J. Med. Chem. 1992, 35, 3873–3878

Synthesis and Biological Activity of 13-*epi*-Avermectins: Potent Anthelmintic Agents with an Increased Margin of Safety[†]

Timothy A. Blizzard,* Gaye M. Margiatto, Helmut Mrozik, Wesley L. Shoop, Robert A. Frankshun, and Michael H. Fisher

Merck Sharp and Dohme Research Laboratories, R50G-231, P.O. Box 2000, Rahway, New Jersey 07065

Received February 6, 1992

Chemical conversion of the potent anthelmintic natural products avermectin B_1 (1) and avermectin B_2 (3) to the corresponding 13-epi analogs (15 and 9) is described. The novel analogs retain the full potency of the natural products but are substantially safer.

Introduction

The avermectins are a family of naturally occurring macrocyclic lactones with important anthelmintic and pesticidal activities.[1] The primary fermentation product, avermectin B_1 (1, Chart I), is an increasingly important agricultural pesticide (Abamectin). Ivermectin (2),[2a] the 22,23-dihydro analog of avermectin B_1, is widely used as an anthelmintic agent in human and animal health.[1c,d] The economic importance of the avermectins has generated considerable interest in their chemical modification[2] and total synthesis.[3] Several articles have described avermectin analogs modified at C-13.[2a,c,h,l] One report in the patent literature[2i] describes 13-β-glycosyloxy milbemycin analogs, including the 13-epimer of the minor component of ivermectin.[1h] However, 13-epimers of natural avermectins have not been previously reported. Since a variety of 13-β-substituted avermectin aglycons have been shown to possess good biological activity[2c,h,i,l] we decided to examine 13-*epi*-avermectins. We describe herein the conversion of two natural avermectins to the corresponding 13-epi analogs. In contrast to epimerization at C-2[2i] or C-19,[2f,g] we have found that inversion of the stereochemistry at C-13 results in derivatives which retain excellent biological activity. In addition, we have discovered that these 13-epi analogs are significantly less toxic than the corresponding natural compounds.

Chemistry

Examination of the avermectin structure suggested that the best approach to 13-epi analogs would be to remove the disaccharide, invert the stereochemistry of the resulting alcohol, and then reattach the disaccharide. Removal of the avermectin disaccharide[2k] and inversion of the C-13 stereochemistry of ivermectin aglycon[2c,l] have been previously reported. Furthermore, recent research on avermectin synthesis has resulted in the availability of the avermectin disaccharide[2b,3b] and an activated disaccharide (8)[3b] as well as important advances in glycosylation of (13-α) avermectin aglycons.[3a–d] We anticipated that we would be able to adapt this methodology to the synthesis of 13-*epi*-avermectins via 13-β-aglycons.

[†] This paper is dedicated to Professor Ralph Hirschmann on the occasion of his 70th birthday.

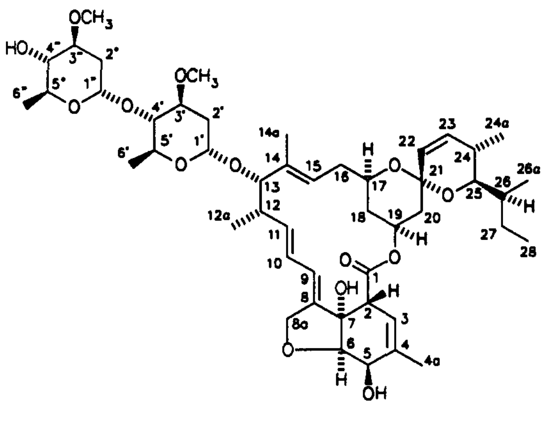
(1) (a) Fisher, M.; Mrozik, H. The Avermectin Family of Macrolide-Like Antibiotics. In *Macrolide Antibiotics*; Omura, S., Ed.; Academic Press: New York, 1984; pp 553–606. (b) Davies, H. G.; Green, R. H. Avermectins and Milbemycins. *Nat. Prod. Rep.* **1986**, *3*, 87–121. (c) Benz, G. W.; Roncalli, R. A.; Gross, S. J. Use of Ivermectin in Cattle, Sheep, Goats, and Swine. In *Invermectin And Abamectin*; Campbell, W. C., Ed.; Springer-Verlag: New York, 1989; pp 215–229. (d) Greene, B. M.; Brown, K. R.; Taylor, H. R. Use of Ivermectin in Humans. In *Ivermectin And Abamectin*; Campbell, W. C., Ed.; Springer-Verlag: New York, 1989; pp 311–323. (e) Dybas, R. A. Abamectin Use in Crop Protection. In *Ivermectin And Abamectin*; Campbell, W. C., Ed.; Springer-Verlag: New York, 1989; pp 287–310. (f) Pulliam, J. D.; Preston, J. M. Safety of Ivermectin in Target Animals. In *Ivermectin And Abamectin*; Campbell, W. C., Ed.; Springer-Verlag: New York, 1989; pp 149–161. (g) Lankas, G. R.; Gordon, L. R. Toxicology. In *Ivermectin And Abamectin*; Campbell, W. C., Ed.; Springer-Verlag: New York, 1989; pp 89–112. (h) Avermectin B_1 is isolated from the fermentation as a mixture of two components. The major (a) component (≥80%) contains a *sec*-butyl side chain at C-25 whereas the minor (b) component (≤20%) has an isopropyl group. Although the components can be separated by HPLC this is not normally done since the a and b isomers have essentially identical biological activities. Thus, all compounds in this paper are actually mixtures of a and b isomers but for the sake of clarity only the a component is shown (i.e. the structure 1 shown for avermectin B_1 is actually the structure of avermectin B_{1a}). Reference 2i describes the synthesis of the b isomer of 13-*epi*-ivermectin from milbemycin D.

