Nodulisporic Acid A, a Novel and Potent Insecticide from a *Nodulisporium* Sp. Isolation, Structure Determination, and Chemical Transformations

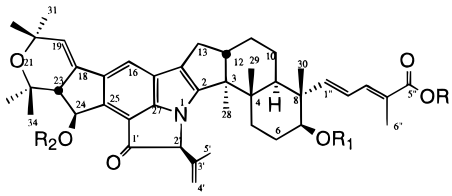
John G. Ondeyka,* Gregory L. Helms,* Otto D. Hensens,* Michael A. Goetz, Deborah L. Zink, Athanasios Tsipouras, Wesley L. Shoop, Lyndia Slayton, Anne W. Dombrowski, Jon D. Polishook, Dan A. Ostlind, Nancy N. Tsou, Richard G. Ball, and Sheo B. Singh*

Contribution from Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065

Received May 21, 1997[⊗]

Abstract: The potent insecticidal agent nodulisporic acid A (**1a**), representative of a new class of indole terpenes, was isolated from fermentations of a *Nodulisporium* sp. Nodulisporic acid A was active against the larvae of the blowfly and mosquito at sub-part-per-million levels. The structure followed mainly from a consideration of spectroscopic evidence, including Dunkel's computerized 2D INADEQUATE analysis. The relative stereochemistry of the eastern and western hemispheres of **1a** and its methyl ester, **1b**, were independently determined on the basis of ROESY, NOESY, NOEDS, and J_{vic} evidence. By employing the same methods, the complete relative stereochemistry was determined by analysis of suitable transformation products, using the reduced β-ketodihydropyrrole ring as a stereochemical bridge between the two zones. These results were confirmed by X-ray analysis of the 7-*p*-bromobenzoate methyl ester derivative, **1c**. The absolute stereochemistry was established by application of the advanced Mosher method to methyl ester **1b**. Of biogenetic interest is the presence of a unique isoprenylated indole moiety not previously found in other indole mycotoxins.

The development of resistance to existing insecticides and the desire to have compounds with less mammalian and environmental toxicity continues to spur the search for novel insecticides. Screening culture extracts using the larvae of the mosquito, *Aedes aegypti*, has been well documented as a viable approach to discovering new insecticidal activities.[1a] A unique macrocyclic lactone, spinosyns, now under development as a crop protection agent, was detected using the mosquito larvae assay and was isolated from *Saccharopolyspora spinosa.*[1c] Another very useful assay involves the larvae of the blowfly, *Lucilia seracata.* Either of the assays can be used to measure insecticidal activities of agents such as avermectins or milbemycins.[1b,d] In our ongoing screening program for biologically active natural products, we have isolated a novel indole terpene designated nodulisporic acid A (**1a**) from a *Nodulisporium* sp. as a potent insecticide. We describe herein the isolation and structure elucidation of **1a**, including the relative and absolute stereochemistry, solution conformation, and biological activity. A number of chemical transformations are also described.



1a: R = R_1 = R_2 = H; nodulisporic acid A
1b: R = CH_3, R_1 = R_2 = H
1c: R = CH_3, R_1 = *p*-BrC_6H_4CO R_2 = H
1d: R = CH_3, R_1 = *p*-BrC_6H_4CO R_2 = MOM
1e: R = CH_3, R_1 = *S*-MTPA R_2 = H
1f: R = CH_3, R_1 = *R*-MTPA R_2 = H

Results and Discussion

Isolation. The *Nodulisporium* sp. (MF 5954, ATCC 74245) is an endophytic fungus isolated from a woody plant. Nodulisporic acid A could be purified from methyl ethyl ketone extracts of the fungus grown either on a solid substrate or in a liquid medium. The extract was dried and partitioned between hexane and MeOH (1:1). The MeOH solubles were first chromatographed over silica gel, followed by a gel filtration step (Sephadex LH20). Final purification was achieved by preparative reverse-phase HPLC, providing nodulisporic acid as a yellow powder in 2 mg/L yield.

