Synthesis of 1,19-Aza-1,19-desoxy-avermectin B_{1a}: The First Avermectin Macrolactam

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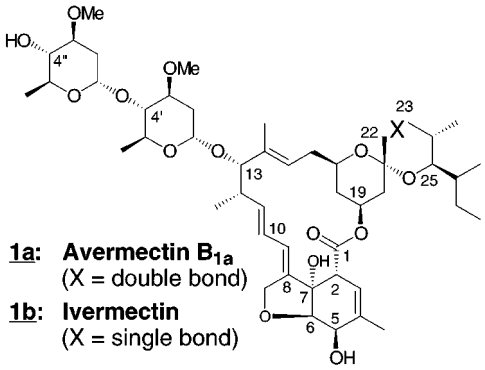
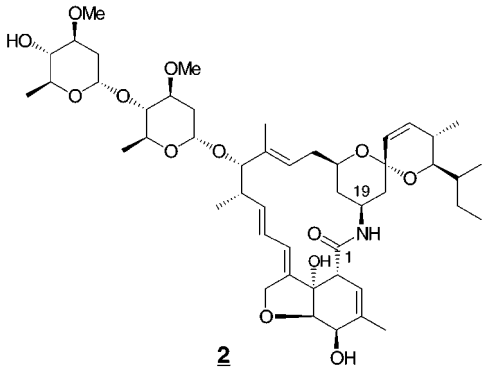
The first synthesis of 1,19-aza-1,19-desoxy-avermectin B_{1a} (**2**) is described. This new macrolactam, prepared efficiently from avermectin B_{1a} (**1a**) in seven steps, was designed to form an intramolecular hydrogen bond between the amide carbonyl and the adjacent C7 tertiary hydroxyl via a six-center hydrogen bonding network. The presence of this intramolecular hydrogen bond is anticipated to confer additional conformational rigidity to the 16-membered macrocycle.

The natural product avermectin B_{1a} (AVM, **1a**) and its semisynthetic analogue, ivermectin (IVM, **1b**), are two of the most important members of the avermectin macrolactone family. Their discovery in the late 1970s ushered in a new era in the treatment of endo- and ectoparasites of livestock and companion animals.[1] Ivermectin additionally has important human clinical uses, specifically for the treatment of individuals afflicted with onchocerciasis[1] (River Blindness). The high intrinsic potency and complex chemical structure of these unusual macrolides sparked intense interest in the scientific community.[2] Major biological advances reported include mode of action studies[3a] as well as the identification[3b] and cloning[3c] of the AVM binding proteins present in *Caenorhabditis elegans*. Extensive synthetic efforts to identify biologically interesting, structurally modified avermectins with enhanced spectrum of parasite control also have been described.[1,2] Our interest in this family of complex natural products has led to the preparation of 1,19-aza-1,19-desoxy-avermectin B_{1a} (**2**), the first example of an avermectin macrolactam and is the subject of the present communication.





2

The impetus for the design and synthesis of macrolactam **2** was provided by conflicting spectroscopic evidence. The presence of an intramolecular hydrogen bond between the C1-carbonyl and the C7-tertiary hydroxyl was inferred by Springer et al. in their study[4] of the single-crystal X-ray structure of avermectin B_{1a}, based on the 2.93 Å distance between the two oxygen atoms in question. The authors also noted that, on the basis of detailed ^{1}H NMR analyses, the solution conformation of the basic avermectin skeleton is "virtually identical" with its solid state conformation. In contrast, Neszmelyi et al.,[5] who studied the solution conformation of AVM by 600 MHz ^{1}H NMR, did not observe this intramolecular hydrogen bond and postulated[5] that the extensive hydrogen-bonding network reported by Springer et al.[4] which fixed the AVM molecules in the crystal lattice was less significant in solution.

While the presence of such an internal hydrogen bond has not been rigorously established, if present it would occur via a highly favorable six-center array. This concept is illustrated schematically in the partial structures shown in Figure 1. A weak intramolecular hydrogen bond would not inhibit rotation of the C1 carbonyl, resulting in an equilibration that could be envisaged as interchanging freely between **3a** and **3b**, subject prima-

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(1) 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; Chapter 21, pp 311−323.

(2) For leading references to chemical and biological studies, see: (a) Blizzard, T. A. *Org. Prep. Proc. Int.* **1994**, *26*, 617−644. (b) Davies, H. G.; Green, R. H. *Chem. Soc. Rev.* **1991**, *20*, 211−270. (c) Davies, H. G.; Green, R. H. *Chem. Soc. Rev.* **1991**, *20*, 271−339.

(3) (a) Arena, J. P.; Liu, K. K.; Paress, P. S.; Schaeffer, J. M.; Cully, D. F. *Mol. Brain Res.* **1992**, *15*, 339−348. (b) Rohrer, S. P.; Meinke, P. T.; Hayes, E. C.; Mrozik, H.; Schaeffer, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4168−4172. (c) Cully, D. F.; Vassilatis, D. K.; Liu, K. K.; Paress, P. S.; Van der Ploeg, L. H. T.; Schaeffer, J. M.; Arena, J. P. *Nature* **1994**, *371*, 707−711.

(4) Springer, J. P.; Arison, B. H.; Hirshfield, J. M.; Hoogsteen, K. *J. Am. Chem. Soc.* **1981**, *103*, 4221−4224.

(5) Neszmelyi, A.; Machytka, D.; Kmety, A.; Sandor, P.; Lukacs, G. *J. Antibiot.* **1989**, *42*, 1494−1500.

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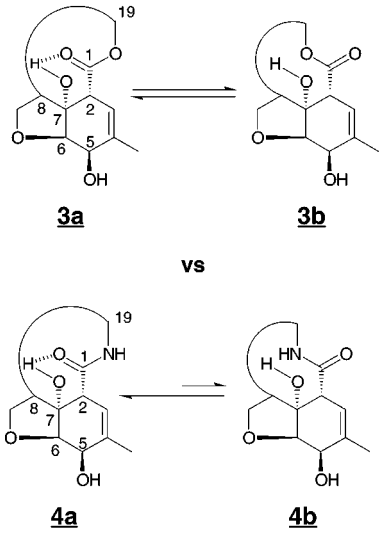


Figure 1. Macrolactone and macrolactam hydrogen bonding equilibria.

