Oxime Formation of 4″-*epi*-(Acetylamino)-5-oxo-4″-deoxyavermectin B_1

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The avermectins[1] are a unique collection of naturally occurring macrocyclic lactones containing an α-L-oleandrosyl-α-L-oleandrose disaccharide appended to the C_{13}-hydroxyl group of the aglycon unit, and they exhibit anthelmintic and insecticidal properties. Since the introduction and expanded use of "abamectin" for the control of a variety of agricultural pests[1] and the commericalization of "ivermectin" in the animal health area (including the use of MECTIZAN for the control of river blindness in humans),[2] a large number of avermectin derivatives have been synthesized seeking potential increases in the spectrum of parasite control possible in plants, animals, and humans. Among these new analogues are the 4″-amino- and 4″-(acylamino)avermectins,[3] including 5-oximino-4″-*epi*-(acetylamino)-4″-deoxyavermectin B_1 (**1**, L-685,869) (Figure 1).[4] We wish to report two high-yielding procedures for the conversion of the α,β-unsaturated enone in the avermectin oxyhydrindane ring system to the α,β-unsaturated oxime.

The requisite enone for oximation was prepared from 4″-*epi*-(acetylamino)-4″-deoxyavermectin B_1 (**2**, MK-397) which has been previously described in the literature.[3] Facile oxidation of **2** with phenyl dichlorophosphate,[5] dimethyl sulfoxide, and triethylamine in isopropyl acetate produced 5-oxo-4″-*epi*-(acetylamino)-4″-deoxyavermectin B_1 (**3**) (Figure 2) in 90% yield as a noncrystalline solid. It was found that exposure of crystalline **2** to air also led to oxidation at the C_5-hydroxyl group to give ketone **3**. This air oxidation was accelerated at elevated temperatures but did not lead to good yields of **3** due to secondary oxidations which degraded the material.

Oximation of enone **3** was expected to result from the usual conditions[6] of hydroxylamine hydrochloride in the presence of base, although 1,4-addition of hydroxylamine to α,β-unsaturated enones has been reported.[7] Oximation of ketone **3** by the action of hydroxylamine hydrochloride in the presence of pyridine or diisopropylethylamine led to poor yields (30–60%) of oxime **1**. The major byproduct found from these reactions was 3-(hydroxylamino)-5-oxime **4** (Figure 3). The stereochemistry at C_3 and C_4 in **4** was determined using NOE difference spectroscopy. The pertinent NOE enhancements are represented by arrows in Figure 3, with the doubled-headed arrow indicating NOE enhancements in both directions.

In attempts to modify the reactivity of hydroxylamine

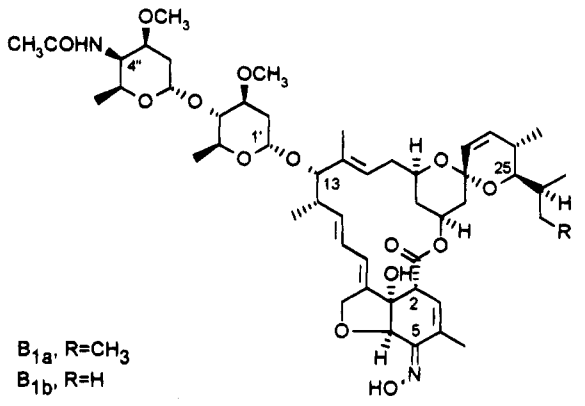
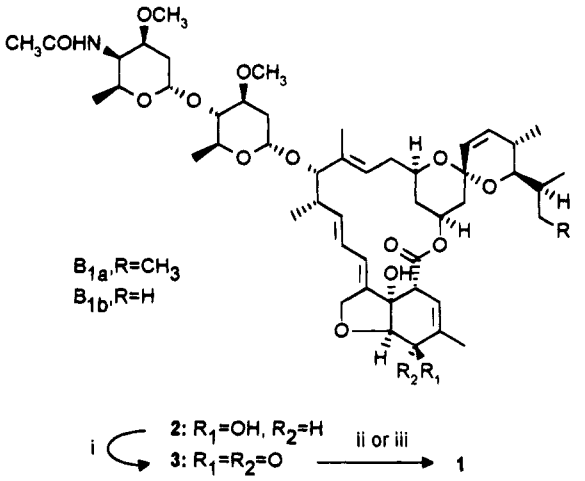


Figure 1.



i: $PhOP(O)Cl_2$, TEA, DMSO, iPrOAc; ii: NH_2OH, iPrOH, H_2O, pH 2.5; iii: NH_2OTMS, $ZnCl_2$.

