophiles. Indeed, **7** could be formed as the predominant reaction product simply by solvolyzing the appropriate triflate in moist DMF containing K_2CO_3.

The reaction generating **7** was shown to be stereospecific: Dess-Martin oxidation[9] of the exocyclic alcohol yielded ketone **8** which had a solitary methyl singlet in the proton NMR. Ketone **8**, when subjected to reduction with $NaBH_4$ in methanol, produced two alcohol diastereomers (**9**). This ring contraction also was observed in the AVM monosaccharide series.



7 8 9

These new alkylthio-substituted mono- and disaccharide AVM derivatives were initially evaluated for biological efficacy using two *in vitro* assays [*Artemia salina* (brine shrimp) immobilization[10a] and *Caenorhabditis elegans* binding[10b]] in addition to an *in vivo Trichostrongylus colubriformis* gerbil assay.[10c] The data presented in **Table I** demonstrate that significant modification of the 4"- or 4'-position is possible while retaining the high biological efficacy characteristic of this class of compounds. The IVM analogs (**4g-j**) were comparable in activity to the related C22,23-unsaturated AVM derivatives (**4b,c,f** and **5e**). On the other hand, analogs bearing somewhat larger alkyl thio groups (e.g. ethyl, 2,2,2-trifluoroethyl, propyl, isopropyl or t-butyl) were poorly active (data not shown). Interestingly, conversion of these relatively inactive lipophilic alkyl sulfides into the corresponding sulfinyl or sulfonyl analogs regenerated AVM's characteristic high biological potency with the sulfoxides exhibiting efficacy comparable to the sulfones. This observation indicates that AVMs lacking polar functionality proximal to the 4"- or 4'-position exhibit reduced biological efficacy. In addition, derivatives bearing larger functional groups generally displayed attenuated biological activity relative to that measured for those with smaller substituents. Surprisingly, comparatively little difference was noted between the anthelmintic efficacies exhibited by the natural α-substituted analogs relative to the corresponding β-isomers. The AVM monosaccharide analogs **6a-d**, while still active, exhibit efficacies that appear somewhat depressed in comparison to that observed for the parent disaccharides (**4a,d,f,l**).

The new thio-substituted avermectin derivatives shown in **Table I** also were evaluated for acaricidal efficacy using a *Tetranychus urticae* (two-spotted spider mite, TSSM) contact assay.[11] While the parent avermectin **1a** is fully active against *T. urticae* at 0.05 ppm, with the exception of **4a** and **4k**, the 4"-β-thio-substituted avermectins exhibited only modest TSSM activity. Similar biological results were observed in the 4"-α-thio series, with only **5a** and **5g** displaying significant potency. Interestingly, the monosaccharides **6a-c** exhibited excellent TSSM activity at this use level.

Thirteen of the most potent derivatives in this new series of AVM analogs were evaluated for oral efficacy against six species of adult gastrointestinal helminths in experimentally infected sheep,[10d] the results of which are shown in **Table II**. Four of these new avermectins (**4b,c,j** and **5b**), at a dosage level of 0.1 mg/kg, exhibited greater than 90% efficacy against *Haemonchus contortus*, *Ostertagia circumcinta*, *Trichostongylus axei*, *T. colubriformis*, *Cooperia curticei* and *Oesphagostomum columbianum*. These results are identical to that determined for AVM at the same dosage.[10d] Two others (**4a**, **5c**), in addition to monosaccharide **6d**, also displayed very good broad spectrum anthelmintic efficacy. Decreased efficacy was observed for the remainder.

Table I: 4"(or 4')-Deoxy-4"(or 4')-Thio-Substituted-Avermectins[12a]