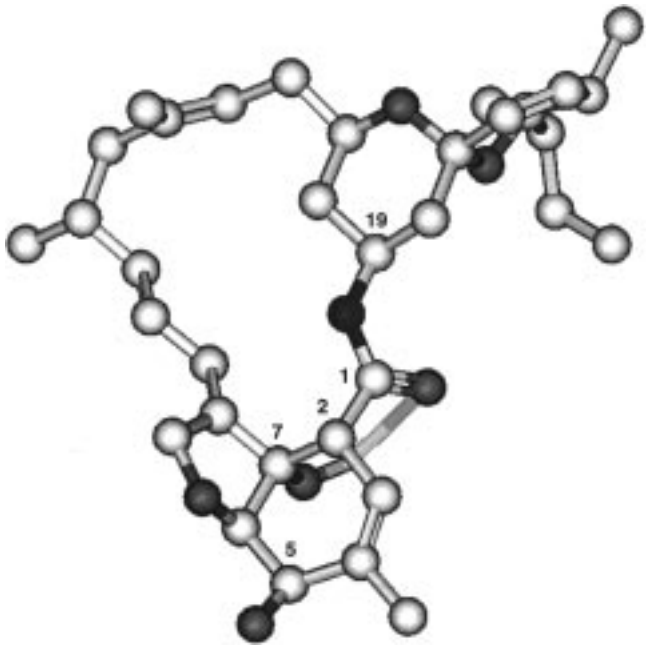


Figure 2. Intramolecular hydrogen bonding network for macrolactam **2**.

rily to nonbonded interactions. Intramolecular hydrogen bonding interactions clearly would be significantly enhanced for macrolactam **2**, introducing a conformational bias favoring **4a** over **4b** by overwhelming other factors. Consequently, it was of interest to synthesize macrolactam **2** and probe the effects induced by amide incorporation into the macrocyle.

Molecular modeling experiments using both single-crystal X-ray coordinates[4] and NMR-derived solution coordinates[5] provided the starting points for calculations which were performed on the 13-desoxy aglycone of **2** (the disaccharide was omitted for computational simplicity). Conformers generated using JIGGLE[6] subsequently were minimized using BATCHMIN.[7] These calculations supported the premise that incorporation of an amide linkage into AVM would result in a structure able to form a strong intramolecular hydrogen bond between the amide carbonyl and the adjacent tertiary hydroxyl; this structure is shown in Figure 2. Inspection of the computationally generated conformers also indicated that no other significant conformational rearrangements occurred upon amide introduction.

The synthetic pathway employed for the preparation of macrolactam **2** is illustrated in Scheme 1. Bis-silylated AVM **5**[8] was subjected to Seebach transesterification[9] with $Ti(OiPr)_4$ in β-(trimethylsilyl)ethanol to generate secoester **6**. The specific reaction conditions employed were those described by Julia et al.[10] during their total synthesis of 22,23-dihydroavermectin B_{1b} aglycone. The yield of this reaction was 77% and was amenable to scale-up (10 g) with only nominal decrease in yield (70%). No loss of stereochemical integrity at the labile C2 stereocenter was detected, nor did desilylation, deglycosylation, or isomerization of the $\Delta^{3,4}$-olefin into conjugation occur. Oxidation of the C19 hydroxyl of **6** subsequently was probed with a variety of oxidants (PCC, PDC, Swern, modified Pfitzner−Moffatt[11] and Dess−Martin periodinane[12]). It was quickly determined that the pyridine-buffered variant[13] of the Dess−Martin oxidation was both the most reproducible and highest yielding of the methods examined, particularly upon scale-up. Ultimately, 5.5 g of the 19-oxo intermediate **7** was synthesized in 78% chemical yield from **6**.

Reductive amination at C19 of **7** proceeded smoothly, presumably via imine **8**, using excess $(TMS)_2NH$ with anhydrous $ZnCl_2$ in EtOAc.[11b] The imine thus generated was not isolated but instead was subjected directly to $NaBH_4$ reduction. The imine reduction produced an inseparable mixture (approximately 1:1) of C19-epimers based on 500 MHz ^1H NMR integration. To ensure that the labile C2 stereocenter was unaffected, a portion of the C19-amino epimeric mixture (**9**) was exhaustively acetylated with acetic anhydride in pyridine yielding diastereomers **10a** and **10b**, which were carefully separated. As shown by 500 MHz ^1H NMR, the C2-methine in each clearly was unchanged.

Originally, all three silyl protecting groups were to be removed in a single chemical transformation. Surprisingly, HF·pyridine,[14] which readily cleaved the 4″- and 5-O-TBDMS ethers of **9**, did not remove the β-silylethyl ester even after protracted reaction times (>4 days). Similarly, buffered $^nBu_4NF{\cdot}3H_2O$ (TBAF, 3:1 $^nBu_4NF{\cdot}3H_2O$:TsOH),[10] which successfully unmasked the C1-

(6) Kearsley, S. JIGGLE − Distance Geometry program, Merck Research Laboratories, unpublished.

(7) Mohamidi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(8) Blizzard, T. A.; Marino, G.; Mrozik, H.; Fisher, M. H. *J. Org. Chem.* **1989**, *54*, 1756−1757.

(9) Seebach, D.; Hungerbuhler, E.; Naef, R.; Schnurrenberger, P.; Weidmann, B.; Zuger, M. *Synthesis* **1982**, 138−141.

(10) (a) Ferezou, J. P.; Julia, M.; Khourzom, R.; Pancrazi, A.; Robert, P. *Synlett* **1991**, 614−617. (b) Ferezou, J. P.; Julia, M.; Liu, L. W.; Pancrazi, A. *Synlett* **1991**, 618−620 (c) Ferezou, J. P.; Julia, M.; Liu, L. W.; Pancrazi, A. *Bull. Soc. Chim. Fr.* **1995**, 428−452.