(2) (a) Chabala, J. C.; Mrozik, H.; Tolman, R. L.; Eskola, P.; Lusi, A.; Peterson, L.; Woods, M. F.; Fisher, M. H.; Campbell, W. C.; Egerton, J. R.; Ostlind, D. A. Ivermectin, a New Broad-Spectrum Antiparasitic Agent. *J. Med. Chem.* **1980**, *23*, 1134–1136. (b) Blizzard, T. A.; Marino, G.; Mrozik, H.; Fisher, M. H. An Improved Preparation of the Avermectin Disaccharide Unit. *J. Org. Chem.* **1989**, *54*, 1756–1757. (c) Mrozik, H.; Linn, B. O.; Eskola, P.; Lusi, A.; Matzuk, A.; Preiser, F. A.; Ostlind, D. A.; Schaeffer, J. M.; Fisher, M. H. Syntheses and Biological Activities of 13-Substituted Avermectin Aglycons. *J. Med. Chem.* **1989**, *32*, 375–381. (d) Mrozik, H.; Chabala, J. C.; Eskola, P.; Matzuk, A.; Waksmunski, F.; Woods, M.; Fisher, M. H. Synthesis of Milbemycins From Avermectins. *Tetrahedron Lett.* **1983**, *24*, 5333–5336. (e) Blizzard, T. A.; Mrozik, H.; Preiser, F. A.; Fisher, M. H. Synthesis, Reactivity, and Bioactivity of Avermectin B_1-3,4-Oxide. *Tetrahedron Lett.* **1990**, *31*, 4965–4968. (f) Blizzard, T. A.; Bostrom, L.; Margiatto, G.; Mrozik, H.; Fisher, M. H. Conversion of Avermectin B_1 to 19-epi-avermectin B_1. *Tetrahedron Lett.* **1991**, *32*, 2723–2726. (g) Hanessian, S.; Chemla, P. Synthesis of 19-epi-Avermectin A_{1a} from Avermectin B_{1a}. *Tetrahedron Lett.* **1991**, *32*, 2719–2722. (h) Blizzard, T.; Margiatto, G.; Linn, B.; Mrozik, H.; Fisher, M. Avermectin Analogs with a Spacer Between the Aglycone and the Disaccharide. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 369–372. (i) Frei, B.; Mereyala, H. B. 13β Sugar Derivatives of Milbemycin, Their Preparation and use as Ecto- and Endoparasiticides for Treatment of Animals and Plants. *Eur. Pat. Appl. EPO* 235085, 1987; *Chem. Abstr.* **1987**, *109*, 110836d. (j) Pivnichny, J. V.; Arison, B. H.; Preiser, F. A.; Shim, J. K.; Mrozik, H. Base Catalyzed Isomerization of Avermectins. *J. Agric. Food Chem.* **1988**, *36*, 826–828. (k) Mrozik, H.; Eskola, P.; Arison, B. H.; Albers-Schonberg, G.; Fisher, M. H. Avermectin Aglycons. *J. Org. Chem.* **1982**, *47*, 489–492. (l) Linn, B. O.; Mrozik, H. Substituted and Unsubstituted 13-(Alkoxy)Methoxy Derivatives of the Avermectin Aglycones, Compositions and Use; Anthelmintic, Parasiticides. U.S. Patent 4,587,247, 1988.

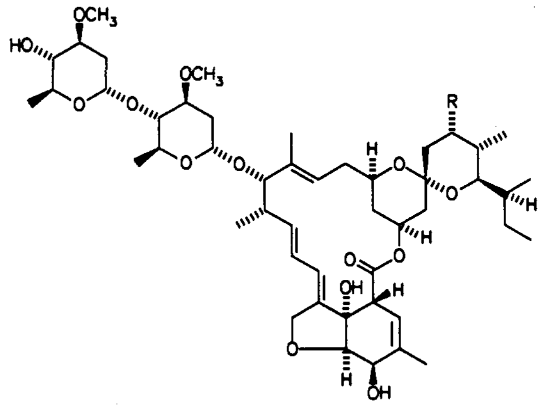
(3) (a) Nicolaou, K. C.; Dolle, R. E.; Papahatjis, D. P.; Randall, J. L. Practical Synthesis of Oligosaccharides. Partial Synthesis of Avermectin B_{1a}. *J. Am. Chem. Soc.* **1984**, *106*, 4189–4192. (b) Hanessian, S.; Ugolini, A.; Dube, D.; Hodges, P. J.; Andre, C. Synthesis of (+)-Avermectin B_{1a}. *J. Am. Chem. Soc.* **1986**, *108*, 2776–2778. (c) Danishefsky, S. J.; Armistead, D. M.; Wincott, F. E.; Selnick, H. G.; Hungate, R. The Total Synthesis of Avermectin A_{1a}. *J. Am. Chem. Soc.* **1989**, *111*, 2967–2980. (d) Ley, S. V.; Armstrong, A.; Diez-Martin, D.; Ford, M. J.; Grice, P.; Knight, J. G.; Kolb, H. C.; Madin, A.; Marby, C. A.; Mukherjee, S.; Shaw, A. N.; Slawin, A. M. Z.; Vile, S.; White, A. D.; Williams, D. J.; Woods, M. Total Synthesis of the Anthelmintic Macrolide Avermectin B_{1a}. *J. Chem. Soc., Perkin Trans. 1* **1991**, 667–692. (e) White, J. D.; Bolton, G. L. Synthesis of Avermectin B_{1a} Aglycon. *J. Am. Chem. Soc.* **1990**, *112*, 1626–1628. (f) For a recent review of avermectin synthesis see: Blizzard, T.; Fisher, M.; Mrozik, H.; Shih, T. Avermectins and Milbemycins. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukacs, G.; Ohno, M., Eds.; Springer-Verlag: Berlin, 1990; pp 65–102.