Figure 2. Preparation of ketone and oxime.

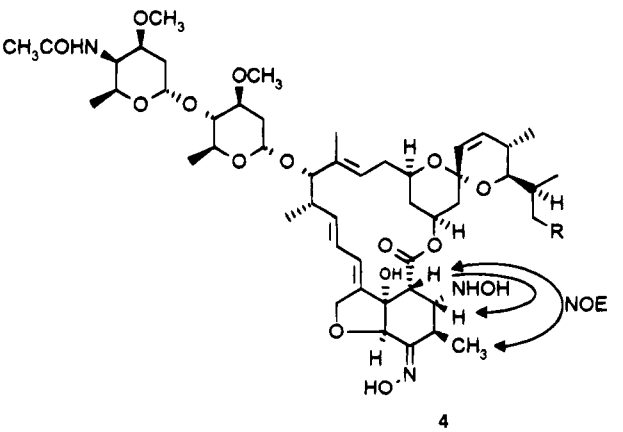


Figure 3.

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and minimize formation of **4**, the action of *O*-(trimethylsilyl)hydroxylamine[8] was examined in nonaqueous systems. With no added base other than excess *O*-(trimethylsilyl)hydroxylamine, little or no oxime formation was detected. Because rates of oxime formation using hydroxylamine are known to be sensitive to acid or base, oximation in the presence of a Lewis acid was explored. Oximation of α,β-unsaturated ketone **3** with *O*-(trimethylsilyl)hydroxylamine/$ZnCl_2$ in isopropyl acetate followed by a hydrolytic workup with 5% aqueous phosphoric acid gave oxime **1** in 90% yield.

Encouraged by this Lewis acid-catalyzed oximation, the action of hydroxylamine hydrochloride upon unsaturated ketone **3** at low pH was examined.[9,10] Addition of hydroxylamine hydrochloride to ketone **3** in aqueous ethanol gave a 70% yield of oxime **1**. The greatest loss of yield resulted from hydrolytic removal of the terminal saccharide unit, and the byproducts of this process were difficult to remove during crystallization of **1**. Monitoring the pH of the reaction mixture showed a continuous drop from a pH of 2.5 to less than 1 as the reaction progressed. When oximation reactions were performed at pH ranges of 3−7 using various buffered systems poor control of 1,4-hydroxylamine addition vs oxime formation resulted. Oxime **1** was shown to be stable to hydroxylamine at pH 4 indicating that (hydroxyamino)oxime **4** arose from initial 1,4 addition to the enone followed by rapid oximation of the ketone. When the reaction medium was changed from aqueous ethanol to aqueous isopropyl alcohol, and the pH was monitored and continuously adjusted to a pH range of 1.8−2.2 by addition of aqueous sodium bicarbonate, formation of (hydroxyamimo)oxime **4** and of hydrolytic byproducts were greatly minimized, and a 90% yield of oxime **1** was attained. This mixture could then be crystallized from ethanol/water in >95 area percent purity by HPLC analysis.

NMR experiments (NOE) determined that the stereochemical orientation of the oxime is as the *Z*-isomer **1-*Z***. HPLC−MS experiments detected the presence of a 3% isomeric component in a typical sample of **1**. Irradiation[11] of an acetone solution of oxime **1-*Z*** at 354 nm changed the ratio of isomers from 97:3 to a maximized ratio of 56:44. This latter mixture of isomers was separated by preparative HPLC, and the minor component required stabilization by storage at <0 °C in polyethylene containers. Comparison of ^{13}C NMR data for these compounds determined that the minor component was the (*E*)-oxime **1-*E***. Reisomerization of solutions (acetonitrile, acetonitrile/water, $CDCl_3$) of **1-*E*** at 25 °C occurred by contact with glass (**1-*E*** was shown to be stable to buffered solution: pH = 2.1, 4.7, 7.0, 9.5 in polyethylene containers) to a 97:3 mixture of **1-*Z***:**1-*E*** and confirmed the assignments of the major and minor component as the oxime isomers.