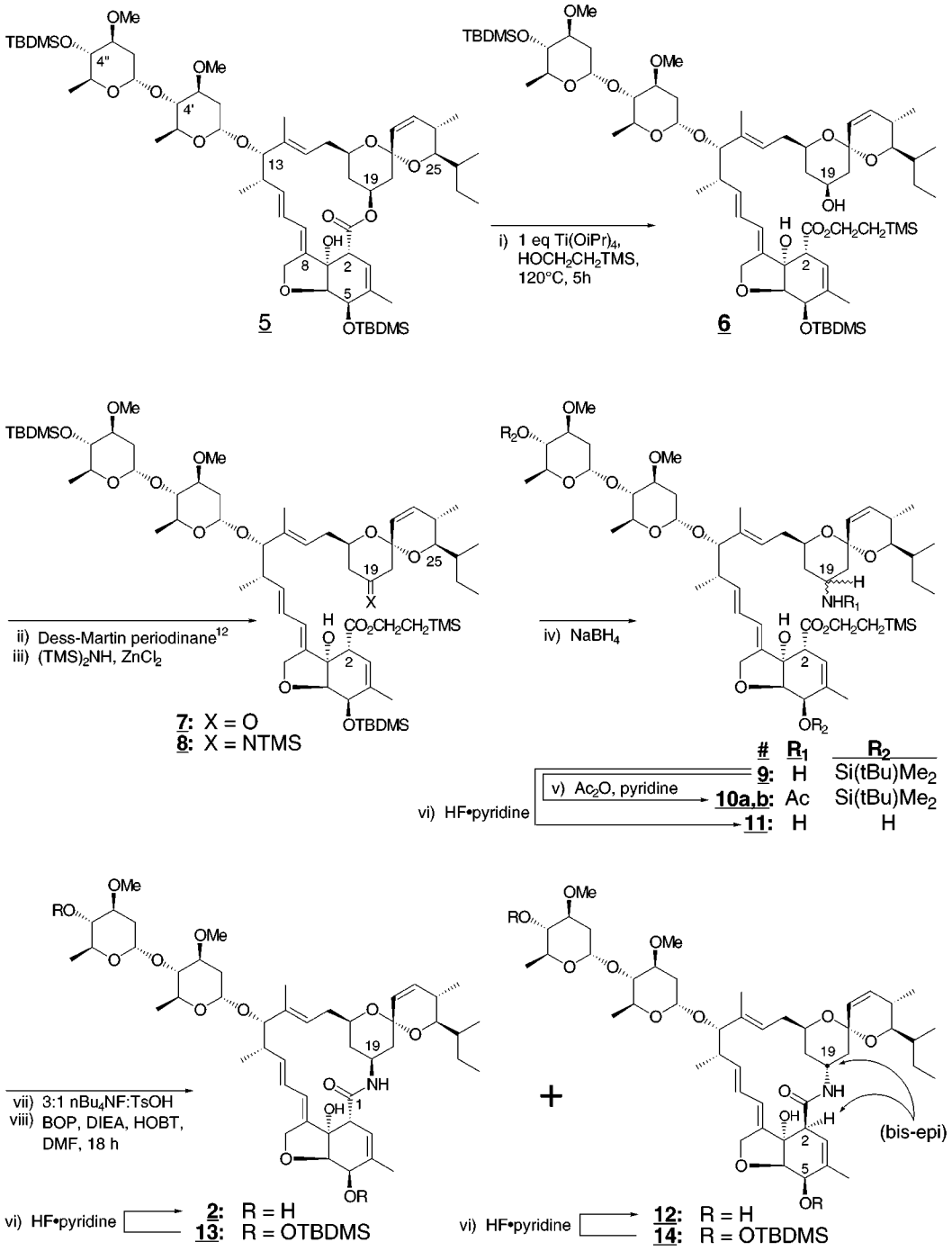
(11) (a) Liu, H.-J.; Nyangulu, J. M. *Tetrahedron Lett.* **1988**, *29*, 3167−3170. (b) Cvetovich, R. J.; Kelly, D. H.; DiMichele, L. M.; Shuman, R. F.; Grabowski, E. J. J. *J. Org. Chem.* **1994**, *59*, 7704−7708.

(12) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155−4166.

(13) Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 7001−7031.

(14) (a) Trost, B. A.; Caldwell, C. G.; Murayama, E.; Heissler, D. *J. Org. Chem.* **1983**, *48*, 3252−3265. (b) Meinke, P. T.; Rohrer, S. P.; Hayes, E. C.; Schaeffer, J. M.; Fisher, M. H.; Mrozik, H. *J. Med. Chem.* **1992**, *35*, 3879−3884.

Scheme 1



carboxylic acid of **9**, did not affect the two TBDMS protecting groups. Consequently, the 4″- and 5-OTBDMS groups of **9** were removed with HF·pyridine, and the C1-carboxylic acid subsequently was liberated with buffered TBAF. The crude carboxylic acid was not purified but instead was subjected directly to macrocyclization with (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP)[15] under high dilution conditions (0.0018 mM in DMF) to form the desired macrolactam **2** (33%) in addition to the undesired 2,19-bis-*epi* analogue **12** (28%). The two other possible epimers (the 2-*epi* analogue and the 19-*epi* analog) and the conjugated $\Delta^{2,3}$-olefin isomers were not observed in the reaction mixture.

Alternatively, the C1-acid of **9**, uncovered using buffered TBAF, could be subjected to BOP-mediated macrocyclization. As before, cyclization yielded two readily separable products, the desired 4″,5-bis-O-TBDMS-1,19-aza-1,19-desoxy-avermectin B_{1a} (**13**) (21%) and its 2,19-bis-*epi* analogue **14** (14%). Deprotection with HF·pyridine[14] subsequently formed 1,19-aza-1,19-desoxy-avermectin B_{1a} (**2**) and 1,19-aza-2,19-bis-*epi*-1,19-desoxy-avermectin B_{1a} (**12**), respectively.