Structure Determination. High-resolution FAB-MS of nodulisporic acid A indicated the molecular formula $C_{43}H_{53}NO_6$ (found *m*/*z* 680.3991, calcd *m*/*z* 680.3951 [M + H]), which was supported by the carbon and carbon-bound proton counts from ^{13}C NMR and DEPT spectra, respectively, assuming three exchangeable protons. The molecule contains one ketone carbonyl, one carboxylic acid group, and eight double bonds (only one of which is isolated), suggesting eight rings to satisfy the 18 double bond equivalents. Three partial structures, **A**−**C** (Figure 1), were proposed on the basis of the COSY, TOCSY, HMQC, and HMBC data, which left seven carbons unaccounted for, six of which were non-protonated (see Table 1).

Three exchangeable groups (OH at C7 and C24 and C5″ COOH) were detected by application of the technique involving deuterium isotope-induced ^{13}C shifts (in CD_2Cl_2 spiked with D_2O), which characteristically affect[2] those carbons α and β to NH/OH groups in alcohols and amines. Additionally, nodulisporic acid A (**1a**), on reaction with diazomethane at −78

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1997.

(1) (a) Henrick, C. A.; Staal, G. B.; Siddall, J. B. *J. Agric. Food Chem.* **1973**, *213*, 354. (b) Putter, I.; MacConnell, J. G.; Prizer, F. A.; Haidri, A. A.; Ristich, S. C.; Dybas, R. A. *Experientia* **1981**, *37*, 963. (c) Kirst, H. A.; Michel, K. H.; Mynderse, J. S.; Chio, E. W.; Yao, R. C.; Nakasukasa, W. M.; Boeck, L. D.; Occlowitz, J. L.; Paschal, J. W.; Deeter, J. B.; Thompson, G. D. *ACS Symp. Ser.* **1992**, *504*, 214−225. (d) Shaw, R. D.; Blackman, G. G. *Aus. Vet. J.* **1971**, *47*, 268.

S0002-7863(97)01664-8 CCC: $14.00

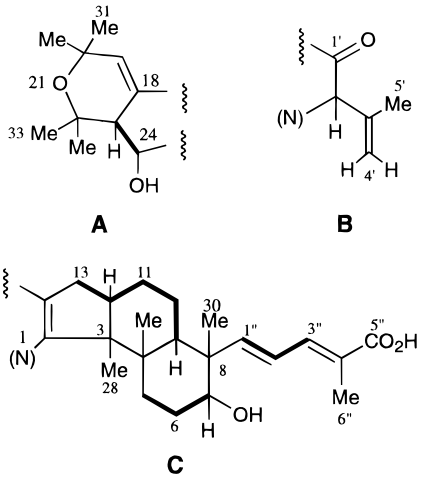


Figure 1. Partial fragments **A**−**C** of nodulisporic acid A (**1**) mainly from COSY/TOCSY (bonds in boldface) and HMBC/HMQC data (see Table 1).

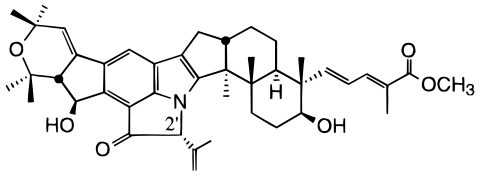
Table 1. ^{1}H and ^{13}C NMR Data of Nodulisporic Acid A (1a) in CD_2Cl_2