0022-2623/92/1835-3873$03.00/0 © 1992 American Chemical Society

Chart I

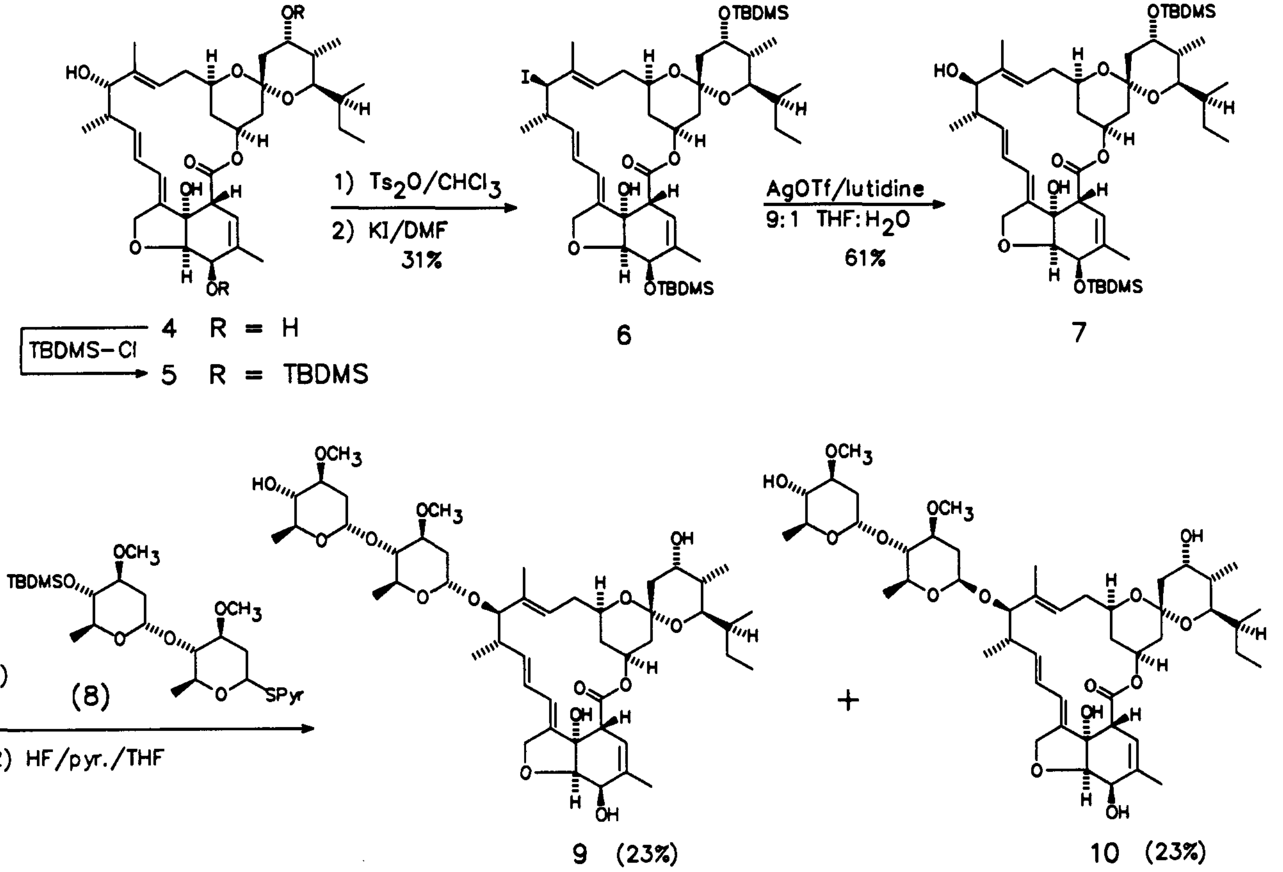


1 AVERMECTIN B_1



2 R = H IVERMECTIN
3 R = OH AVERMECTIN B_2

Scheme I



The synthesis of 13-*epi*-avermectin B_2 (**9**) is outlined in Scheme I. Silylation of avermectin B_2 aglycon (**4**)[2k] afforded the bis-silyl ether **5**. This was converted to the 13-epi aglycon **7** using a modification of the literature procedure for the corresponding ivermectin analog.[2c] Thus, tosylation of **5** followed by displacement of the 13-*O*-tosylate with potassium iodide in DMF afforded the 13-β-iodo derivative (**6**). Silver-catalyzed solvolysis of **6** afforded the 13-epi aglycon **7** along with a small amount of the corresponding 15-hydroxy-Δ-13,14 analog.[2c] Glycosylation of **7** with disaccharide **8**[3b] followed by deprotection using HF/pyridine/THF proceeded smoothly to afford 13-*epi*-avermectin B_2 **9** and the corresponding 1′-β-epimer **10**.

Application of the same sequence of reactions to avermectin B_1 aglycon (**11**)[2k] afforded 13-*epi*-avermectin B_1 (**15**) and the corresponding 1′-β-isomer **16** (Scheme II). Although most of the reactions in this sequence were comparable to the reactions in Scheme I, the glycosylation reaction was a noteworthy exception. Glycosylation of **7** (B_2 series) was much cleaner and resulted in a higher yield of the anomeric glycosides than glycosylation of **14** (B_1 series) under the same conditions. However, we found that glycosylation of **14** could be cleanly accomplished provided that the reaction was run at higher concentration (0.33 M for **14** vs 0.06 M for **7**). It is possible that the glycosylation reaction of **7** could also be improved by running the reaction at a higher concentration but since

Scheme II

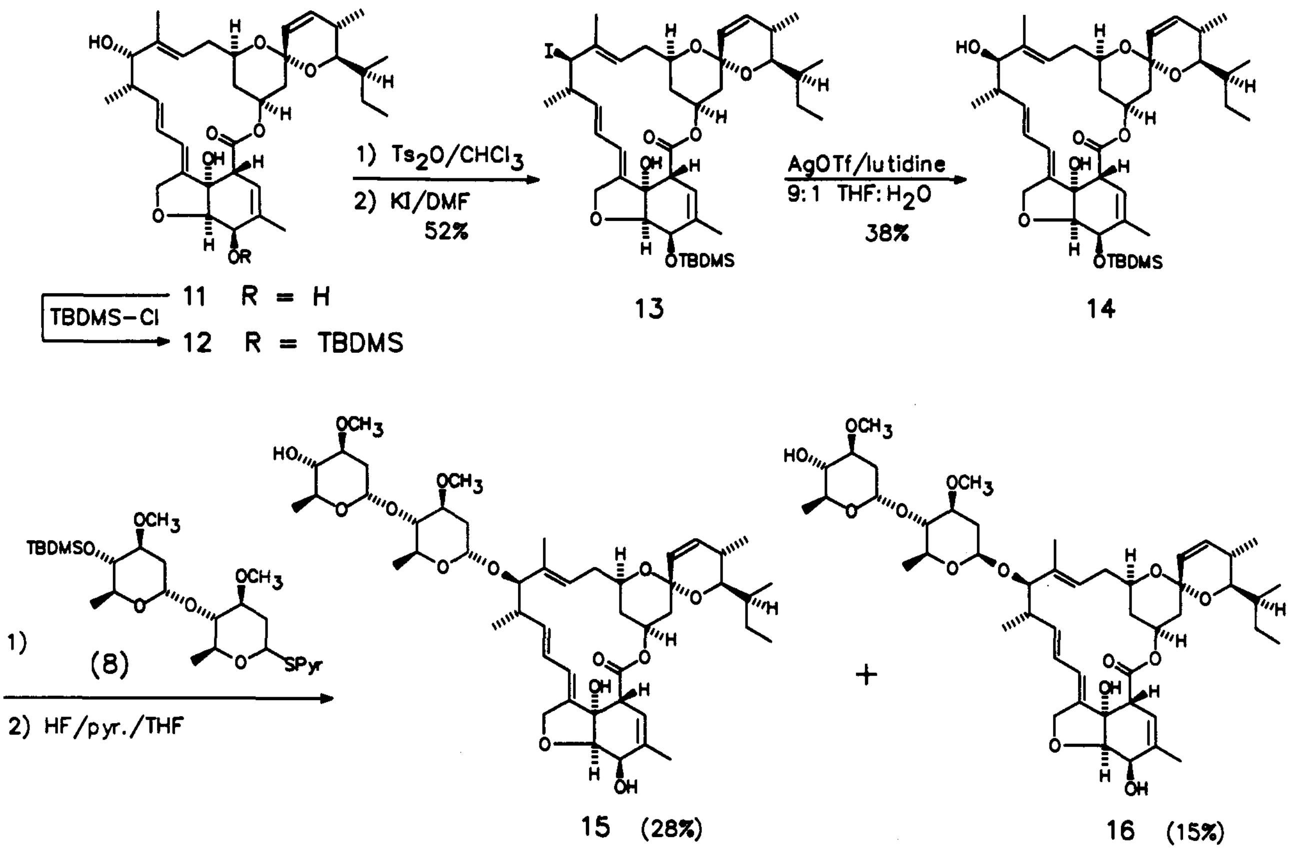


Table I. Brine Shrimp (*Artemia salina*) Activity of Avermectin Analogs[a]

compd	structure	IC_{100}, ng/mL	compd	structure	IC_{100}, ng/mL
3	avermectin B_2	220	1	avermectin B_1	356[b]
9	13-*epi*-AVM B_2	650	15	13-*epi*-AVM B_1	870
10	1′,13-bis-*epi*-AVM B_2	20850	16	1′,13-bis-*epi*-AVM B_1	10415

[a] Brine shrimp data obtained as described in ref 4, average of 2 assays unless otherwise noted. [b] Average of 191 assays.