The structure and stereochemical assignments of the product macrolactams **2** and **12** were determined following examination of their 500 MHz proton NMR spectra.

(15) (a) Felix, A. M.; Wang, C.-T.; Heimer, E. P.; Fournier, A. *Int. J. Peptide Res.* **1988**, *31*, 231−238. (b) Castro, B.; Dormoy, J. R.; Evin, G.; Sleve, C. *Tetrahedron Lett.* **1975**, *16*, 1219−1222.

For example, the presence of the amide NH of **2** was an unmistakable doublet centered at 7.25 ppm. The C7 tertiary hydroxyl proton, typically a sharp singlet at 4.5 ppm in AVM, was shifted 2.3 ppm downfield to 6.8 ppm, strongly indicative of the formation of the desired intramolecular hydrogen bonding between the amide carbonyl and the proximal hydroxyl.

The stereochemistry at C19 was established by measuring the width at half-height of the C19-methine proton in a decoupling experiment. In **2**, for instance, the width at half-height was 27 Hz, clearly indicating an axial proton with three large couplings and the natural stereochemistry. This is in direct contrast to macrolactam **12**, where in an identical experiment the presence of an epimeric, equatorial hydrogen at C19 was inferred by the narrow, 11 Hz peak width that was observed after removal of the NH coupling during a double irradiation experiment. The latter experiment indicated that all of the couplings between C19 and its C18 and C20 neighbors are small.

Similarly, the C2 stereochemistry of **2** and **12** also was deduced from their proton NMR spectra. In **2**, the series of small coupling constants that characterize the C2-multiplet closely resembles that of AVM B_{1a}, strongly suggesting that the natural stereochemistry had been retained. In addition, the 4a-methyl singlet of macrolactam **2** is at 1.80 ppm versus 1.95 ppm for **12**. In 2-*epi*-AVM analogues, this downfield shift is consistent with inversion of stereochemistry at C2. Compelling evidence for the presence of an epimeric C2-methine in macrolactam **12** also was provided by the 3.3 ppm downfield displacement of the 5-hydroxy signal relative to its chemical shift in AVM B_{1a}. This indicated that the hydroxyl participates in a strong intramolecular hydrogen bond which can only occur when the hydroxyl and the amide carbonyl are on the same side of the six-membered ring. The appearance of H-2 as a slightly broadened doublet ($J = 6.5$ Hz) further supports the 2-epi assignment since the increase in $J_{2,3}$ from about 2 Hz in AVM B_{1a} translates to approximately a 25−35° change in the H2−H3 dihedral angle. Additional confirmatory evidence for the inversion at C2 was obtained from NOE difference experiments. Irradiation of H5 elicited an NOE signal from H2 and irradiation of H2 resulted in a NOE signal from H6. These findings necessitate that H2 is on the same side of the six-membered ring as H5 and H6. Molecular modeling experiments indicated that in the 2,19-bis-*epi* analogue **12**, an intramolecular hydrogen bond indeed would form between the amide carbonyl and the C5-hydroxyl, as illustrated in Figure 3.

The absence of 2-*nat*-1,19-aza-1,19-desoxy-19-*epi*-AVM B_{1a} from the cyclization reaction is not unprecedented. Hanessian and Chemla synthesized 19-*epi*-AVM A_1 and noted that its C2 center was much more prone to epimerization than the parent avermectin.[16] They estimated, using computational techniques, that 2,19-bis-*epi*-AVM A_1 aglycone was at least 5 kcal/mol more stable than the corresponding 19-*epi* analogue. Similar calculations indicated that bis-*epi* **12** was more stable than its 2-*nat*-19-*epi* antipode by approximately 2 kcal/mol[17] and ostensibly **12** formed after macrolactamization. In addition, no $\Delta^{2,3}$-conjugated products were isolated from this reaction.

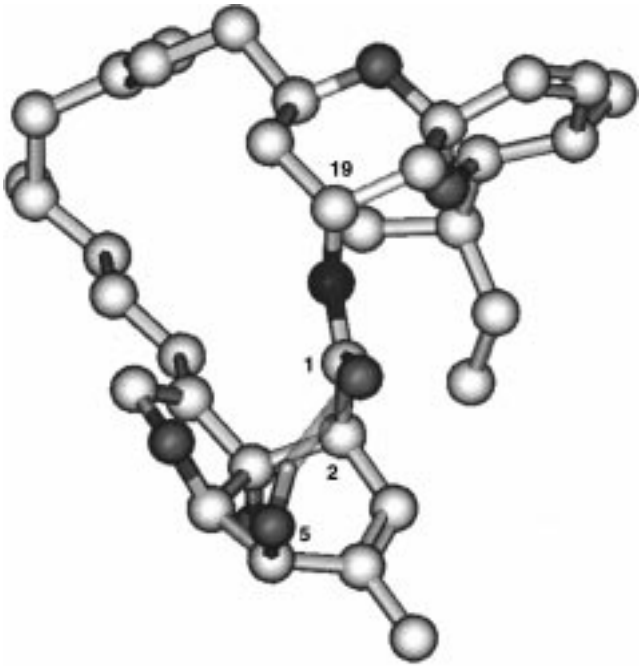


Figure 3. Intramolecular hydrogen bonding network of macrolactam **12**.

In summary, an efficient seven-step synthesis of 1,19-aza-1,19-desoxy-avermectin B_{1a} from avermectin B_{1a} has been described. Macrolactam **2**, the first example of an avermectin macrolactam, formed a strong intramolecular hydrogen-bonding network between the amide carbonyl and the adjacent tertiary C7-hydroxy, imparting additional conformational rigidity to the macrocyle.