Entry	Compound[a,b]	Yield[c] (%)	*A. salina*[a] IC_{100} (ng/mL)	*C. elegans* Binding[a] IC_{50} (ng/mL)	*T. colub.*[a] ED_{85} (mg/kg)	*T. urticae* % Mortality[a] (0.05 ppm)
1a	Avermectin	---	400	0.073	0.031	95
4a	4"-β-MeS-AVM	88	1730	0.060	0.063	92
4b	4"-β-MeSO-AVM	---	870	0.262	0.031	64
4c	4"-β-$MeSO_2$-AVM	---	430	0.132	0.063	45
4d	4"-β-($HOCH_2CH_2S$)-AVM	74	650	0.784	0.063	55
4e	4"-β-($HOCH_2CH_2SO$)-AVM	---	1300	>1.275	0.125	13
4f	4"-β-($HOCH_2CH_2SO_2$)-AVM	---	870	0.363	0.063	34
4g	4"-β-MeSO-IVM	---	870	0.108	0.063	77
4h	4"-β-$MeSO_2$-IVM	---	870	0.084	0.125	64
4i	4"-β-($HOCH_2CH_2SO$)-IVM	---	870	0.188	0.250	31
4j	4"-β-($HOCH_2CH_2SO_2$)-IVM	---	650	0.119	0.125	48
4k	4"-β-($MeCH(OH)CH_2S$)-AVM	34	870	0.216	0.063	89
4l	4"-β-($AcNHCH_2CH_2S$)-AVM	44	870	0.127	0.031	29
4m	4"-β-($EtSO_2$)-AVM	---	650	0.128	0.031	68
4n	4"-β-($AcOCH_2CH_2S$)-AVM	85	1730	0.525	0.125	68
5a	4"-α-MeS-AVM	75	3470	0.226	0.250	98
5b	4"-α-MeSO-AVM	---	430	-------	0.016	----
5c	4"-α-$MeSO_2$-AVM	---	430	0.098	0.125	85
5d	4"-α-($HOCH_2CH_2S$)-AVM	72	870	0.091	0.063	70
5e	4"-α-($HOCH_2CH_2SO$)-AVM	---	870	0.469	0.063	69
5f	4"-α-($HOCH_2CH_2SO_2$)-AVM	---	870	0.351	0.063	64
5g	4"-α-($MeCH(OH)CH_2S$)-AVM	40	870	0.153	0.125	88
5h	4"-α-($AcNHCH_2CH_2S$)-AVM	61	870	0.165	0.063	63
5i	4"-α-($HOCH_2CH_2CH_2S$)-AVM	85	870	0.153	0.250	53
5j	4"-α-($EtSO_2$)-AVM	---	870	0.123	0.063	64
6	Avermectin MS	---	430	0.810	0.075	93
6a	4'-β-MeS-AVM MS	59	6930	0.422	0.125	100
6b	4'-β-($HOCH_2CH_2S$)-AVM MS	74	870	-------	0.063	94
6c	4'-β-($HOCH_2CH_2SO_2$)-AVM MS	---	870	-------	>0.250	94
6d	4'-β-($AcNHCH_2CH_2S$)-AVM MS	53	430	-------	0.063	----

(a) Bioactivities were determined for deprotected (HF.pyridine[5d]) avermectin derivatives.
(b) MS = monosaccharide
(c) Yields refer to the triflate displacement reaction.

Table II: Alkylthio-Substituted AVM Sheep Anthelmintic Data[a,b]

Entry	*H. contortus*	*Os. circumcincta*	*T. axei*	*T. colubriformus*	*C. curticei*	*Oe. columbianum*
1a	3	3	3	3	3	3
4a	3	2	3	2	3	3
4b	3	3	3	3	3	3
4c	3	3	3	3	3	3
4d	3	3	3	2	0	3
4f	3	3	3	2	1	3
4j	3	3	3	3	3	3
4l	3	3	3	1	3	3
4n	3	3	2	3	0	3
5a	3	1	2	1	2	3
5b	3	3	3	3	3	3
5c	3	2	3	3	3	3
5d	3	0	1	2	0	3
6c	2	2	3	3	3	0
6d	3	3	3	3	0	3

(a) Efficacy (at 0.1 mg/kg) as % reduction from control: 0 = < 50%, 1 = 51-75%, 2 = 76-90%, 3 = 91-100%.
(b) Bioactivites were determined for deprotected (HF.pyridine[5d]) avermectin derivatives.
(c) Evaluated at 0.15 mg/kg.

In summary, the facile triflate synthesis, thiol displacement and subsequent oxidation protocol represents an attractive and efficient method for the synthesis of new, heteroatom-substituted avermectin derivatives under mild conditions. Avermectin analogs thus modified at the 4"- and 4'-positions exhibit potent, broad spectrum anthelmintic and acaricidal activity.