Table II. Anthelmintic Activity of Avermectin Analogs in Sheep[a]

compd[b]	*H. c.*[c]	*Os. c.*[c]	*T. a.*[c]	*T. c.*[c]	*C. spp.*[c]	*Oe. c.*[c]
3 (α)	3	3	3	3	3	3
9 (β)	3	3	3	3	3	3
1 (α)	3	3	3	3	3	3
15 (β)	3	3	3	3	2	3

[a] Sheep data was obtained as described in the Experimental Section. All compounds were tested at 0.1 mg/kg. Efficacy as % reduction from control: 0 = <50%, 1 = 51–75%, 2 = 76–95%, 3 = >95%. [b] C-13 stereochemistry indicated in parentheses. [c] *H. c.* = *Haemonchus contortus*. *Os. c.* = *Ostertagia circumcincta*. *T. a.* = *Trichostrongylus axei*. *T. c.* = *Trichostrongylus colubriformis*. *C.* spp. = *Cooperia* species. *Oe. c.* = *Oesophagostomum columbianum*.

Table III. Acute Toxicity of Avermectin Analogs in Mice[a]

compd	structure	LD_{50}, mg/kg	compd	structure	LD_{50}, mg/kg
3	avermectin B_2	50	1	avermectin B_1	19[1g]
9	13-*epi*-AVM B_2	540	15	13-*epi*-AVM B_1	160

[a] Estimated mouse LD_{50} data obtained as described in the Experimental Section.

we already had adequate supplies of **9** in hand this experiment was not done.

Biological Results and Discussion

The novel avermectin analogs were initially evaluated in an in vitro brine shrimp assay (Table I).[4] The 13-epi analogs with the natural (α) glycoside stereochemistry (**9** and **15**) retained good activity while the 1′-β analogs (**10** and **16**) were clearly less active. Analogs **9** and **15** (the 13-epimers of avermectins B_2 and B_1) were evaluated for in vivo activity against several parasites in sheep (Table II). Both **9** and **15** retain excellent antiparasitic activity in sheep. Thus, in contrast to epimerization at C-2[2i] or at C-19,[2f,g] epimerization at C-13 affords analogs which retain the full biological activity of the natural products. Surprisingly, evaluation of the 13-epi analogs in a mouse LD_{50} assay showed that they are substantially safer than the natural compounds in mammals (Table III) and thus have a much greater margin of safety.[5] Although ivermectin has been used safely for years in the treatment of millions of animals and humans, a compound with an improved therapeutic index has obvious advantages. For example, a safer compound could be given in higher dosages to gain efficacy against resistant parasites and could potentially be useful in those few animal species which are unusually sensitive to ivermectin.[1f]

Conclusion

Avermectin analogs with inverted stereochemistry at C-13 are substantially safer than the corresponding natural products. However, the 13-epi analogs retain the full

---

(4) Blizzard, T. A.; Ruby, C. L.; Mrozik, H.; Preiser, F. A.; Fisher, M. H. Brine Shrimp (*Artemia Salina*) as a Convenient Bioassay for Avermectin Analogs. *J. Antibiot.* **1989**, *42*, 1304.

(5) This observation appears to be general. We have prepared several 13-*epi*-avermectin derivatives in addition to the compounds described herein and have yet to find an exception to this rule.

anthelmintic activity of the natural compounds. The improved therapeutic index of 13-epi-avermectins may allow their use in a broader range of applications. Ongoing work in this area will be described in future reports from this laboratory.

Experimental Section

General. ^{1}H NMR spectra were obtained at 200, 300, or 400 MHz on Varian XL-200, XL-300, or XL-400 NMR spectrometer, respectively.[6] ^{13}C NMR spectra were obtained at 75.4 or 100.5 MHz. Yields are unoptimized. All title compounds were judged to be at least 95% pure by ^{1}H NMR analysis. Satisfactory elemental analyses (±0.4%) were obtained for all test compounds and for key synthetic intermediates. Elemental analyses were performed by the Merck analytical chemistry department or by Robertson Microlit Laboratories, Inc. Analytical thin-layer chromatography (TLC) was performed on 2.5- × 10-cm plates coated with 0.25-mm thickness of silica gel containing PF254 indicator (Analtech). Preparative TLC was performed on 20- × 20-cm plates coated with 0.5, 1.0, or 1.5 mm of silica gel containing PF254 indicator (Analtech). Compounds were visualized with shortwave UV light. For preparative TLC compounds were eluted from the silica gel with ethyl acetate. Flash chromatography was performed using EM Science Silica Gel 60 (230–400 mesh).

Sheep Assay. Anthelmintic efficacy of the compounds was determined in sheep raised helminth free and experimentally infected with the parasites listed in Table II. When the infections were patent the sheep were randomly assigned to a treatment or control group. Each compound was tested in one sheep while two sheep served as controls. The compounds were administered in a single oral dosage of 0.1 mg/kg. Control animals were given only the vehicle. Seven days after dosing the animals were necropsied, and the residual worm burdens were determined. The efficacies of the test compounds were recorded as described in footnote *a* of Table II.

Estimated Mouse LD_{50} Assay. Acute toxicity of the compounds was evaluated by calculating the LD_{50} for male CD-1 mice. Each compound was evaluated at several doses in treatment groups of five mice per dose allocated at random from a pool of mice. The compounds were administered orally in a vehicle via a calibrated syringe with a blunt tipped needle. Seven days after dosing the number of deaths was determined and the estimated LD_{50} values were determined by the method of dose–pair responses. This method effectively emulates linear regression while minimizing the number of animals required.