Experimental Section

General Experimental. All moisture- and air-sensitive reactions were performed under a positive pressure of purified nitrogen. All solvents and reagents were dried or distilled prior to use. Analytical thin-layer chromatography (TLC) was conducted using Analtech (250 μm) plates. Preparative thin-layer chromatography was conducted using Analtech (1500 or 2000 μm) plates. Flash chromatography was performed using 230−400 mesh silica gel (EM Merck). HPLC chromatography was performed using a Zorbax RX-8 4.6 × 250-mm column. All compounds were purified to homogeneity as determined by TLC and/or reversed-phase HPLC.

4″,5-Bis-*O*-(*tert*-butyldimethylsilyl)-1-*O*-(2-(trimethylsilyl)ethyl)avermectin B_{1a} 1,19-Secoester (6). To **5** (600 mg, 545 μmol) in $HOCH_2CH_2SiMe_3$ (6 mL) at room temperature was added $Ti(OiPr)_4$ (162 μL, 545 μmol). The solution was heated at 120 °C for 6 h. The reaction crude was partially purified on a 3 in. plug of silica gel using 1:1 hexanes/EtOAc as eluant. The solvent and excess $HOCH_2CH_2SiMe_3$ were removed in vacuo. Secoester **6** (602 mg, 77%) was obtained following flash chromatography on silica gel using 1:1 hexanes: EtOAc as eluant. TLC: 85:15 hexanes/EtOAc, $R_f = 0.28$. MS ($M + NH_4$): 1237.1.

^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 6.05 (1H, br s), 5.83 (2H, m), 5.69 (1H, dd, $J = 1.7$, 10.0 Hz), 5.52 (1H, dd, $J = 2.5$, 9.9 Hz), 5.37 (1H, br s), 5.30 (2H, m), 4.85 (1H, br s), 4.70 (1H, d, $J = 3.0$ Hz), 4.61 (2H, s), 4.49 (1H, br s), 4.14−4.25 (3H, m), 4.00−4.07 (1H, m), 3.88 (1H, d, $J = 4.8$ Hz), 3.77 (1H, d, $J = 6.4$ Hz), 3.62−3.72 (3H, m), 3.52−3.60 (1H, m), 3.35 (3H, s), 3.30 (3H, s), 3.17 (1H, t, $J = 9.1$ Hz), 3.11 (1H, t, $J = 8.8$ Hz), 1.77 (3H, s), 1.50 (3H, s), 1.19 (3H, d, $J = 6.2$

(16) Hanessian, S.; Chemla, P. *Tetrahedron Lett.* **1991**, *32*, 2719−2722.

(17) Similar calculations[7] performed in this laboratory indicated that 2,19-bis-*epi*-AVM was approximately 2 kcal/mol (not 5 kcal/mol) more stable than 19-*epi*-AVM, a value consistent with that determined for bis-*epi* macrolactam **12**.

Hz), 1.18 (3H, d, J = 6.3 Hz), 0.90 (9H, s), 0.87 (9H, s), 0.10 (6H, s), 0.07 (3H, s), 0.05 (3H, s), 0.02 (9H, s).

4″,5-Bis-*O*-(*tert*-butyldimethylsilyl)-19-oxo-1-*O*-(2-(trimethylsilyl)ethyl)avermectin B_{1a} 1,19-Secoester (7). To alcohol **6** (1.15 g, 955 μmol) in CH_2Cl_2 (10 mL) at 25 °C buffered with pyridine (500 μL) was added Dess−Martin reagent (485 mg, 1.15 mmol).[12] After 30 min, additional Dess−Martin reagent (485 mg, 1.15 mmol) was added. After 2 h, the solution was poured into 1:1 saturated aqueous $NaHCO_3$/saturated aqueous $Na_2S_2O_3$, the aqueous layer extracted with CH_2Cl_2, and the organic layer dried ($MgSO_4$). The solution was filtered and concentrated in vacuo. The crude mixture was purified by flash chromatography on silica gel using 1:9 acetone/hexanes as eluant to yield ketone **7** (892 mg, 78%) as a pale yellow foam. TLC: 85:15 hexanes/EtOAc, R_f = 0.44. MS (M − 2Si(tBu)Me_2 + NH_4): 1006.9.

^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 6.06 (1H, br s), 5.85 (1H, s), 5.84 (1H, d, J = 8.2 Hz), 5.76 (1H, dd, J = 1.6, 10.1 Hz), 5.61 (1H, dd, J = 2.5, 9.9 Hz), 5.37 (2H, br s), 5.31 (1H, d, J = 3.7 Hz), 4.69 (2H, s), 4.61 (2H, s), 4.49 (1H, br s), 4.12−4.25 (2H, m), 4.04−4.12 (1H, m), 3.89 (1H, d, J = 4.8 Hz), 3.62−3.72 (3H, m), 3.51−3.58 (1H, m), 3.35 (3H, s), 3.30 (3H, s), 3.17 (1H, t, J = 9.0 Hz), 3.10 (1H, t, J = 8.7 Hz), 1.77 (3H, s), 1.56 (3H, s), 1.19 (3H, d, J = 5.9 Hz), 1.17 (3H, d, J = 5.7 Hz), 0.90 (9H, s), 0.87 (9H, s), 0.81 (3H, d, J = 6.6 Hz), 0.10 (6H, s), 0.07 (3H, s), 0.05 (3H, s), 0.02 (9H, s).

19-Amino-4″,5-bis-*O*-(*tert*-butyldimethylsilyl)-19-desoxy-1-*O*-(2-(trimethylsilyl)ethyl)avermectin B_{1a} 1,19-Secoester (9). To 19-oxo **7** (200 mg, 165 μmol) in dry EtOAc (5 mL, 38 μg H_2O/mL) at 25 °C were added sequentially $(Me_3Si)_2NH$ (165 μL, 787 μmol) and $ZnCl_2$ (29 mg, 215 μmol). The solution was heated at 45 °C for 75 min and then cooled to 0 °C, and $NaBH_4$ (25 mg, 665 μmol) was added. After 5 min at 0 °C and 30 min at room temperature, the solution was poured into saturated aqueous NaCl and extracted with EtOAc and the organic layer dried ($MgSO_4$). The solution was filtered and concentrated under reduced pressure. Amines **9** (151 mg, 76%) were obtained as an inseparable mixture of C19-epimers (∼1:1 by 500 MHz ^{1}H NMR) following flash chromatography on silica gel using a gradient solvent system (1:3, then 4:6, then 1:1 acetone/hexanes, ∼200 mL of each). TLC: 1:3 acetone/hexanes, R_f = 0.33. MS (M + 1): 1219.2.