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Experimental Section

General. HPLC analyses were performed using a Spectra-Physics SP8700 ternary solvent delivery system with a Vydac C18 Protein/Peptide (218TP54) reversed phase column, at 25 °C, UV detection at 245 nm, with the solvent systems described in each experimental procedure. All reactions were carried out under an atmosphere of N_2, and the solvents and reagents were used as received or were dried over 3 Å molecular sieves prior to use as needed. Karl Fisher water analyses were performed with a Metrohm 684 KF Coulometer. Pure samples of each isolated compound were obtained by silica gel chromatography (230-400 mesh), eluting with mixtures of ethyl acetate:hexanes or methanol:ethyl acetate. Infrared spectra were recorded on a Perkin-Elmer 1420 ratio recording infrared spectrophotometer. Melting points were determined using a DuPont 9900 DSC (2 °C/min, under N_2 in an open cup) and are reported as a range from the DSC extrapolated onset temperature to the peak temperature. Proton and carbon-13 spectra were recorded in $CDCl_3$ on a Bruker AM-400 at a frequency of 400.13 and 100.61 MHz, respectively. The chemical shifts are reported in ppm relative to residual $CHCl_3$ for proton (δ = 7.27 ppm) and $CDCl_3$ for carbon (δ = 77.0 ppm). All coupling constants are reported in Hz, and the following proton multiplicites are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, om = overlapping multiplets, br = broad. High-resolution mass spectroscopy studies were performed in the FAB mode. MK-397 was used as the mixture of B_{1a} and B_{1b} components.[3]

5-Oxo-4″*epi*-(acetylamino)-4″-deoxyavermectin B_1 (3). To a solution of **2** (25.0 g, 25.8 mmol), DMSO (7.5 mL), and triethylamine (18.5 mL) in *i*-PrOAc (175 mL) at −20 °C was added phenyl dichloro phosphate (7.55 mL) over 30 min. After a 90 min age at −10 °C, the reaction was quenched with saturated aqueous NaCl (100 mL), and the organic phase was washed with a 1:1 mixture of saturated aqueous $NaHCO_3$ and saturated aqueous NaCl (100 mL). The solvent was removed *in vacuo* (40 °C, 50 Torr) to give **3** as a solid foam (24.6 g) which was used as is for the oximation steps. HPLC assay: gradient, acetonitrile:water (0.1% H_3PO_4), 50:50 to 90:10 over 30 min; (**2**) t_R: B_{1b} = 5.18 min, B_{1a} = 6.86 min; (**3**) t_R: B_{1b} = 10.3 min, B_{1a} = 12.4 min.