5,23-Bis-*O*-(*tert*-butyldimethylsilyl)avermectin B_2 Aglycon (5). Imidazole (399 mg, 6.25 equiv) and *tert*-butyldimethylsilyl chloride (353 mg, 2.5 equiv) were added to a solution of avermectin B_2 aglycone (4, 565 mg)[2k] in 6 mL of dry DMF. The resulting yellow solution was stirred at 35–40 °C for 24 h, then cooled to room temperature, and partitioned between water (50 mL) and ether (50 mL). The aqueous layer was extracted with ether (2 × 25 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to a yellow oil (1.11 g). The crude product was purified by flash chromatography on silica gel eluted with 3:1 hexane/ether to afford 607 mg (76%) of 5 as a white foam. Partial ^{1}H NMR data (300 MHz, $CDCl_3$): δ 5.80–5.60 (3 H, m, H_9, H_{10}, and H_{11}), 5.32–5.16 (3 H, m, H_3, H_{15}, and H_{19}), 4.64 and 4.54 (2 H, 2 d, J = 15 Hz, H_{8a}), 4.40 (1 H, br s, H_5), 4.10 (1 H, s, 7-OH), 3.97 (1 H, br s, H_{13}), 3.82–3.77 (2 H, m, H_6 and H_{23}), 3.71–3.61 (1 H, m, H_{17}), 3.69 (1 H, d, J = 9 Hz, H_{25}), 3.32 (1 H, br s, H_2), 2.56–2.42 (1 H, m, H_{12}), 1.75 (3 H, br s, H_{4a}), 1.50 (3 H, s, H_{14a}), 0.90 (9 H, s, ^{t}BuSi), 0.86 (9 H, s, ^{t}BuSi), 0.12 (6 H, s, $Si(CH_3)_2$), 0.02 (3 H, s, $SiCH_3$), –0.03 (3 H, s, $SiCH_3$).

5,23-Bis-*O*-(*tert*-butyldimethylsilyl)-13-*β*-iodo-13-deoxyavermectin B_2 Aglycon (6). *p*-Toluenesulfonic anhydride (3.0 g, 5.1 equiv) was added to a solution of 5 (1.5 g), (dimethylamino)pyridine (1.1 g, 5.0 equiv), and diisopropylethylamine (2.2 mL, 7.0 equiv) in 15 mL of deuterochloroform. (Note that deuterochloroform was used as the solvent so that the reaction could be followed easily by NMR; alternatively chloroform can be used as the solvent and the reaction allowed to proceed for a predetermined time.) The mixture was stirred at room temperature for 16 h and then partitioned quickly between dichloromethane (25 mL) and water (25 mL). The aqueous layer was extracted with dichloromethane (3 × 25 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The resulting orange oil was dissolved in 25 mL of dry dimethylformamide, and then potassium iodide (3.3 g, 11.0 equiv) was added. The mixture was stirred at 60 °C for 75 min, then cooled to room temperature, and partitioned between ether (50 mL) and water (50 mL). The aqueous layer was extracted with ether (3 × 50 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash chromatography on silica gel eluted with 4% acetone in hexane to afford 520 mg (31%) of 6 as a white foam (R_f 0.20). Partial ^{1}H NMR data (300 MHz, $CDCl_3$): δ 5.86–5.68 (2 H, m, H_9 and H_{10}), 5.42 (1 H, dd, J = 5, 10 Hz, H_{15}), 5.30 (1 H, br s, H_3), 5.28 (1 H, dd, J = 10, 15 Hz, H_{11}), 5.25–5.12 (1 H, m, H_{19}), 4.65 and 4.55 (2 H, 2 d, J = 15 Hz, H_{8a}), 4.57 (1 H, d, J = 11 Hz, H_{13}), 4.40 (1 H, br s, H_5), 3.99 (1 H, s, 7-OH), 3.82–3.75 (2 H, m, H_6 and H_{23}), 3.69 (1 H, d, J = 9 Hz, H_{25}), 3.63–3.52 (1 H, m, H_{17}), 3.34 (1 H, br s, H_2), 2.69–2.52 (1 H, m, H_{12}), 1.76 (3 H, br s, H_{4a}), 1.70 (3 H, s, H_{14a}), 0.93 (9 H, s, ^{t}BuSi), 0.86 (9 H, s, ^{t}BuSi), 0.10 (6 H, s, $Si(CH_3)_2$), 0.02 (3 H, s, $SiCH_3$), –0.03 (3 H, s, $SiCH_3$). Anal. ($C_{46}H_{77}O_8Si_2I$) C, H.

5,23-Bis-*O*-(*tert*-butyldimethylsilyl)-13-*epi*-avermectin B_2 Aglycon (7). Silver trifluoromethanesulfonate (410 mg, 2.9 equiv) was added to a solution of 6 (520 mg) and 2,6-lutidine (0.37 mL, 5.75 equiv) in 9 mL of 9:1 tetrahydrofuran/water. The mixture (yellow-white precipitate) was stirred at room temperature for 45 min and then partitioned between ether (50 mL) and 0.1 N HCl (25 mL). The layers were separated, and the organic layer was washed with 25 mL of 5% aqueous $NaHCO_3$, then dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on four 1.5-mm silica gel plates eluted twice with 33% ether in hexane to afford 280 mg (61%) of 7 as a white foam (R_f 0.45). Partial ^{1}H NMR data (300 MHz, $CDCl_3$): δ 5.82–5.66 (2 H, m, H_9 and H_{10}), 5.38–5.12 (4 H, m, H_3, H_{11}, H_{15}, and H_{19}), 4.65 and 4.57 (2 H, 2 d, J = 15 Hz, H_{8a}), 4.40 (1 H, br s, H_5), 4.02 (1 H, s, 7-OH), 3.83–3.77 (2 H, m, H_6 and H_{23}), 3.69 (1 H, d, J = 9 Hz, H_{25}), 3.68 (1 H, d, J = 10 Hz, H_{13}), 3.65–3.54 (1 H, m, H_{17}), 3.33 (1 H, br s, H_2), 2.40–2.38 (1 H, m, H_{12}), 1.76 (3 H, br s, H_{4a}), 1.56 (3 H, s, H_{14a}), 0.90 (9 H, s, ^{t}BuSi), 0.85 (9 H, s, ^{t}BuSi), 0.11 (6 H, s, $Si(CH_3)_2$), 0.02 (3 H, s, $SiCH_3$), –0.02 (3 H, s, $SiCH_3$). Anal. ($C_{46}H_{78}O_9Si_2$) C, H.

13-*epi*-Avermectin B_2 (9) and 1′,13-Bis-*epi*-avermectin B_2 (10). A solution of disaccharide 8[2h] (560 mg, 1.8 equiv) in 4 mL of dry acetonitrile was added slowly dropwise (over a period of 30 min) to a cold (0 °C), rapidly stirring, solution of aglycone 7 (500 mg) and silver trifluoromethanesulfonate (270 mg, 1.75 equiv) in 6 mL of dry acetonitrile. The resulting mixture (gummy precipitate) was stirred vigorously at 0 °C for 3 h and then partitioned between ethyl acetate (15 mL) and 5% aqueous $NaHCO_3$ (10 mL). The layers were separated with the aid of a centrifuge. The aqueous layer was extracted with ethyl acetate (4 × 6 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed on a silica gel column eluted with 9% acetone in hexane to afford 4″,5,23-tris-*O*-(*tert*-butyldimethylsilyl)-13-*epi*-avermectin B_2 (white foam, 270 mg, 36%) as the major product. The isomeric 4″,5,23-tris-*O*-(*tert*-butyldimethylsilyl)-1′,13-bis-*epi*-avermectin B_2 (250 mg, 34%) was also obtained as a byproduct of the reaction. A deprotection reagent solution was prepared by cautiously adding 25 g of hydrogen fluoride–pyridine complex to a cold (0 °C) mixture of pyridine (12.5 mL) and tetrahydrofuran (27.5 mL). A portion (4.2 mL) of the resulting reagent solution was added to a cold (0 °C) solution of the major glycosylation product [4″,5,23-tris-*O*-(*tert*-butyldimethylsilyl)-13-*epi*-avermectin B_2, 778 mg (product of several glycosylation reactions, combined for deprotection)] in 14 mL of dry tetrahydrofuran. The resulting solution was stirred at room temperature for 5 days and then