position	δC[a]	δH[b]	HMBC C no.[c]
1			
2	154.7		
3	56.1		
4	39.1		
5	32.3	1.93 (m), 1.76 (m)	
6	25.95	1.78 (m), 1.81 (m)	
7	76.8	3.45 (m)	
8	47.8		
9	45.2	1.65 (m)	
10	24.7	1.47 (m), 1.47 (m)	
11	25.7	1.55 (m), 1.49 (m)	
12	48.0	2.85 (m)	13
13	27.75	α: 2.33 (dd, J = 10.8, 13.9 Hz)	11, 12
		β: 2.76 (dd, J = 6.5, 13.9 Hz)	2, 3, 12,
14	122.7		
15	121.8		
16	116.7	7.73, (s)	14, 18, 25, 27
17	134.0		
18	135.9		
19	122.0	6.07 (d, J = 3.2 Hz)	17, 20, 23, 31, 32
20	72.6		
21			
22	73.9		
23	58.2	2.82 (dd, J = 3.0, 6.2 Hz)	17, 22, 24, 25, 33, 34
24	75.3	5.24 (d, J = 6.2 Hz)	18, 22, 25, 26
25	138.4		
26	113.1		
27	162.0		
28	15.1	0.97 (s)	2, 3, 4, 12
29	19.6	1.15 (s)	3, 4, 5, 9
30	11.24	1.07 (s)	7, 8, 9, 1″
31	29.9	1.34 (s)	19, 20, 32
32	31.95	1.31 (s)	19, 20, 31
33	23.45	1.13 (s)	22, 23, 34
34	30.1	1.43 (s)	22, 23, 33
1′	198.0		
2′	76.4 (br)	5.11 (s)	27, 1′, 3′, 4′
3′	140.0 (br)		
4′	117.5 (br)	5.23 (br s), 4.99 (br s)	2′, 5′
5′	18.1 (br)	1.48 (br s)	2′, 3′, 4′
1″	154.6	5.96 (d, J = 15.2 Hz)	7, 8, 9, 30, 3″
2″	125.9	6.42 (dd, J = 11.2, 15.2 Hz)	8, 3″, 4″
3″	140.8	7.34 (br d, J = 11.2 Hz)	1″, 2″, 4″, 5″, 6″
4″	125.1		
5″	172.6		
6″	12.65	1.96 (d, J = 1.2 Hz)	3″, 4″, 5″

[a] 125 MHz. [b]500 MHz. [c] Optimized for J = 7 Hz.

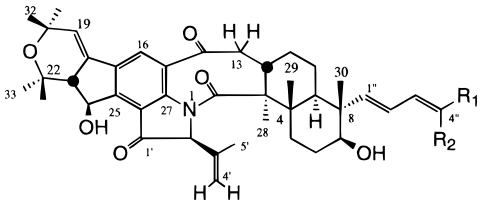
°C, formed exclusively a monomethyl ester (**1b**), which confirmed the presence of a carboxyl group.

These experiments account for all six oxygens in the three fragments **A**−**C** (Figure 1). By default, the nitrogen must, therefore, be placed adjacent to the allylic C2′ carbon in fragment **B** to account for the H2′ chemical shift (δ 5.11) and the corresponding ^{13}C resonance (δ 76.4). A significant number of plausible structures were generated, of which **1a** appeared the most likely on the basis of the two- and three-bond HMBC correlations (optimized for 7 Hz) and ^{13}C chemical shift assignments (Table 1). The only carbon not assigned by analysis of the HMBC data was the signal at 121.8 ppm; this could be ascribed to the indole β carbon C15 because of its similar chemical shift to C14 (122.7 ppm). Moreover, the structure adequately accounts for the low-field position of C27 (162.0 ppm), which is simultaneously α to the indole nitrogen and β to the carbonyl group. This assignment was further substantiated by its HMBC correlation to H2′. Of interest is the exchange-broadening phenomenon involving the ^{1}H and ^{13}C resonances of the isopropenyl group at C2′ but no longer present in the 2′-epimeric compound **2** (see Solution Conformation, below).



2

During the course of NMR experiments in CD_3CN, it was observed that **1a** degrades to two compounds, **3a** and **3b**, as seen by both NMR and HPLC. After isolation by preparative HPLC, the structure of the major transformation product, **3a**, was determined by the previous NMR protocol. All proton and



3a: R_1 = COOH R_2 = CH_3
3b: R_1 = CH_3 R_2 =COOH

carbon resonances of **1a** were readily accounted for in the spectra of **3a**, except for two of the quaternary sp^2 carbons, which had moved significantly downfield to 174.5 (C2) and 197.8 ppm (C14), indicative of amide and ketone carbonyl functionalities. ^{1}H NMR comparison of **3a** and **3b** showed the products to differ only in the dienoic acid side chain. Whereas the 1″*E*,3″*E* configuration is retained in **3a**, it is isomerized to the 1″*E*, 3″*Z* geometry in **3b**. Both products are the result of oxidation of one of the indole double bonds (C2−C14) in a manner similar to that previously reported for paxilline[3] and shearinine B[4] and recently observed in our laboratory with a paxilline derivative by allowing it to stand in CD_2Cl_2 indefinitely at −20 °C. The mode of cleavage and in-depth structure

(2) (a) Hansen, P. E. In *Progress in NMR Spectroscopy*; Elmley, J. W., Feeney, J., Sutcliffe, L. H., Eds.; Pergamon Press: Oxford, 1983; Vol. 15, pp 105−234. (b) Hansen, P. E. In *Progress in NMR Spectroscopy*; Elmley, J. W., Feeney, J., Sutcliffe, L. H., Eds.; Pergamon Press: Oxford, 1988; Vol. 20, pp 207−255.

