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Synthesis and Biological Activities of Novel 4″-Alkylidene Avermectin Derivatives

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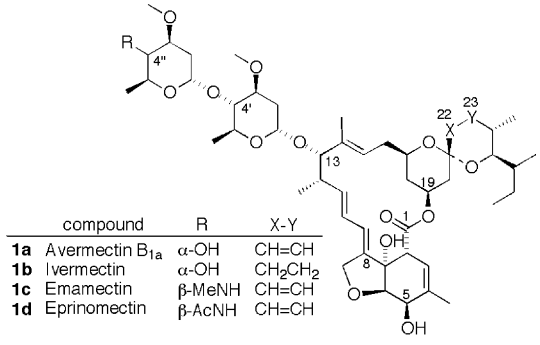
Abstract—Horner–Emmons reaction of 4″-dehydro-5-*O*-TBDMS-avermectin B_{1a} with a variety of phosphorus ylides using LHMDS gave novel 4″-alkylidene avermectin derivatives in high yields. Further modifications led to derivatives bearing diverse functional groups. The new avermectin derivatives showed potent growth inhibitory activity against *Artemia salina* and *Caenorhabditis elegans*.

Avermectins, isolated originally from the culture broth of *Streptomyces avermitilis*, are a series of unique 16-membered ring macrolides and have been known to exhibit exceptionally potent anthelmintic, acaricidal, and insecticidal activities.[1] Avermectin B_1 is the most effective avermectin for insects and mites, and has been commercialized for agricultural use.[2,3] Ivermectin, 22,23-dihydroavermectin B_1 prepared by regioselective hydrogenation using Wilkinson's catalyst,[4] has been widely used as an anthelmintic drug in animals, while ivermectin is also used in humans for onchocerciasis,[5] strongyloidiasis[6] and lymphatic filariasis.[7] Since the discovery of ivermectin, various derivatives have been synthesized so far to provide avermectin derivatives having higher and broader spectrum of activity.[8] In our search for new synthetic avermectins, we focused on chemical modification of the 4″-position on L-oleandrose to improve the activities and pharmacokinetic profiles, because introduction of acyloxy, amino and thio groups at this moiety was found to be effective in terms of solubility, distribution, stability, and diversity of spectrum with potent activity.[9] Among them, emamectin having an *epi* methylamino group at the 4″-position was developed as an agricultural insecticide.[10] Eprinomectin in which the 4″-hydroxyl group is replaced by an *epi* acetylamino group exhibits potent endectocidal activity with minimal residues in milk, and is used for treatment of lactating dairy cattle parasites.[11] To develop avermectins modified at the 4″-position, we attempted Horner–Emmons reaction of the 4″-dehydro derivative to introduce alkylidene groups. The Horner–Emmons reaction would be synthetically useful for introduction of a variety of functional groups. In this letter, we report the synthesis of new 4″-alkylidene derivatives and their biological activities (Fig. 1).

Avermectin B_{1a} was used as the starting material. 4″-Dehydro-5-*O*-TBDMS-avermectin B_{1a} (**2**) was prepared by selective silylation of the C5-hydroxyl group followed by oxidation of the C4″-hydroxyl group with



compound		R	X-Y
1a	Avermectin B_{1a}	α-OH	CH=CH
1b	Ivermectin	α-OH	CH_2CH_2
1c	Emamectin	β-MeNH	CH=CH
1d	Eprinomectin	β-AcNH	CH=CH

Figure 1. Structures of avermectins.

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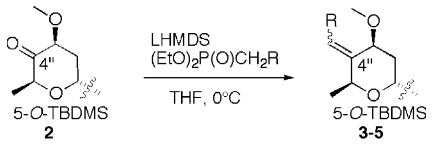
Table 1. Horner–Emmons reaction