---

(6) For complete ^{1}H NMR assignments for avermectin B_1 see the following references: (a) Neszmelyi, A.; Machytka, D.; Kmety, A.; Sandor, P.; Lukacs, G. Solution Conformation of the Disaccharide of Avermectin B_{1a} Examined by NMR Spectroscopy and Nuclear Overhauser Enhancement Restrained Hard-Sphere Exo-Anomeric Effect Calculation. *J. Antibiot.* **1989**, *42*, 1494–1501. (b) Diez-Martin, D.; Grice, P.; Klob, H. C.; Ley, S. V.; Madin, A. Synthesis of a C16-C28 Fragment of Avermectin B_{1a} and Reassignment of Some ^{1}H and ^{13}C Resonances of Avermectin B_{1a}. *Tetrahedron Lett.* **1990**, *31*, 3445–3448.

cooled in an ice bath as pyridine (12 mL) was added followed by ethyl acetate (20 mL) and 5% aqueous $NaHCO_3$ (24 mL). The layers were separated with the aid of a centrifuge, and the aqueous layer was extracted with ethyl acetate (3 × 12 mL). The combined organic layers were dried over $MgSO_4$ and K_2CO_3, filtered, and evaporated to a light yellow oil (547 mg). The crude product was purified by flash chromatography on silica gel eluted with 3:1 hexane/acetone to afford 370 mg (65%) of **9** as a white foam (R_f 0.09) (23% overall yield from **7**). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.80–5.68 (2 H, m, H_9 and H_{10}), 5.38 (1 H, br s, H_3), 5.35–5.10 (3 H, m, H_{11}, H_{15}, and H_{19}), 5.30 (1 H, d, J = 3 Hz, $H_{1''}$), 4.85 (1 H, d, J = 3 Hz, $H_{1'}$), 4.66 (2 H, br s, H_{8a}), 4.26 (1 H, br t, J = 6 Hz, H_5), 3.92 (1 H, d, J = 6 Hz, H_6), 3.85 (1 H, s, 7-OH), 3.78–3.58 (3 H, m, $H_{5''}$, H_{17}, and H_{23}), 3.55–3.35 (4 H, m, $H_{3'}$, $H_{3''}$, $H_{5'}$, and H_{13}), 3.47 (1 H, d, J = 10 Hz, 23-OH), 3.43 (1 H, d, J = 10 Hz, H_{25}), 3.38 (3 H, s, OCH_3), 3.32 (3 H, s, OCH_3), 3.24 (1 H, br s, H_2), 3.18 (1 H, t, J = 9 Hz, $H_{4'}$), 3.12 (1 H, br t, J = 9 Hz, $H_{4''}$), 2.57 (1 H, br s, 4″-OH), 2.40 (1 H, d, J = 6 Hz, 5-OH), 2.55–2.15 (5 H, m, H_{12}, H_{16}, $H_{2'eq}$, and $H_{2''eq}$), 2.00 (1 H, dd, J = 5, 12 Hz, H_{20eq}), 1.94 (1 H, dd, J = 3, 14 Hz, H_{22}), 1.84 (3 H, br s, H_{4a}), 1.80–1.30 (8 H, m, $H_{2'ax}$, $H_{2''ax}$, H_{18eq}, H_{20ax}, H_{24}, H_{26}, and H_{27}), 1.63 (1 H, dd, J = 4, 14 Hz, H_{22}), 1.55 (3 H, br s, H_{14a}), 1.23 (3 H, d, J = 6 Hz, $H_{6''}$), 1.10 (3 H, d, J = 6 Hz, $H_{6'}$), 1.03 (3 H, d, J = 7 Hz, H_{12a}), 0.94 (3 H, t, J = 7 Hz, H_{28}), 0.95–0.8 (1 H, m, H_{18ax}), 0.88 (3 H, d, J = 7 Hz, H_{26a}), 0.83 (3 H, d, J = 7 Hz, H_{24a}). Anal. ($C_{48}H_{74}O_{15}$) C, H. Deprotection of a portion of the minor glycosylation product (147 mg, 4″,5,23-tris-*O*-(*tert*-butyldimethylsilyl)-1′,13-bis-*epi*-avermectin B_2) using the same procedure afforded 106 mg of a crude product which was purified by preparative TLC on two 1.0-mm silica gel plates eluted with 1:1 hexane/acetone to afford 71 mg (67%) of ***10*** (R_f 0.38) (23% overall yield from **7**). Partial ^{1}H NMR data (300 MHz, $CDCl_3$): δ 5.81–5.69 (2 H, m, H_9 and H_{10}), 5.38 (1 H, br s, H_3), 5.35 (1 H, d, J = 3 Hz, $H_{1''}$), 5.34–5.10 (3 H, m, H_{11}, H_{15}, and H_{19}), 4.66 (2 H, br s, H_{8a}), 4.27 (1 H, br t, J = 6 Hz, H_5), 4.23 (1 H, dd, J = 9, 1 Hz, $H_{1'}$), 3.92 (1 H, d, J = 6 Hz, H_6), 3.80–3.58 (3 H, m, $H_{5''}$, H_{17}, and H_{23}), 3.77 (1 H, s, 7-OH), 3.77 (1 H, d, J = 10 Hz, H_{13}), 3.53 (1 H, d, J = 10 Hz, 23-OH), 3.50 (1 H, d, J = 10 Hz, H_{25}), 3.45–3.15 (3 H, m, $H_{3'}$, $H_{3''}$, and $H_{5'}$), 3.35 (3 H, s, OCH_3), 3.30 (3 H, s, OCH_3), 3.21 (1 H, br s, H_2), 3.12 (1 H, t, J = 9 Hz, $H_{4'}$), 3.12 (1 H, br t, J = 9 Hz, $H_{4''}$), 3.59 (1 H, br s, 4″-OH), 2.39 (1 H, d, J = 6 Hz, 5-OH), 2.45–2.10 (5 H, m, H_{12}, H_{16}, $H_{2'eq}$, and $H_{2''eq}$), 2.03 (1 H, dd, J = 5, 12 Hz, H_{20eq}), 1.96 (1 H, dd, J = 3, 14 Hz, H_{22}), 1.84 (3 H, br s, H_{4a}), 1.80–1.30 (8 H, m, $H_{2'ax}$, $H_{2''ax}$, H_{18eq}, H_{20ax}, H_{24}, H_{26}, and H_{27}), 1.64 (1 H, dd, J = 4, 14 Hz, H_{22}), 1.45 (3 H, br s, H_{14a}), 1.28 (3 H, d, J = 6 Hz, $H_{6'}$), 1.24 (3 H, d, J = 6 Hz, $H_{6''}$), 1.10 (3 H, d, J = 7 Hz, H_{12a}), 0.95–0.8 (1 H, m, H_{18ax}), 0.97 (3 H, t, J = 7 Hz, H_{28}), 0.89 (3 H, d, J = 7 Hz, H_{26a}), 0.84 (3 H, d, J = 7 Hz, H_{24a}). Anal. ($C_{48}H_{74}O_{15}\cdot H_2O$) C, H.

