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4"-DEOXY-4"-AMINOAVERMECTINS WITH POTENT BROAD SPECTRUM ANTIPARASITIC ACTIVITIES

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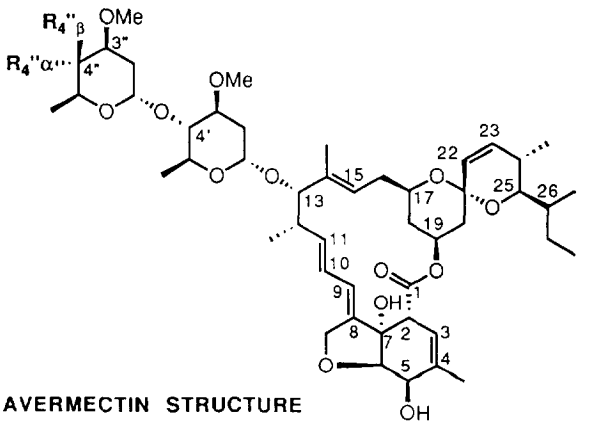
Abstract: Reductive amination of 4"-oxo-5-O-tert-butyldimethylsilyl-avermectins with sodium cyanoborohydride and ammonium acetate gave an epimeric mixture of 4"-deoxy-4"-amino analogs with the epimeric, axial 4"-ß-amino derivative as the major component. Acylation of the amino substituent gave highly active broad spectrum antiparasitic compounds, as determined in a sheep anthelmintic assay. 4"-Epi-acetylamino-4"-deoxyavermectin B_1 (**12**) was selected for further antiparasitic studies and is currently under development as a novel avermectin endectocide.

Ivermectin is a highly potent antiparasitic drug which is used extensively as both a veterinary and human parasiticide. Its commercialization in 1980 followed the discovery of the anthelmintic activities of the avermectins and milbemycins at the Merck Research Laboratories in 1975. Ivermectin is effective against a wide spectrum of parasitic nematodes. More importantly and in contrast to all other important anthelmintic agents the avermectins are also highly active against many ectoparasitic pests such as grubs, lice, mites, ticks, and bots following oral, subcutaneous, or topical application. This antiparasitic spectrum makes them especially useful for a wide range of applications.[1]



AVERMECTIN STRUCTURE

While avermectin aglycones have poor anthelmintic activities, 13-deoxyaglycones retain many of the anthelmintic properties of the avermectins.[2] Although the hydrophobic C-13-bis-oleandroside substituent is apparently not obligatory for antiparasitic activity, it contributes desirable properties to the macrocyclic lactone, and is therefore a prime target for chemical modification.[3,4] 4"-Deoxy-4"-epi-N-methylaminoavermectin B_1, for instance, is distinguished by a 1000 fold increase in potency against the lepidopteran pest *S. eridania* (southern armyworm).[5,6] We now describe additional 4"-substituted avermectin derivatives which exhibit highly desirable anthelmintic properties as novel avermectin endectocides.

The introduction of an amino substituent into the avermectins was originally prompted by the observation that most antibacterial macrolide antibiotics contain a basic amine. It was also of considerable interest to examine the effect of this substitution on aqueous solubility, distribution, stability and antiparasitic spectrum. The 4"-position of avermectin B_1 and ivermectin appeared to be best suited for the introduction of an amino group because of ready accessibility. Previous work had shown that modifications at this position are compatible with high bioactivities, while variations at most other chemically accessible positions often result in inferior compounds.[7,8]

Chemistry. Mono 4"-O-acyl analogs were obtained previously by acylation after protection of the more reactive C-5-alcohol group.[3] Likewise protection of the 5-hydroxy usually as the O-tert-butyldimethylsilyl-ether and subsequent oxidation gave the 4"-oxo analog in high yield. This oxidation was carried out conveniently with DMSO - oxalyl chloride (Swern). Milder oxidation reagents such as pyridinium dichromate do not oxidize the hindered 4"-alcohol group. Interestingly, pyridinium dichromate rapidly oxidizes unprotected avermectin B_1 or ivermectin in good yield to the 5-oxo analog, otherwise obtained via manganese(IV) oxide oxidation of the allylic C-5 alcohol.[9] Prolonged oxidation of 5-O-TBDMS avermectin B_1 with pyridinium dichromate leads to a modest yield of an allylic oxidation product identified as the 8a-oxo analog.[10]