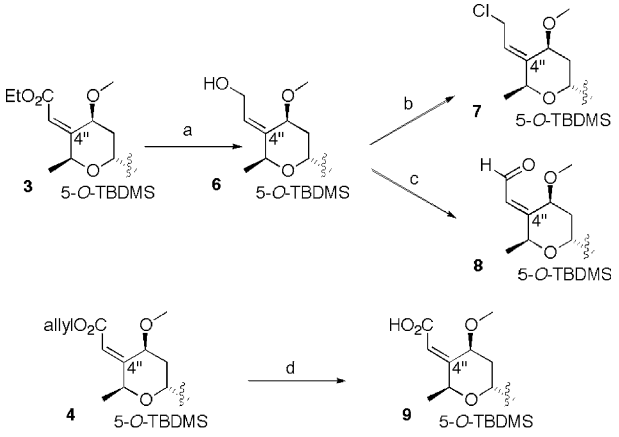


Compd	*R*	Yield (%)	*E*/*Z*
3	CO_2Et	89	7.4
4	CO_2Allyl	85	5.2
5	CN	quant.	3.7

$DMSO/SO_3$-pyridine/Et_3N. Though the Wittig reaction of **2** was unsuccessful, Horner–Emmons reaction in THF at 0 °C using LHMDS as a base proceeded smoothly to give 4″-alkylidene derivatives in high yields (Table 1). Use of *t*-BuOK or LDA instead of LHMDS reduced the yields. Although the C2-proton is known to be sensitive under basic conditions, 2-epimeric or 2,3-conjugated products were not observed under this condition. Similar olefination of 4′-dehydroavermectin B_{1a} monosaccharide was carried out using this procedure ($R = CO_2Allyl$, 98%, only *E*; $R = CN$, 98%, $E/Z = 2.3$). But 13-dehydroavermectin B_{1a} aglycone was not affected probably due to steric hindrance at the C13 position and stability of the conjugated carbonyl group. The *E*/*Z* isomers of 4″-alkylidene were easily separated by silica gel column chromatography.[12–14] The *E* isomers were used further to modify the functional group.

Key intermediates were prepared in excellent yields as shown in Scheme 1. Reduction of ethyl ester (**3**) with DIBAL gave the corresponding alcohol (**6**), which was converted to chloride (**7**) with TsCl/*i*-Pr_2NEt/DMAP and aldehyde (**8**) with MnO_2, respectively. Deprotection of allyl ester (**4**) with $Pd(PPh_3)_4$ and $NaBH_4$ afforded carboxylic acid (**9**).[15]

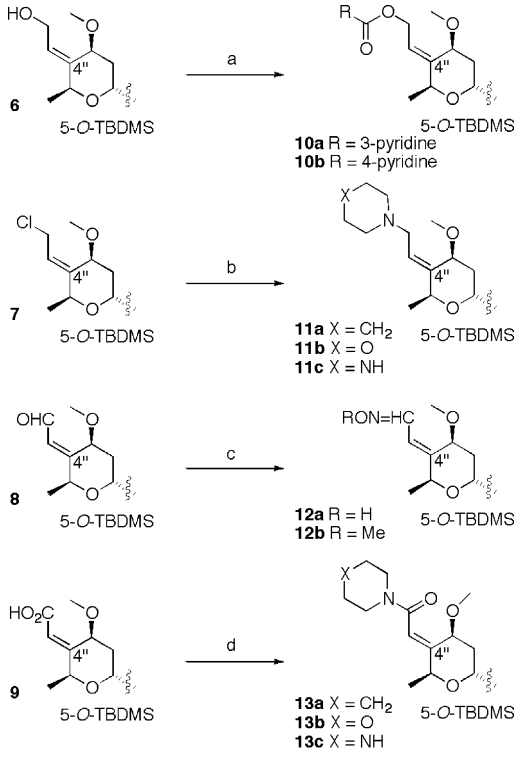
Scheme 2 illustrates the procedures leading to a variety of derivatives to examine the roles of this moiety. Treatment of **6** with acyl chlorides in the presence of DMAP gave acyloxy derivatives (**10a**,**b**). Amino derivatives (**11a**–**c**) were prepared by reaction of **7** with respective amines. Treatment of **8** with hydroxylamine or methoxylamine afforded oxime derivatives (**12a**,**b**) as a 1:1 mixture of *E*/*Z* isomers. Amides (**13a**–**c**) were synthesized by condensation of **9** with respective amines under the EDCI/HOBt condition. The products were obtained in good to moderate yields. These synthetic derivatives were applied to biological assays, after deprotection of 5-*O*-TBDMS by HF-pyridine solution.[16]



Scheme 1. (a) DIBAL, CH_2Cl_2, −78 °C, 89%; (b) TsCl, *i*-Pr_2NEt, DMAP, CH_2Cl_2, rt, quant.; (c) MnO_2, CH_2Cl_2, rt, 84%; (d) $Pd(Ph_3P)_4$, $NaBH_4$, EtOH, 0 °C, 90%.