5-*O*-(*tert*-Butyldimethylsilyl)avermectin B_1 Aglycon (12). Imidazole (9.67 g, 4.5 equiv) and *tert*-butyldimethylsilyl chloride (9.50 g, 2.0 equiv) were added to a solution of avermectin B_1 aglycon (**11**, 18.38 g)^{2k} in 150 mL of dry DMF. The resulting orange solution was stirred at 25 °C for 1.75 h and then partitioned between water (1 L) and ether (400 mL). The aqueous layer was extracted twice with ether, and then the combined organic layers were washed three times with water, dried over Na_2SO_4, filtered, and evaporated to a yellow oil (24 g). The crude product was purified by flash chromatography on silica gel eluted with a gradient of 10% ethyl acetate in hexane to 20% ethyl acetate in hexane to afford 17.55 g (80%) of **12** as a foam. Partial ^{1}H NMR data (200 MHz, $CDCl_3$): δ 5.85–5.65 (4 H, m, H_9, H_{10}, H_{11}, and H_{22}), 5.53 (1 H, dd, J = 10, 3 Hz, H_{23}), 5.40–5.20 (3 H, m, H_3, H_{15}, and H_{19}), 4.69 and 4.55 (2 H, 2 d, J = 15 Hz, H_{8a}), 4.42 (1 H, br s, H_5), 3.99 (1 H, s, 7-OH), 3.99 (1 H, br s, H_{13}), 3.94–3.77 (1 H, m, H_{17}), 3.80 (1 H, d, J = 6 Hz, H_6), 3.45 (1 H, d, J = 9 Hz, H_{25}), 3.36 (1 H, br s, H_2), 2.60–2.40 (1 H, m, H_{12}), 1.77 (3 H, br s, H_{4a}), 1.53 (3 H, s, H_{14a}), 0.92 (9 H, s, ^{t}BuSi), 0.12 (6 H, s, $Si(CH_3)_2$).

5-*O*-(*tert*-Butyldimethylsilyl)-13-*β*-iodo-13-deoxyavermectin B_1 Aglycon (13). *p*-Toluenesulfonic anhydride (32.64 g, 4 equiv) was added to a solution of **12** (17.55 g), (dimethylamino)pyridine (15.27 g, 5 equiv), and diisopropylethylamine (30.5 mL, 7 equiv) in 250 mL of dichloromethane. The mixture was stirred at room temperature for 23 h and then partitioned quickly between dichloromethane and water (1 L). The aqueous layer was extracted twice with dichloromethane, and the combined organic layers were washed with water, dried over Na_2SO_4, filtered, and evaporated. The resulting brown foam was dissolved in 250 mL of dry dimethylformamide and then potassium iodide (49.8 g, 12 equiv) was added. The mixture was stirred at 60 °C for 1 h, then cooled to room temperature, and partitioned between ether (500 mL) and water (1 L). The aqueous layer was extracted with ether (3×), and the combined organic layers were washed with water, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography on silica gel eluted with a gradient of 50% dichloromethane in hexane gradually increasing to 100% dichloromethane to afford 10.41 g of **13** (52%) as a foam. Partial ^{1}H NMR data (200 MHz, $CDCl_3$): δ 5.95–5.70 (4 H, m, H_9, H_{10}, H_{11}, and H_{22}), 5.52 (1 H, dd, J = 10, 3 Hz, H_{23}), 5.50–5.20 (3 H, m, H_3, H_{15}, and H_{19}), 4.67 and 4.57 (2 H, 2 d, J = 15 Hz, H_{8a}), 4.57 (1 H, d, J = 10 Hz, H_{13}), 4.42 (1 H, br s, H_5), 3.92 (1 H, s, 7-OH), 3.88–3.68 (1 H, m, H_{17}), 3.80 (1 H, d, J = 6 Hz, H_6), 3.43 (1 H, d, J = 9 Hz, H_{25}), 3.37 (1 H, br s, H_2), 2.70–2.52 (1 H, m, H_{12}), 1.77 (3 H, br s, H_{4a}), 1.70 (3 H, s, H_{14a}), 0.90 (9 H, s, ^{t}BuSi), 0.12 (6 H, s, $Si(CH_3)_2$).

5-*O*-(*tert*-Butyldimethylsilyl)-13-*epi*-avermectin B_1 Aglycon (14). Silver trifluoromethanesulfonate (3.32 g, 1.0 equiv) was added to a solution of **13** (10.41 g) and 2,6-lutidine (2.3 mL, 1.5 equiv) in 150 mL of 9:1 tetrahydrofuran/water. The mixture (precipitate) was stirred at room temperature for 1 h then diluted with ether (150 mL), and filtered. The filtrate was washed sequentially with water, 0.1 N HCl, and water, then dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography on silica gel eluted with a gradient of 10% acetone in hexane to 15% acetone in hexane to afford 6.53 g of a yellow foam which consisted of a mixture of **14** and the 15-hydroxy-Δ-13,14 isomer. Repeated chromatography of this mixture on silica gel eluted with 40% ether in hexane afforded 3.39 g (38%) of pure **14** as a foam. Partial ^{1}H NMR data (300 MHz, $CDCl_3$): δ 5.83–5.69 (3 H, m, H_9, H_{10}, and H_{22}), 5.52 (1 H, dd, J = 10, 3 Hz, H_{23}), 5.38–5.14 (4 H, m, H_3, H_{11}, H_{15}, and H_{19}), 4.67 and 4.57 (2 H, 2 d, J = 15 Hz, H_{8a}), 4.40 (1 H, br s, H_5), 3.92 (1 H, s, 7-OH), 3.82–3.70 (1 H, m, H_{17}), 3.78 (1 H, d, J = 6 Hz, H_6), 3.69 (1 H, d, J = 10 Hz, H_{13}), 3.43 (1 H, d, J = 9 Hz, H_{25}), 3.35 (1 H, br s, H_2), 2.40–2.26 (1 H, m, H_{12}), 1.76 (3 H, br s, H_{4a}), 1.57 (3 H, s, H_{14a}), 0.92 (9 H, s, ^{t}BuSi), 0.12 (6 H, s, $Si(CH_3)_2$).