^1H NMR: δ 6.58 (br s, 1H), 5.93 (dm, J = 10.3, 1H), 5.82−5.69 (om, 3H), 5.60 (d, J = 10.0, NH), 5.56 (dd, J = 9.9, 2.5, 1H), 5.42 (m, 1H), 5.38 (d, J = 3.8, 1H), 4.98 (m, 1H), 4.78 (br d, J = 3.1, 1H), 4.73 (m, 2H), 4.44 (dd, J = 10.0, 2.9, 1H), 4.06 (m, 1H), 4.02 (s, OH), 3.94 (br s, 1H), 3.93−3.77 (om, 2H), 3.85 (s, 1H), 3.71−3.57 (om, 3H), 3.48 (d, 9.5, 1H), 3.43, 3.39 (s's, 6H), 3.21 (t, J = 9.0, 1H), 2.53 (m, 1H), 2.37−2.18 (om, 4H), 2.06 (s, 3H), 2.06−2.00 (om, 2H), 1.88 (s, 3H), 1.80 (m, 1H), 1.67−1.41 (om, 6H), 1.49 (br s, 3H), 1.23 (d, J = 6.2, 3H), 1.16 (d, J = 6.7, 3H), 1.13 (d, J = 6.5, 3H), 0.98−0.89 (om, 10H). ^{13}C NMR: δ 192.1, 172.2, 170.8, 139.0, 138.1, 137.9, 136.8, 136.4, 135.2, 127.5, 124.6, 121.8, 118.1, 98.7, 95.8, 94.9, 82.0, 81.9, 81.0, 80.8, 79.3, 74.9, 73.3, 69.9, 69.1, 68.4, 67.1, 65.5, 56.4, 55.9, 48.4, 46.6, 40.5, 39.9, 36.6, 35.2, 34.5, 34.2, 31.9, 30.6, 27.6, 23.5, 20.1, 18.3, 17.1, 16.4, 15.5, 15.1, 13.0, 12.1. HRMS: $[MH]^+$ = 912.5087 (calcd = 912.5108). IR (CCl_4): λ_{max} = 3440, 2980, 2940, 1735, 1712, 1685, 1500, 1450, 1370, 1120, 980 cm^{-1}. Anal. Calcd for $C_{50}H_{73}NO_{14}$: C, 65.84; H, 8.07; N, 1.54. Found: C, 65.85; H, 8.30; N, 1.90.

5-Oximino-4″-*epi*-(Acetylamino)-4″-deoxyavermectin B_1 (1). Procedure A: Hydroxylamine Hydrochloride. Ketone **3** (21.6 g, 23.7 mmol) was dissolved in 2-propanol (400 mL), and a solution of hydroxylamine hydrochloride (15.0 g, 220 mmol) in water (40 mL) was added. The pH of the solution was maintained at 1.8−2.1 by the addition of saturated aqueous $NaHCO_3$ via a syringe pump controlled by a pH meter/controller during a 10 h reaction age. After a final adjustment to pH = 4, the mixture was diluted with *tert*-butyl methyl ether (500 mL) and H_2O (500 mL). The organic phase was washed with H_2O (2 × 250 mL), concentrated *in vacuo* (25 °C, 50 Torr) and dissolved in ethanol (170 mL). The solution was warmed to 60 °C, H_2O (75 mL) was added, and the product crystallized upon cooling to 20−25 °C. The slurry was cooled to 0 °C, filtered, washed (2:1 ethanol:water), and dried to give **1** (18.5 g, 82% yield), mp = 185−191 °C. HPLC assay: gradient, acetonitrile: water (0.1% H_3PO_4), 50:50 to 88:12 over 15 min, 2.0 mL/min;

(**4**) t_R: B_{1b} = 4.0 min, B_{1a} = 4.76 min; (**1a**) t_R: B_{1b} = 7.95 min, B_{1a} = 9.38 min; (**1b**) t_R: B_{1a} = 9.68 min; (**3**) t_R: B_{1b} = 9.41 min, B_{1a} = 10.89 min.