Insecticidal and nematocidal activities of avermectin derivatives were evaluated by a microplate assay against brine shrimp *Artemia salina,* and free living-nematode *Caenorhabditis elegans*.[17] The results are listed in Table 2. The new avermectin derivatives showed good to moderate growth inhibitory activity. Among them, nitriles **5*E*** and **5*Z*** had the same activity as avermectin B_{1a} and ivermectin. The geometry of the olefin does not seem to have a crucial effect on biological activities (**5*E*** and **5*Z***, **9*E*** and **9*Z***).

In the preliminary results, the avermectin derivatives showed good efficacy against *Haemonchus contortus* and *Trichostrongylus colubriformis* in in vivo trials with sheep (s.c.). **5*E***, **9*E*** and **10a** exhibited more potent activity than ivermectin against *Trichostrongylus colubriformis* at a dosage of 0.01 mg/kg. In vitro trials against *Boophilus microplus*, *Musca domestica*, *Lucilia cuprina*, *Ctenocephalides felis* and *Periplaneta americana* demonstrated a higher overall arthropodicidal potency for **9*E***, **11c**, **12a** and **12b** if compared to ivermectin. The



Scheme 2. (a) RCOCl, DMAP, CH_2Cl_2, rt, 60–65%; (b) amine, EtOH, rt, 71–99%; (c) NH_2OR, EtOH, pyridine, rt, 60–69%; (d) EDCI, HOBt, CH_2Cl_2, rt, 74–99%.

Table 2. In vitro activities of the alkylidene derivatives against *C. elegans* and *A. salina*

Compd	MIC (ng/mL)	
	C. elegans	*A. salina*
Avermectin B1a	2	0.5
Ivermectin	2	2
3	10	10
4	10	2
5*E*	0.5	0.5
5*Z*	2	2
6	10	10
7	10	10
8	10	10
9*E*	10	2
9*Z*	10	2
10a	10	2
10b	50	10
11a	50	10
11b	10	10
11c	10	2
12a	10	2
12b	10	10
13a	10	2
13b	10	10
13c	10	2

efficacy of these derivatives against fleas was higher than that of ivermectin. In vivo **9*E*** was fully active against the cattle tick on cattle at 100 ppm. Further in vitro and in vivo studies on new avermectin derivatives are in progress.

Acknowledgements

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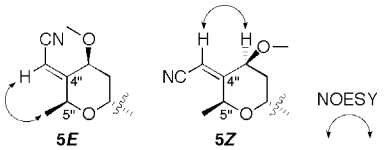
12. To a solution of lithium bis(trimethylsilyl)amide (162 μL, 162 μmol, 1 M in tetrahydrofuran) in tetrahydrofuran (0.3 mL) at 0 °C was added diethyl cyanomethylphosphonate (26 μL, 162 μmol) and the mixture was stirred at 0 °C for 30 min. To the resulting mixture was added a solution of **2** (80.0 mg, 81.1 μmol) in tetrahydrofuran (0.5 mL) dropwise and the mixture was stirred for 1 h. The mixture was poured into saturated aqueous NH_4Cl and extracted with ethyl acetate (5 mL×3). The combined organic layer was washed with saturated aqueous $NaHCO_3$ (5 mL) and brine (5 mL), dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by preparative TLC (toluene–ethyl acetate = 2:1) to provide **5*E*** (64.5 mg, 79%) and **5*Z*** (17.6 mg, 21%) as a white powder.
13. Selected data for deprotected **5*E***: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.85 (m, 1H), 5.75 (dd, J = 10.0, 2.0 Hz, 1H), 5.70 (m, 2H), 5.53 (dd, J = 10.0, 2.0 Hz, 1H), 5.44 (t, J = 4.5 Hz, 1H), 5.40 (s, 1H), 5.38 (m, 1H), 5.34 (s, 1H), 4.97 (m, 1H), 4.76 (d, J = 3.0 Hz, 1H), 4.69 (dd, J = 14.5, 2.5 Hz, 1H), 4.64 (dd, J = 14.5, 2.5 Hz, 1H), 4.46 (q, J = 6.5 Hz, 1H), 4.30 (dd, J = 7.5, 4.5 Hz, 1H), 4.28 (d, J = 6.5 Hz, 1H), 3.95 (d, J = 6.5 Hz, 1H), 3.91 (br.s, 1H), 3.85 (m, 1H), 3.83 (dq, J = 9.0, 6.0 Hz, 1H), 3.61 (ddd, J = 11.5, 9.0, 4.5 Hz, 1H), 3.47 (d, J = 8.5 Hz, 1H), 3.46 (s, 3H), 3.43 (s, 3H), 3.28 (q, J = 2.0 Hz, 1H), 3.26 (t, J = 9.0 Hz, 1H), 2.50 (m, 1H), 2.30–2.21 (m, 4H), 2.16 (ddd, J = 14.0, 7.5, 4.5 Hz, 1H), 2.06 (dt, J = 14.0, 4.5 Hz, 1H), 2.00 (ddd, J = 12.0, 4.0, 1.0 Hz, 1H), 1.85 (s, 3H), 1.76 (m, 1H), 1.64–1.41 (om, 8H), 1.34 (d, J = 6.5 Hz, 3H), 1.22 (d, J = 6.0 Hz, 3H), 1.14 (d, J = 6.5 Hz, 3H), 0.92 (t, J = 8.0 Hz, 3H), 0.91 (d, J = 6.5 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H), 0.86 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.7, 163.4, 139.6, 137.9 (×2), 136.2, 135.0, 127.7, 124.7, 120.3, 118.3, 118.0, 116.0, 97.1, 95.7, 95.0, 94.0, 82.0, 80.7, 80.3, 79.1, 79.0, 75.4, 74.8, 68.4, 68.3 (×2), 67.6, 67.1, 66.7, 57.8, 56.7, 45.6, 40.4, 40.0, 36.9, 36.6, 35.1, 34.5, 34.2, 30.5, 27.4, 20.2, 19.9, 18.1, 17.6, 16.3, 15.1, 12.9, 12.0. Data for deprotected **5*Z***: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.85 (m, 1H), 5.76 (dd, J = 10.0, 2.0 Hz, 1H), 5.70 (m, 2H), 5.54 (dd, J = 10.0, 2.5 Hz, 1H), 5.51 (s, 1H), 5.46 (t, J = 3.5 Hz, 1H), 5.41 (s, 1H), 5.40 (m, 1H), 4.98 (m, 1H), 4.77 (d, J = 3.0 Hz, 1H), 4.70 (dd, J = 14.5, 2.5 Hz, 1H), 4.68 (q, J = 6.5 Hz, 1H), 4.65 (dd, J = 14.5, 2.5 Hz,