(3) (a) Mantle, P. G.; Burt, S. J.; MacGeorge, K. M.; Bilton, J. N.; Sheppard, R. N. *Xenobiotica* **1990**, *20*, 809. (b) Springer, J. P.; Clardy, J.; Wells, J. M.; Cole, R. J.; Kirksey, J. W. *Tetrahedron Lett.* **1975**, 2531.

(4) Belofsky, G. N.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron* **1995**, *51*, 3959.

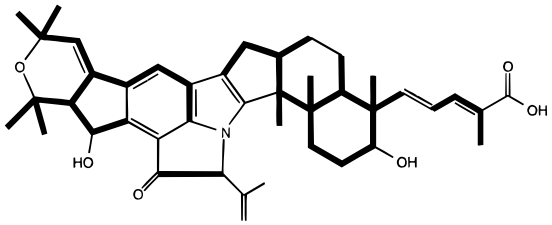


Figure 2. $^{13}C-^{13}C$ bonds (in boldface) in **1a** directly detected by the INADEQUATE analysis.

elucidation of **3a** provides independent support for the proposed structure (**1a**) of nodulisporic acid A. Sensitivity toward light and oxygen, apparently independent of the nature of the solvent, accounts for these oxidation reactions.

INADEQUATE Analysis. To complete the spectroscopic structure determination of **1a**, several INADEQUATE experiments were conducted to directly detect $^{13}C-^{13}C$ connectivities in the molecule to confirm its carbon skeleton. Both natural abundance and ^{13}C-enriched material were used in these experiments, and the spectral data were subjected to the computerized CCBond analysis technique proposed by Dunkel and colleagues.[5] The procedure detects the absence or presence of bonds between a pair of ^{13}C nuclei whose double-quantum frequencies can be generated from the ^{13}C frequencies obtained from a simple 1D ^{13}C NMR experiment. The dramatically reduced sample requirement of the method is a distinct advantage under conditions when signals are too weak to be identified visually.[6] In the natural abundance case, 32 out of a possible 47 connectivities were identified on 26 mg of **1a** in 3−5 day experiments optimized for a low (40 Hz) and high J_{CC} value (60 Hz) using a 3 mm dedicated ^{13}C microprobe. Several milligrams of doubly labeled ^{13}C compound were isolated from a fermentation experiment, using doubly labeled [^{13}C] acetate. Despite a wide range of incorporation efficiencies (0.1−1.0%) at different sites in the molecule, only two additional connectivities were found in the INADEQUATE experiment, one being the C1′−C2′ bond. All bonds detected in this manner are consistent with the proposed structure. Figure 2 depicts a composite drawing of all bonds in **1a** detected with both the natural abundance and the ^{13}C-enriched material.

Structure and Relative Stereochemistry of C7 *p*-Bromobenzoate Derivative 1c by X-ray Crystallography. Two independent approaches (NMR and X-ray) were simultaneously taken to deduce the relative stereochemistry of nodulisporic acid A. The X-ray is described here, and the NMR approach follows.

The methyl ester **1b** was reacted with 1.5 equiv of *p*-bromobenzoyl chloride in the presence of 4-(dimethylamino)-pyridine (DMAP) in CH_2Cl_2 to give **1c** in excellent yield. No epimerization was observed in the absence of CH_3CN as a solvent with the DMAP concentration kept low. Crystallization of **1c** from MeOH−CH_2Cl_2 at room temperature gave crystals suitable for X-ray crystallography. The structure and the relative stereochemistry of nodulisporic acid were confirmed by an X-ray crystallographic analysis[7] of crystals of **1c** (Figure 3). Due to poor crystal quality, however, the absolute configuration could not be independently established.