13-*epi*-Avermectin B_1 (15) and 1′,13-Bis-*epi*-avermectin B_1 (16). A solution of silver trifluoromethanesulfonate (150 mg, 2.7 equiv) in 0.25 mL of dry acetonitrile was added dropwise slowly (over 25 min) to a solution of aglycon **14** (150 mg) and disaccharide **8**^{2h} (330 mg, 3 equiv) in 0.4 mL of dry acetonitrile. About 15 min after the addition was complete, the reaction mixture was diluted with 5 mL of ethyl acetate then 3 mL of 5% aqueous sodium bicarbonate was added (voluminous white precipitate). The mixture was centrifuged, and the supernatant layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 3 mL), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to a yellow oil (432 mg). The crude product was chromatographed on three 2.0-mm silica gel plates eluted with 1:1 hexane/ether to afford 127 mg (54%) of 4″,5-bis-*O*-(*tert*-butyldimethylsilyl)-13-*epi*-avermectin B_1 (mixture of α and β anomers at C-1′). This material was combined with an additional 8 mg isolated from a previous experiment and dissolved in 2 mL of dry tetrahydrofuran. The solution was cooled in an ice bath as 1 mL of the deprotection reagent (prepared as described above; see procedure for **9**) was added. The resulting solution was stirred at room temperature for 62 h then cooled in an ice bath as pyridine (4 mL) was added followed by ethyl acetate (4 mL) and 2% aqueous $NaHCO_3$ (4 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 3 mL). The combined organic layers were dried over $MgSO_4$ and K_2CO_3, filtered, and evaporated to a yellow oil (94 mg). The crude product was chromatographed on a 1.5-mm silica gel plate eluted three times with ether to afford 55 mg (51%) of **15** (28% overall yield from **14**) as a white foam (R_f 0.24) and 29 mg (27%) of **16** (15% overall yield from **14**) as a white foam (R_f 0.29). Data for **15** ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.80–5.74 (2 H, m, H_9 and H_{10}), 5.73 (1 H, dd, J = 10, 2 Hz, H_{22}), 5.52 (1 H, dd, J = 10, 3 Hz, H_{23}), 5.40 (1 H, br s, H_3), 5.38–5.28 (2 H, H_{11} and H_{19}), 5.33 (1 H, d, J = 3 Hz, $H_{1''}$), 5.19 (1 H, t, J = 7 Hz, H_{15}), 4.87 (1 H, d, J = 3 Hz, $H_{1'}$), 4.70 and 4.65 (2 H, 2 d, J = 14 Hz, H_{8a}), 4.28 (1 H, t, J = 7 Hz, H_5), 3.94 (1 H, d, J = 7 Hz, H_6), 3.94 (1 H, s, 7-OH), 3.70–3.60 (1 H, m, H_{17}), 3.70 (1 H, dq, J = 9, 6 Hz, $H_{5''}$), 3.55–3.30 (3 H, m, $H_{3'}$, $H_{3''}$, and $H_{5'}$), 3.44 (1 H, d, J = 10 Hz, H_{13}), 3.43 (1 H, dd, J = 10, 2 Hz, H_{25}), 3.39 (3 H, s, OCH_3), 3.33 (3 H, s, OCH_3), 3.26 (1 H, q, J = 2 Hz, H_2), 3.17 (1 H, t, J = 9 Hz, $H_{4'}$), 3.13 (1 H, t, J = 9 Hz, $H_{4''}$), 2.48 (1 H, br s, 4″-OH), 2.44–2.34 (1 H, m, H_{12}), 2.34 (1 H, d, J = 7 Hz, 5-OH), 2.30–2.20 (5 H, m, H_{16}, H_{24}, $H_{2'eq}$, and $H_{2''eq}$), 2.01 (1 H, ddd, J = 12, 5, 1 Hz, H_{20eq}), 1.85 (3 H, br s, H_{4a}), 1.71–1.66 (1 H, m, H_{18eq}), 1.56 (3 H, br s, H_{14a}), 1.60–1.40 (6 H, m, H_{20ax}, H_{26}, H_{27}, $H_{2'ax}$, and $H_{2''ax}$), 1.23 (3 H, d, J = 6 Hz, $H_{6''}$), 1.08 (3 H, d, J = 6 Hz, $H_{6'}$), 1.04 (3 H, d, J = 6 Hz, H_{12a}), 0.94 (3 H, t, J = 7 Hz, H_{28}), 0.89 (3 H, d, J = 7 Hz, H_{24a}), 0.89 (3 H, d, J = 7 Hz, H_{26a}). Anal. ($C_{48}H_{72}O_{14}\cdot H_2O$) C, H. **Data for 16:** Partial ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.80–5.70 (3 H, m, H_9, H_{10}, and H_{22}), 5.52 (1 H, dd, J = 10, 3 Hz, H_{23}), 5.39 (1 H, br s, H_3), 5.37 (1 H, d, J = 3 Hz, $H_{1''}$), 5.36–5.24 (2 H, H_{11} and H_{19}), 5.19 (1 H, dd, J = 9, 7 Hz, H_{15}), 4.65 (2 H, br s, H_{8a}), 4.26 (1 H, br t, J = 7 Hz, H_5), 4.25 (1 H, br d, J = 9 Hz, $H_{1'}$), 3.94 (1 H, d, J = 7 Hz, H_6), 3.82–3.56 (2 H, m, $H_{5''}$ and H_{17}), 3.75 (1 H, s, 7-OH), 3.74 (1 H, d, J = 10 Hz, H_{13}), 3.47–3.17 (3 H, m, $H_{3'}$, $H_{3''}$, and $H_{5'}$), 3.44 (1 H, dd, J = 10, 2 Hz, H_{25}), 3.35 (3 H, s, OCH_3), 3.30 (3 H, s, OCH_3), 3.25 (1 H, q, J = 2 Hz, H_2), 3.22 (1 H, t, J = 9 Hz, $H_{4'}$), 3.12 (1 H, t, J = 9 Hz, $H_{4''}$), 1.75 (3 H, br s, H_{4a}), 1.47 (3 H, br s, H_{14a}), 1.28 (3 H, d, J = 6 Hz, $H_{6''}$), 1.24 (3 H, d, J = 6 Hz, $H_{6'}$), 1.08 (3 H, d, J = 6 Hz, H_{12a}), 0.95 (3 H, t, J = 7 Hz, H_{28}), 0.90 (3 H, d, J = 7 Hz, H_{24a}), 0.90 (3 H, d, J = 7 Hz, H_{26a}). Anal. ($C_{48}H_{72}O_{14}\cdot H_2O$) C, H.

Acknowledgment. The authors thank Dr. Phil Eskola, Mr. Joe Leone, Dr. Dong Ok, and Mr. Frank Waksmunski for technical assistance. We thank Dr. Larry Colwell for mass spectra.