1H), 4.28 (d, $J=6.0$ Hz, 1H), 3.98 (dd, $J=8.0$, 5.0 Hz, 1H), 3.96 (d, $J=6.0$ Hz, 1H), 3.93 (br.s, 1H), 3.86 (m, 1H), 3.84 (dq, $J=9.0$, 6.5 Hz, 1H), 3.62 (ddd, $J=11.5$, 9.0, 5.0 Hz, 1H), 3.48 (dd, $J=9.5$, 1.5 Hz, 1H), 3.44 (s, 3H), 3.36 (s, 3H), 3.29 (q, $J=2.0$ Hz, 1H), 3.26 (t, $J=9.0$ Hz, 1H), 2.51 (m, 1H), 2.30–2.24 (m, 3H), 2.23 (dd, $J=12.0$, 5.0 Hz, 1H), 2.20 (ddd, $J=13.0$, 5.0, 3.5 Hz, 1H), 2.01 (ddd, $J=12.0$, 5.0, 1.5 Hz, 1H), 1.95 (ddd, $J=13.0$, 8.0, 3.5 Hz, 1H), 1.86 (s, 3H), 1.77 (ddd, $J=10.5$, 4.5, 2.0 Hz, 1H), 1.64 (d, $J=6.5$ Hz, 3H), 1.62–1.43 (m, 8H), 1.25 (d, $J=6.5$ Hz, 3H), 1.15 (d, $J=7.0$ Hz, 3H), 0.93 (t, $J=8.0$ Hz, 3H), 0.92 (d, $J=6.0$ Hz, 3H), 0.90 (d, $J=7.0$ Hz, 3H), 0.87 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.8, 161.8, 139.6, 138.0 (×2), 136.2, 135.1, 127.7, 124.7, 120.3, 118.3, 118.0, 116.0, 96.9, 95.7, 95.0, 92.8, 82.0, 81.0, 80.4, 79.1, 79.0, 76.9, 74.9, 68.4, 68.3 (×2), 67.7, 67.4, 67.1, 56.9, 56.7, 45.7, 40.4, 39.7, 38.6, 36.6, 35.1, 34.5, 34.2, 30.5, 27.4, 20.2, 19.9, 18.4, 18.2, 16.3, 15.1, 12.9, 12.0.

14. The E/Z configuration of nitriles (**5**) was determined by NOESY experiments.



5E **5Z**

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