19-(N-Acetylamino)-4″,5-bis-*O*-(*tert*-butyldimethylsilyl)-19-desoxy-1-*O*-(2-(trimethylsilyl)ethyl)avermectin B_{1a} 1,19-Secoester (10a and 10b). To amine **9** (20 mg, 16 μmol) in methylene chloride (2 mL) at 25 °C were added pyridine (250 μL) and DMAP (2 mg) followed by acetic anhydride (100 μL). After 15 min, the reaction was quenched with saturated aqueous $NaHCO_3$ (2 mL), extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by preparative thin-layer chromatography (2000 μm silica gel plate) using 15:85 acetone/hexanes as eluant (three developments). Two C19-diastereomers were obtained from this reaction: mobile product **10a** (9 mg, 39%, R_f = 0.46) and polar product **10b** (8 mg, 38%, R_f = 0.43, MS (M + NH_4): 1278.2) in 2:8 acetone/hexanes, two developments.

^{1}H NMR (500 MHz, $CDCl_3$, partial data for **10a**) δ 7.24 (1H, d, J = 7.5 Hz), 6.08 (1H, d, J = 10.1 Hz), 5.80−5.91 (2H, m), 5.70 (1H, d, J = 9.8 Hz), 5.48 (1H, dd, J = 2.5, 10.1 Hz), 5.42 (1H, t, J = 7.1 Hz), 5.30 (2H, br s), 4.72 (1H, d, J = 5.5 Hz), 4.69 (1H, d, J = 3.5 Hz), 4.54 (1H, d, J = 13.9 Hz), 4.39 (1H, d, J = 13.9 Hz), 4.20−4.30 (2H, m), 4.05−4.18 (2H, m), 3.81−3.87 (1H, m), 3.54−3.68 (3H, m), 4.95−3.52 (3H, m), 3.32 (3H, s), 3.28 (3H, s), 3.15 (1H, t, J = 9.1 Hz), 3.10 (1H, t, J = 8.7 Hz), 1.93 (3H, s), 1.73 (3H, s), 1.47 (3H, s), 1.18 (3H, d, J = 6.2 Hz) 1.17 (3H, d, J = 6.1 Hz), 0.96 (3H, t, J = 7.4 Hz), 0.91 (9H, s), 0.86 (9H, s), 0.12 (3H, s), 0.10 (3H, s), 0.06 (9H, s), 0.02 (6H, s).

^{1}H NMR (500 MHz, $CDCl_3$, partial data for **10b**) δ 7.41 (1H, d, J = 7.3 Hz), 6.04 (1H, br s), 5.81−5.88 (2H, m), 5.79 (1H, d, J = 10.0 Hz), 5.48 (1H, dd, J = 2.5, 101 Hz), 5.35−5.44 (1H, m), 5.36 (1H, br s), 5.30 (1H, d, J = 3.7 Hz), 4.79 (1H, s), 4.66 (1H, d, J = 3.2 Hz), 4.61 (2, br s), 4.48 (1H, br s), 4.25 (1H, br s), 4.08−4.22 (2H, m), 3.89 (1H, d, J = 5.0 Hz), 3.84 (1H, m), 3.45−3.68 (5H, m), 3.33 (3H, s), 2.89 (3H, s), 3.15 (1H, t, J = 9.1 Hz), 3.09 (1H, t, J = 8.9 Hz), 1.92 (3H, s), 1.76 (3H, s), 1.17 (3H, d, J = 6.2 Hz), 1.16 (3H, d, J = 6.2), 0.95 (3H, t, J = 7.4 Hz), 0.89 (9H, s), 0.86 (9H, s), 0.09 (3H, s), 0.08 (3H, s), 0.06 (3H, s), 0.04 (3H, s), 0.01 (9H, s).

19-Amino-19-desoxy-1-*O*-(2-(trimethylsilyl)ethyl)avermectin B_{1a} 1,19-Secoester (11). To bis-silyl ether **9** (234 mg, 195 μmol) in THF (5 mL) at 25 °C was added HF·pyridine solution (1 mL, prepared using 25 g of HF·pyridine, 10 mL of pyridine, 25 mL of THF). The solution was stirred for 48 h and then cooled to 0 °C. Pyridine (3 mL) was added and the solution then poured into 1:1 H_2O/Et_2O (50 mL). The layers were separated and neutralized separately with saturated aqueous $NaHCO_3$. The combined aqueous layers were extracted with Et_2O, and then the combined organic layers were washed with saturated aqueous NaCl and dried ($MgSO_4$). The solution was filtered and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel using 1:4:95 NH_4OH/MeOH/$CHCl_3$ as eluant to yield **11** (155 mg, 83%) as a colorless glass. TLC: 1:9:90 NH_4OH/MeOH/$CHCl_3$, R_f = 0.28. MS (M + 1): 991.0.

^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 6.08 (m), 5.86 (m), 5.68 (d, J = 8.5 Hz), 5.47 (d, J = 9.9 Hz), 5.42 (t, J = 7.2 Hz), 5.37 (s), 5.31 (s), 4.72 (br s), 4.61 (s), 4.54 (d, J = 13.7 Hz), 4.49 (br s), 4.41 (d, J = 10.1 Jz), 4.24 (d, 5.9 Hz), 4.18 (m), 4.11 (q, J = 9.6 Hz), 4.01 (m), 3.88 (d, J = 4.8 Hz), 3.60−3.71 (m), 3.50−3.58 (m), 3.47 (s), 3.45 (s), 3.35 (s), 3.34 (s), 3.30 (s), 3.28 (s), 3.24 (br s), 3.17 (t, J = 9.4 Hz), 3.11 (t, J = 8.7), 1.77 (s), 1.74 (s), 1.69 (br s), 1.49 (s), 1.19 (m), 0.91 (s), 0.90 (s), 0.87 (s), 0.86 (s), 0.10 (s), 0.09 (s), 0.07 (s), 0.06 (s), 0.05 (s), 0.02 (s), 0.01 (s).