The 5-O-tert-butyldimethylsilyl-4"-oxo avermectin derivatives gave upon reductive amination with $NaCNBH_3$ and ammonium or alkylammonium acetates a mixture of the two epimeric 4"-deoxy-4"-amino compounds mostly with a large excess of the axial epi amino analog. A solution of 5-O-TBDMS-4"-oxoavermectin B_{1a} (3.85 g, 3.9 mmole), NH_4OCOCH_3 (3.1 g, 39 mmole), $NaCNBH_3$ (245 mg, 3.9 mmole), in anhydrous methanol (60 ml) was stirred 20 min at room temperature to give after workup and silica gel chromatography 1.6 g (41 %) of 4"-deoxy-4"-β-amino-, 0.38 g (9.8 %) of the epimeric -4"-α-amino-, and 0.44 g (11 %) of 4"-β-(hydroxy)-5-O-TBDMS avermectin B_{1a}. Deprotection with 1% p-toluenesulfonic acid in methanol gave the amino derivatives **3** and **4**. Substitution of alkylammonium acetates for NH_4OCOCH_3 gave the monoalkyl analogs **6** and **8** to **11**. Alternatively reductive alkylation of the 4"-amino compounds **3** or **4** with $NaCNBH_3$ or $NaBH_4$ and an aldehyde resulted in the substituted analogs. The acylated amino derivatives were then obtained by conventional methods. Under certain conditions of reductive amination an additional isomer was observed, which was identified after acetylation and deprotection as 3"-epi-4"-acetylamino-4"-deoxy

avermectin B_{1a} **16** through ^1H NMR and mass spectra. The stereochemistry at C3" was determined by examination of coupling constants in the 300 MHz ^1H NMR of **16** (proton assignments made with COSY). For example, H3" appears as a ddd with J = 3, 3, 3 Hz (consistent only with an equatorial H3"). The stereochemistry at C4" was assigned as α (normal stereochemistry) based on the chemical shift of H4" (3.88 ppm compared to 4.4 ppm for H4" of **12**). Reaction of amine **3** with methylisocyanate gave the urea **27**, and the amidine **29** was obtained by reaction with excess N,N-dimethylformamide dimethyl acetal. Reductive amination with dimethylhydrazine gave the hydrazine derivative **28**, and reaction of the 4"-ketone with semicarbazide gave the semicarbazone **30**. After removal of the 5-O-TBDMS protecting group these amino analogs were submitted for biological testing (Table).

Biology. The new compounds were tested initially in an *in vitro* brine shrimp assay[11] and several model assays for their antiparasitic[12] and insecticidal[13] activities. Since efficacy against a wide spectrum of parasites is required for a commercially useful antiparasitic agent and since the model assays did not predict this required efficacy adequately, the anthelmintic activities of the more important compounds were then confirmed in sheep experimentally infected with a spectrum of seven gastrointestinal parasites. The effect of an oral dose of 0.05 to 0.2 mg/kg was compared directly to avermectin B_1 (**2**) and ivermectin (**1**).[14] The data (Table) show that acylation of the basic amino compounds **3** and **4** to the neutral 4"-acylamino analogs (**12** to **27**) resulted in consistantly improved broad spectrum anthelmintic activities. Little difference in bioactivities was seen with the epimeric 4"-acetyl- (**12, 13, 16**), or -formyl- (**19**), and -propionylamino (**20**) derivatives, while the monosaccharide analog (**18**) was less potent. Good activities were also observed for hydrazine **28** and semicarbazone **30**, but not for the amidine **29**. Of the alkylated amines (**6** - **11**) mostly the smaller substituents, in particular mono and dimethyl analogs **6** and **7** show the best biological activity. 4"-Epi-acetylamino-4"-deoxyavermectin B_1 (**12**) had one of the best overall activities and was selected, on the basis of its high anthelmintic and ectoparasiticidal activities , for additional detailed antiparasitic evaluation. It is currently under development as a novel avermectin endectocide.[15]