^{1}H NMR: δ = 8.93 (br, NOH), 5.94 (m, 1H), 5.81 (m, 1H), 5.77 (dd, J = 9.9, 1.6, 1H), 5.75 (om, 2H), 5.66 (d, J = 9.9, NH), 5.56 (dd, J = 9.9, 2.8, 1H), 5.44 (m, 1H), 5.39 (d, J = 4.0, 1H), 4.98 (br dd, J = 9.5, 4.8, 1H), 4.80–4.66 (om, 3H), 4.67 (s, 1H), 4.44 (dd, J = 10.3, 3.6, 1H), 4.07 (qd, J = 6.3, 1.2, 1H), 3.94 (br s, 1H), 3.87 (om, 3H), 3.70 (m, 1H), 3.63 (ddd, J = 11.5, 8.7, 4.8, 1H), 3.49 (dd, J = 9.9, 1.2, 1H), 3.44 (s, 3H), 3.42 (m, 1H), 3.40 (s, 3H), 3.22 (t, J = 9.1, 1H), 2.53 (m, 1H), 2.35–2.20 (om, 4H), 2.07 (s, 3H), 2.03 (om, 2H), 1.94 (dd, J = 2.4, 1.2, 3H), 1.80 (m, 1H), 1.67–1.44 (om, 6H), 1.50 (br s, 3H), 1.24 (d, J = 6.3, 3H), 1.17 (d, J = 6.7, 3H), 1.13 (d, J = 6.3, 3H), 0.98–0.89 (om, 9H), 0.89 (om, 1H). ^{13}C NMR: δ 173.2, 170.9, 151.4, 138.2, 138.1, 136.3, 135.1, 132.2, 127.7, 125.0, 124.9, 121.3, 118.3, 98.7, 95.8, 94.9, 82.0, 81.1, 79.3, 78.6, 74.9, 73.3, 72.9, 68.7, 68.5, 68.4, 67.0, 65.5, 56.6, 56.1, 48.4, 46.4, 40.5, 39.9, 36.6, 35.2, 34.5, 34.2, 31.8, 30.6, 27.5, 23.4, 20.2, 18.3, 17.5, 17.0, 16.4, 15.1, 13.0, 12.0. HRMS: $[M + Li]^+$ = 933.5315 (calcd = 933.5299). IR ($CHCl_3$): λ_{max} = 3660, 3450, 3010, 2990, 2940, 1710, 1665, 1505, 1450, 1370, 1340, 1190, 1120, 1050, 990 cm^{-1}. Anal. Calcd for $C_{50}H_{74}N_2O_{14}$: C, 64.77; H, 8.05; N, 3.02. Found: C, 64.55; H, 8.31; N, 2.89.

Procedure B: ***O*-(Trimethylsilyl)hydroxylamine/$ZnCl_2$.** To a solution of ketone **3** (23.4 g, 24.2 mmol) in *i*-PrOAc (100 mL) was added $ZnCl_2$ (3.87 g, 28.4 mm) and *O*-(trimethylsilyl)-hydroxylamine (4.9 mL, 39.7 mmol). The mixture was aged for 4 h at 25 °C. Saturated aqueous NaCl (20 mL) and 5% aqueous phosphoric acid (20 mL) were added, and the mixture was aged for 40 min. The organic phase was washed with a mixture of saturated aqueous NaCl (20 mL) and saturated aqueous $NaHCO_3$ (20 mL) and then washed with saturated aqueous NaCl (20 mL). The organic phase was concentrated *in vacuo* and crystallized as above to give 18.7 g of **1** (80% yield).