1,19-Aza-1,19-desoxy-avermectin B_{1a} (2) and 1,19-Aza-2,19-bis-*epi*-1,19-desoxy-avermectin B_{1a} (12). Secoester **11** (100 mg, 101 μmol) was dissolved in THF (5 mL) at 0 °C. To this was added a stock solution of *n*-$Bu_4NF·3H_2O$ (95 mg, 303 μmol) and *p*-toluenesulfonic acid (17.5 mg, 101 μmol) in THF (2 mL) to achieve a 1:1:3 ratio of secoester/TsOH/TBAF. After 15 min at 0 °C and 11 h at 25 °C, the reaction crude was poured into 1:1 saturated aqueous NaCl/saturated aqueous $NaHCO_3$ (20 mL), extracted with EtOAc, and dried ($MgSO_4$). The solvent was removed at ambient temperature under reduced pressure, and the crude product was lyophilized from benzene. The lyophilate was dissolved in dry DMF (55 mL, 113 μg H_2O/mL) and cooled to 0 °C. To the crude secoacid was sequentially added HOBT (68 mg, 505 μmol), DIEA (90 μL, 505 μmol), and BOP (207 mg, 207 μmol). The solution was aged for 5 h at 0 °C, and then 16 h at 25 °C. The solution was poured into 1:1 saturated aqueous NaCl/saturated aqueous $NaHCO_3$ (100 mL), extracted with CH_2Cl_2, and dried (Na_2SO_4). The organic layer was filtered, and solvents were removed at ambient temperature under reduced pressure (high vacuum was used to remove residual DMF). The crude was purified by flash chromatography on silica gel using gradient elution (0.3:2.7:97, 0.5:4.5:95, and then 0.7:5.3:93 NH_4OH/MeOH/$CHCl_3$, one column volume each) to yield macrolactam **2** (29 mg, 33%) and bis-*epi*-analogue **12** (24 mg, 28%).

Data for 1,19-Aza-1,19-desoxy-avermectin B_{1a} (2): HPLC: 8:2 MeOH/H_2O, 2 mL/min, t_R = 6.8 min; TLC: 0.5:4.5:95 NH_4OH/MeOH/$CHCl_3$, R_f = 0.19; HRMS: calcd for $C_{48}H_{73}NO_{13}$ (M + Na): 894.4980, found 894.5056; $[\alpha]_D$ = +20.04° ± 0.1° (c = 0.815 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 7.43 (1H, d, J = 10.6 Hz), 6.39 (1H, s), 5.97 (1H, d, J = 10.3 Hz), 5.76 (1H, dd, J = 1.7, 10.1 Hz), 5.55−5.68 (2H, m), 5.47 (1H, dd, J = 2.6, 9.9 Hz), 5.37 (1H, d, J = 3.4 Hz), 5.21 (1H, s), 4.92 (1H, t, J = 7.7 Hz), 4.73 (1H, d, J = 3.2 Hz), 4.64 (2H, dq, J = 2.3, 14.4 Hz), 4.33 (1H, br s), 3.98 (1H, d, J = 5.9 Hz), 3.69−3.94 (5H, m), 3.54−3.66 (2H, m), 3.52 (1H, d, J = 10.5 Hz), 3.41 (3H, s), 3.40 (3H, s), 3.21 (1H, t, J = 9.0 Hz), 3.14 (1H, t, J = 9.1 Hz), 2.22−2.39 (4H, m), 2.15 (1H, dd, J = 1.8, 6.1 Hz), 1.91 (1H, m), 1.81 (3H, s), 1.41−1.79 (4H, m), 1.46 (3H, s), 1.24 (3H, d J = 7.5 Hz), 1.22 (3H, d, J = 6.8 Hz), 1.14 (1H, d, J = 7.9 Hz), 0.98 (3H, t, J = 8.1 Hz), 0.91 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.67, 144.43, 141.35, 139.28, 135.84, 134.53, 127.88, 125.70, 122.49, 121.61, 120.23, 98.68, 95.76, 94.05, 88.14, 81.10, 80.78, 79.51, 78.75, 78.29, 76.12, 74.61, 71.24, 69.22, 68.20, 67.28, 66.25,

57.76, 56.50, 56.48, 42.23, 39.46, 37.40, 35.42, 35.11, 35.05, 34.72, 34.32, 30.66, 28.16, 22.07, 19.21, 18.60, 17.77, 16.41, 14.60, 14.24, 12.37.