TABLE OF BIOLOGICAL ACTIVITIES OF AMINOSUBSTITUTED AVERMECTIN DERIVATIVES

	AVERMECTIN STRUCTURES			BRINE SHRIMP		SHEEP ANTHELMINTIC TEST					
	$R_{4"}\alpha$ =	$R_{4"}\beta$ =	-C_{22}-C_{23}-	IC_{100} ng/ml	DOSE (mg/kg)	*H.c.* [d]	*O.c.*	*T.a.*	*T.c.*	*C.c.*	*C.o.*	*Oe.c.*
1	OH	H	$-CH_2-CH_2-$ [a]	430	0.10	3 [e]	3	3	3	-	3	3
2	OH	H	-CH=CH- [b]	296	0.10	3	3	3	3	-	3	3
3	H	NH_2	-CH=CH-	1730	0.10	3	3	0	1	3	-	3
4	NH_2	H	-CH=CH-	2600	0.10	3	3	0	0	3	-	3
5	H	NH2	$-CH_2-CH_2-$	1730	0.10	3	3	1	2	3	-	3
6	H	$NHCH_3$	-CH=CH-	1730	0.10	3	3	3	2	3	-	3
7	H	$N(CH_3)_2$	-CH=CH-	1300	0.04	3	3	0	1	1	-	3
8	H	$NHCH(CH_3)_2$	-CH=CH-	1730	0.05	2	0	0	1	1	1	3
9	H	$NH(CH_2)_7CH_3$	-CH=CH-	>55500	0.10	0	0	0	0	0	-	0
10	H	$NHCH_2C_6H_5$	-CH=CH-	>55500	0.20	3	3	0	3	3	3	3
11	H	$NHCH_2CH_2OH$	-CH=CH-	3470	0.10	3	3	0	2	3	-	3
12	H	$NHCOCH_3$	-CH=CH-	540	0.05	3	3	3	3	3	-	3
13	$NHCOCH_3$	H	-CH=CH-	325	0.05	3	0	3	0	2	3	3
14	H	$NHCOCH_3$	$-CH_2-CH_2-$	650	0.10	3	3	3	3	3	3	3
15	H	$NHCOCH_3$	$-CH_2-CH(OH)\alpha-$	870	0.10	0	3	3	3	2	3	3
16	$NHCOCH_3$	H (3"-EPI)	-CH=CH-	430	0.05	3	3	3	3	0	2	3
17	H	$N(CH_3)COCH_3$	-CH=CH-	650	0.05	3	3	3	3	3	3	3
18	(4'α=H)	(4'β=$NHCOCH_3$)	-CH=CH- [c]	1300	0.10	0	3	3	3	0	-	2
19	H	NHCHO	-CH=CH-	380	0.05	3	3	3	3	3	3	3
20	H	$NHCOCH_2CH_3$	-CH=CH-	430	0.05	3	3	3	3	3	3	3
21	H	$NHCOCH_2OCH_3$	$-CH_2-CH_2-$	870	0.10	3	3	3	2	3	-	3
22	H	$NHCOCF_3$	-CH=CH-	27800	0.10	3	0	3	3	3	-	3
23	H	$NHCOC(CH_3)_3$	-CH=CH-	1730	0.10	3	2	2	2	3	-	3
24	H	$NHCOC_6H_5$	-CH=CH-	28000	0.05	2	2	0	0	3	3	3
25	H	$NHSO_2CH_3$	-CH=CH-	430	0.05	3	3	3	3	3	3	3
26	H	$NHCOOCH_3$	-CH=CH-	430	0.10	3	3	3	3	3	3	3
27	H	$NHCONHCH_3$	-CH=CH-	430	0.10	3	1	3	2	3	-	3
28	H	$NHN(CH_3)_2$	-CH=CH-	430	0.10	3	3	3	3	3	3	3
29	H	$N=CH-N(CH_3)_2$	-CH=CH-	870	0.10	3	3	1	0	2	0	3
30	4"α & β =$N-NHCONH_2$		-CH=CH-	650	0.08	3	3	3	3	0	-	3

[a](IVERMECTIN), [b](AVERMECTIN B1a), [c](MONOSACCHARIDE), [d] *H. c. = Haemonchus contortus, O. c. = Ostertagia circumcincta, T. a. = Trichostrongylus axei, T. c. = Trichostrongylus colubriformis, C. c. = Cooperia circumcincta, C. o. = Cooperia oncophora, Oe. c. = Oesophagostomum columbianum,* [e] Efficacy as % reduction from control: 0 = <50%, 1 = 51-75%, 2 = 76-95%, 3 = >95%.

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