3(*R*)-(Hydroxyamino)-4(*R*)-methyl-5-oximino-4″-*epi*-(acetylamino)-4″-deoxy-3,4-dihydroavermectin B_1 (4). Oximation with NH_2OH/Diisopropylethylamine. Ketone **3** (1.25 g, 1.27 mmol) in MeOH (12 mL) was treated with hydroxylamine hydrochloride (0.9 g, 12.9 mmol) and diisopropylethylamine (1.0 mL). The mixture was aged for 2 h and then poured into H_2O (20 mL) and *i*-PrOAc (20 mL). The organic phase was washed with H_2O (20 mL) and evaporated *in vacuo* to a crude solid. Purification by column chromatography gave 0.7 g of **4** which was crystallized from 1:1 EtOH:H_2O to give 0.45 g of crystalline **4**, mp = 251.0–252.9 °C (exothermic). ^{1}H NMR: δ 9.53 (br, N-OH), 5.94 (m, 1H), 5.77–5.68 (om, 4H), 5.54 (dd, J = 9.8, 2.2, 1H), 5.39 (d, J = 3.5, 1H), 5.33 (m, 1H), 4.97 (m, 1H), 4.77 (br s, 1H), 4.59 (m, 2H), 4.44 (dd, J = 10.0, 3.2, 1H), 4.13–4.03 (om, 2H), 3.98 (s, 1H), 3.93 (br s, 1H), 3.90–3.78 (om, 2H); 3.70 (m, 1H), 3.62 (m, 1H), 3.47 (d, J = 10.2, 1H), 3.43 (s, 3H), 3.42 (om, 1H), 3.40 (s, 3H), 3.21 (t, J = 8.9, 1H), 3.10 (d, J = 1.9, 1H), 2.51 (m, 1H), 2.35–2.20 (om, 4H), 2.06 (s, 3H), 2.03 (om, 2H), 1.83 (br d, J = 8.8, 1H), 1.67–1.41 (om, 6H), 1.50 (br s, 3H), 1.24, 1.23 (od's, 6H), 1.15, 1.13 (od's, 6H), 0.97–0.89 (om, 9H), 0.89 (om, 1H). ^{13}C NMR: δ 171.5, 171.1, 155.6, 138.4, 138.0, 136.3, 135.1, 127.7, 125.2, 122.1, 118.5, 98.8, 95.9, 95.0, 83.0, 82.2, 81.2, 79.7, 79.4, 74.9, 73.4, 68.6, 68.4, 67.6, 67.1, 65.6, 64.3, 56.7, 56.2, 48.5, 43.6, 40.8, 40.3, 36.3, 35.2, 34.6, 34.1, 31.8, 30.6, 28.2, 27.5, 23.5, 20.2, 18.3, 17.0, 16.4, 15.6, 15.2, 13.0, 12.1. HRMS: $[MH]^+$ = 960.5428 (calcd = 960.5432). IR ($CHCl_3$): λ_{max} = 3560, 3440, 3300, 3005, 2985, 2920, 2900, 1715, 1665, 1515, 1450, 1375, 1340, 1250, 1120, 980 cm^{-1}. Anal. Calcd for $C_{50}H_{77}N_3O_{15}$: C, 62.55; H, 8.08; N, 4.38. Found: C, 62.34; H, 8.01; N, 4.29.

Photolytic Isomerization of Oxime 1-*Z*. Isolation of Oxime 1-*E*. A solution of oxime **1** (2.00 g) in acetone (500 mL) in a Pyrex flask was inserted into a Rayonet photoreactor and irradiated at 350 nm at 25 °C for 1.5 h. The mixture was separated by reversed phase preparative chromatography by means of peak shaving/recycling technique. Fractions were collected in polyethylene vessels, concentrated with a stream of N_2, and then evaporated to a solid by freeze-drying. HPLC: Vydac C18 Protein/Peptide reversed phase column; acetonitrile: methanol:water (48:10:42), 3.0 mL/min; (**1-Z**) t_R: B_{1b} = 11.43 min, B_{1a} = 17.26 min; (**1-E**) t_R: B_{1b} = 12.63 min, B_{1a} = 17.17 min. ^{1}H NMR: (selected data) δ 8.10 (v br, NOH), 5.94 (m, 1H), 5.82 (dq, J = 2.8, 1.6, 1H), 5.61 (d, J = 10.3, NH), 5.56 (dd, J = 9.9, 2.4, 1H), 5.46 (m, 1H), 5.40 (d, J = 4.4, 1H), 4.96 (m, 1H), 4.77 (d, J = 3.2, 1H), 4.68 (br s, 2H), 4.45 (m, 1H), 4.19 (s, 1H), 3.94 (br s, 1H), 3.83 (br s, OH), 3.47 (s, 3H), 3.41 (s, 3H), 3.26 (m, 1H), 2.52 (m, 1H), 2.27 (dd, J = 2.4, 1.6, 3H), 2.08 (s, 3H). ^{13}C NMR: δ 172.9, 170.8, 148.2, 138.4, 138.3, 136.4, 135.1, 130.7, 128.9, 127.6, 124.8, 121.4, 118.2, 98.8, 95.7, 94.9, 82.1, 81.2, 80.1, 79.6, 79.1, 74.8, 73.2, 68.4, 67.0, 65.5, 56.7, 56.1, 48.6, 46.9, 40.4, 39.9, 36.5, 35.0, 34.5, 34.1, 31.7, 30.5, 27.5, 23.6, 22.4, 20.2, 18.3, 17.1, 16.4, 15.1, 13.0, 12.1.

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