Preparation of Triflate 3a and Sulfide 4l: To a solution containing 7.20 g 5-OTBDMS-AVM (**2**), 1.78 g 4-dimethylaminopyridine and 2.62 mL diisopropylethylamine in 50 mL CH_2Cl_2 at 0°C was added (dropwise!) 3.41 mL $(CF_3SO_2)_2O$. After 15 min at 0°C and 30 min at RT, the deep red solution was diluted with 50 mL hexanes, poured onto a 3" silica gel plug and eluted with 3:1 hexanes:EtOAc. The solvent was removed under reduced pressure at ambient temperature and the resulting pale yellow oil lyophilized from benzene to yield 6.74 g (83%) triflate **3a**[12b] as a pale yellow powder. To 570 mg $HSCH_2CH_2NHAc$, 780 mg K_2CO_3 and 20 mg 18-crown-6 in 5 mL DMF (KF = 84 μg H_2O/mL) at RT was added 770 mg triflate **3a**. After 30 min, the solution was poured into saturated $NaHCO_3$, extracted (EtOAc) and dried ($MgSO_4$). Pure **4l**[12c] (333 mg, 44%) was obtained following flash chromatography on silica gel using 1:3 hexanes:EtOAc as eluant.

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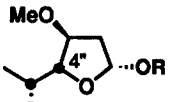
8) A similar oleandrosyl ring-contraction reaction was observed upon reacting F_3SNEt_2 with **2**, resulting in the stereospecific formation of fluoride **10** (Mrozik, H. In *Natural and Derived Pest Management Agents*, Hedin, P., Ed.; Am. Chem. Soc. Books, 1993, in press). Additional related stereoselective ring-contraction reactions of oleandrose (Nagel, A. A.; DiBrino, J.; Vincent, L. A.; Retsema, *J. Med. Chem.* **1982**, 25, 881-884 and references therein) or of pyranosyl sulfonates (Fleet, G. W. J.; Seymour, L. C. *Tetrahedron Lett.* **1987**, *28*, 3015-3018) have been reported.



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12) (a) All compounds were characterized by NMR and mass spectra. Yields were not optimized. (b) Data for **3a**: Partial ^1H NMR (300 MHz, $CDCl_3$, δ): 5.65 - 5.82 (m, 4H, H_9, H_{10}, H_{11}, H_{23}), 5.52, (dd, J_1 = 2.6 Hz, J_2 = 9.8 Hz, 1H, H_{22}), 5.39 (d, J = 3.3 Hz, $H_{1''}$), 5.33 (m, 1H, H_{19}), 5.30 (br s, 1H, H_3),4.95 (br t, 1H, H_{15}), 4.75 (d, J = 3.1 Hz, 1H, $H_{1'}$), 4.61 (AB, J_{AB} = 14.5 Hz, 2H, H_{8a}), 4.41 (br s, 1H, H_{15}), 4.36 (t, J = 9.6 Hz, 1H, $H_{4''}$), 4.11 (s, 1H, 7-OH), 3.90 (br s, 1H), 3.79 (d, J = 5.5 Hz, 1H, H_6), 3.41 (s, 3H, OMe), 3.40 (s, 3H, OMe), 3.36 (br s, 1H, H_2), 3.19 (t, J = 9.0 Hz, $H_{4'}$), 1.76 (s, 3H, H_{4a}), 1.47 (s, 3H, H_{14a}), 1.28 (d, J = 6.4 Hz, 3H), 1.21 (d, J = 6.3 Hz, 3H), 1.14 (d, J = 6.8 Hz, 3H), 0.90 (s, 9H, SitBu), 0.10 (s, 6H, $SiMe_2$). (c) Data for **4l** (HF.pyridine[5d] deprotected form): Partial ^1H NMR (300 MHz, $CDCl_3$, δ): 6.58 (br t, 1H, NH), 5.68 - 5.87 (m, 4H, H_9, H_{10}, H_{11}, H_{23}), 5.53 (dd, J_1 = 2.5 Hz, J_2 = 9.9 Hz, 1H, H_{22}), 5.40 (s, 1H, H_3), 5.38 (m, 1H, H_{19}), 5.34 (d, J = 3.5 Hz, 1H, $H_{1''}$), 4.95 (m, 1H, H_{15}), 4.73 (d, J = 2.7 Hz, 1H, $H_{1'}$), 4.66 (s, 2H, H_{8a}), 4.27 (br t, 1H, H_5), 4.04 (s, 1H, 7-OH), 3.94 (d, J = 6.2 Hz, 1H, H_6), 3.91 (s, 1H), 3.42 (s, 3H, OMe), 3.41 (s, 3H, OMe), 3.27 (br s, 1H, H_2), 3.19 (t, J = 9.1 Hz, 1H, $H_{4'}$), 2.94 (br s, 1H), 1.97 (s, 3H, Ac), 1.85 (s, 3H, H_{4a}), 1.66 (s, 3H, H_{14a}), 1.30 (d, J = 6.5 Hz, 3H), 1.20 (d, J = 6.2 Hz, 3H), 1.14 (d, J = 6.8 Hz, 3H).