Data for 1,19-Aza-2,19-bis-*epi*-1,19-desoxy-avermectin B_{1a} (12): HPLC: 8:2 MeOH/H_2O, 2 mL/min, t_R = 7.1 min; TLC: 0.5:4.5:95 NH_4OH/MeOH/$CHCl_3$, R_f = 0.48; HRMS: calcd for $C_{48}H_{73}NO_{13}$ (M + H): 872.5160, found 872.5135; $[\alpha]_D$ = +43.96° ± 0.4° (c = 0.169 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$, partial data): δ 7.57 (1H, d, J = 8.9 Hz), 5.96 (1H, d, J = 10.8 Hz), 5.70−5.80 (2H, m), 5.54−5.66 (3H, m), 5.50 (1H, dd, J = 2.8, 10.1 Hz), 5.34 (1H, d, J = 3.5 Hz), 5.23 (1H, t, J = 8.1 Hz), 4.76 (1H, d, J = 2.5 Hz), 4.67 (2H, dq, J = 1.6, 13.5 Hz), 4.24 (1H, br s), 4.09 (1H, dd, J = 6.4, 11.0 Hz), 4.02 (1H, d, J = 6.2 Hz), 3.88 (1H, d, J = 9.4 Hz), 3.56−3.78 (5H, m), 3.37 (3H, s), 3.36 (3H, s), 3.20 (1H, t, J = 9.0 Hz), 3.16 (1H, d, J = 6.4 Hz), 3.13 (1H, t, J = 8.9 Hz), 2.62 (1H, br s), 2.47 (2H, m), 2.24−2.36 (3H, m), 2.12−2.21 (2H, m), 1.89 (3H, s), 1.71−1.78 (2H, m), 1.47 (3H, s), 1.24 (3H, d, J = 6.4 Hz), 1.23 (3H, d, J = 6.4 Hz), 1.15 (3H, d, J = 7.0 Hz), 1.10 (3H, t, J = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.89, 141.95, 137.93, 137.52, 136.49, 134.86, 127.60, 125.85, 125.44, 119.44, 119.28, 118.82, 98.53, 95.39, 94.21, 83.18, 82.09, 80.48, 79.56, 79.46, 79.37, 78.15, 76.06, 75.24, 73.49, 68.54, 68.01, 67.85, 67.13, 64.92, 56.60, 56.38, 46.24, 40.23, 38.80, 38.17, 35.73, 35.15, 34.96, 34.54, 34.15, 30.61, 29.65, 29.08, 28.05, 23.24, 23.13, 19.60, 19.53, 19.43, 18.40, 17.64, 16.51, 16.35, 15.42, 13.87, 12.12.

1,19-Aza-4″,5-bis-*O*-(*tert*-butyldimethylsilyl)-1,19-desoxy-avermectin B_{1a} (13) and 1,19-Aza-4″,5-bis-*O*-(*tert*-butyldimethylsilyl)-2,19-bis-*epi*-1,19-desoxy-avermectin B_{1a} (14). A stock solution of n-$Bu_4NF{\cdot}3H_2O$ (671 mg, 2.13 mmol) and p-toluenesulfonic acid (122 mg, 709 μmol), a 3:1 ratio of the two reagents, in anhydrous THF (10.5 mL) was prepared. To β-silylethyl ester **9** (151 mg, 126 μmol) in THF (7.5 mL) at 25 °C was added freshly prepared TsOH/TBAF stock solution (1.85 mL) to achieve a 1:1:3 ratio of silane/TsOH/TBAF. The solution, which turned red upon addition of the stock solution, was stirred for 15 h. The resultant pale yellow solution was poured into 1:1 saturated aqueous NaCl/saturated aqueous $NaHCO_3$ and extracted with EtOAc and the organic layer dried (Na_2SO_4). The organic layer was filtered and concentrated under reduced pressure at ambient temperature and the residue lyophilized from benzene. The lyophilate, without purification, was cooled to 0 °C in anhydrous DMF (75 mL, 218 μg H_2O/mL). To this solution were added sequentially HOBT (85 mg, 630 μmol), DIEA (113 μL, 630 μmol), and BOP (258 mg, 630 μmol). The solution was stirred at 0 °C for 4 h and then an additional 21 h at 25 °C. The DMF was removed at ambient temperature under high vacuum, and the crude was purified by preparative TLC (2 × 2000 μm, silica gel) using 1:3 acetone/hexanes as eluant to yield macrolactam **13** (29 mg, 21%) and 2,19-bis-*epi*-analogue **14** (19 mg, 14%) as colorless glasses. TLC: 3:7 acetone/hexanes, R_f = 0.60 for **13**, R_f = 0.38 for **14**.

1,19-Aza-4″,5-bis-*O*-(*tert*-butyldimethylsilyl)-1,19-desoxy-avermectin B_{1a} (13): ^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 7.04 (1H, d, J = 8.2 Hz), 5.92 (1H, d, J = 10.7 Hz), 5.74 (2H, m), 5.52 (2H, m), 5.41 (1H, br s), 5.27 (1H, d, J = 3.3 Hz), 5.23 (1H, t, J = 7.4 Hz), 4.76 (1H, d, J = 2.7 Hz), 4.48 (2H, q, J = 13.7 Hz), 4.46 (1H, s), 4.34 (1H, br s), 3.89 (1H, d, J = 3.9 Hz), 3.35 (3H, s), 3.31 (3H, s), 3.18 (1H, t, J = 9.1 Hz), 3.11 (1H, J = 8.8 Hz), 2.91 (1H, br s), 1.82 (3H, s), 1.48 (3H, s), 0.91 (9H, s), 0.87 (9H, s), 0.10 (3H, s), 0.09 (3H, s), 0.07 (3H, s), 0.05 (3H, s).

1,19-Aza-1,19-desoxy-avermectin B_{1a} (2). Bis-silyl ether **13** (10 mg, 12 μmol) in THF (4 mL) at 25 °C was deprotected using HF·pyridine solution (1 mL) as described above. The crude was purified by flash chromatography on silica gel using 9:1 EtOAc/acetone as eluant to yield **2** (5 mg, 63%) as a white powder following lyophilization from benzene. Macrolactam **2** was identical in all respects to the product previously obtained.

1,19-Aza-2,19-bis-*epi*-1,19-desoxy-avermectin B_{1a} (12). Bis-silyl ether **14** (24 mg, 22 μmol) in THF (4 mL) at 25 °C was deprotected using HF·pyridine solution (1 mL) as described above. The crude was purified by flash chromatography on silica gel using 9:1 EtOAc/acetone as eluant to yield **12** (8 mg, 43%) as a white powder following lyophilization from benzene. Macrolactam **12** was identical in all respects to the product previously obtained.

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Supporting Information Available: 500 MHz ^{1}H NMR spectra for compounds **2**, **6**, **7**, **9**, **10a**, **10b**, and **12** and 125 MHz ^{13}C NMR spectra for compounds **2** and **12** are available in addition to Cartesian coordinates for Figures 2 and 3 (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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