Relative and Absolute Stereochemistry and Solution Conformation of Nodulisporic Acid A by NMR. Nodulisporic acid A (**1a**) contains two separate stereochemical hemispheres. The relative stereochemistries of the eastern and western hemispheres of **1a** were independently determined on the basis of ROESY, NOESY, NOEDS, and $^3J_{vic}$ evidence. Nodulisporic acid A (**1a**) and the derivatives revealed strong NOESY correlations at 400 MHz but very weak correlations at 500 MHz. This is a result of the frequency dependence of the NOE intensity in the particular molecular weight range of these compounds (679−900), which at 500 MHz falls in the NOE zero-crossing region from positive to negative. As ROEs are always positive, the largely complementary NOESY and ROESY data of **1a** were collected at 400 and 500 MHz, respectively.

(a) Relative Stereochemistry of Eastern Hemisphere. The *trans*−*anti*−*trans* fusion of the three cyclic rings was determined on the basis of ROESY and NOESY correlations of **1a** and **1b** (Figure 4). The correlations between H9 and both H7 and 28-H_3 established their 1,3-diaxial relationship. The angular methyl 29-H_3 showed strong ROE/NOE correlations to H12 and 30-H_3, also confirming their 1,3-diaxial configuration. The olefinic side-chain proton H1″ gave strong correlations to $H7_{ax}$, $H9_{ax}$, and H3″, thus placing the 7-OH and the side chain at the respective equatorial positions. The H13β methylene exhibited NOE correlations to H12, whereas H13α gave correlations to 28-H_3.

(b) Relative Stereochemistry of Western Hemisphere. The relative stereochemistry of the eastern hemisphere cannot be directly correlated to that of the western hemisphere because of their separation by a number of sp^2 centers. The problem is all the more challenging as the two vicinal asymmetric (C23/C24) centers are located in a five-membered ring, where coupling constants are seldom definitive in determining relative configurations. The NOE/ROE correlations from the methyl groups to H23/H24 or *vice versa* could not be relied on for the H23/H24 stereochemical assignment due to the conformational flexibity of the dihydropyran ring. The vicinal coupling constant (J = 6.2 Hz) between H23 and H24 was not diagnostic, since the predicted J value for both *cis* and *trans* hydrogens is the same. Therefore, it became important to find a derivative which not only allows correlation of the stereochemistry at C24 to the eastern hemisphere but also provides a handle for establishing its orientation with respect to H23. Encouraged by the observation of NOEs from H16 to both H19 and H13 in the northern half of the molecule, we reasoned that a hydrogen introduced at C1′ in the southern half (preferably by nonste-

(5) (a) Dunkel, R.; Mayne, C. L.; Curtis, J.; Pugmire, R. J.; Grant, D. M. *J. Magn. Reson.* **1990**, *90*, 290. (b) Dunkel, R.; Mayne, C. L.; Pugmire, R. J.; Grant, D. M. *Anal. Chem.* **1992**, *64*, 3133. (c) Dunkel, R.; Mayne, C. L.; Foster, M. P.; Ireland, C. M.; Li, D.; Owen, D. L.; Pugmire, R. J.; Grant, D. M. *Anal. Chem.* **1992**, *64*, 3150.

(6) (a) Foster, M. P.; Mayne, C. L.; Dunkel, R.; Pugmire, R. J.; Grant, D. M.; Kornprobst, J.-M.; Verbist, J.-F.; Biard, J.-F.; Ireland, C. M. *J. Am. Chem. Soc.* **1992**, *114*, 1110−1111. (b) Perera, P.; Andersson, R.; Bohlin, L.; Andersson, C.; Li, D.; Owen, N. L.; Dunkel, R.; Mayne, C. L.; Pugmire, R. J.; Grant, D. M.; Cox, P. A. *Magn. Reson. Chem.* **1993**, *31*, 472.

(7) Crystal structure details for **1c**: $C_{51}H_{60}BrNO_8$, M_r = 894.958, monoclinic, space group $C2$, a = 38.26(1), b = 9.709(2), and c = 12.729(4) Å, β = 99.52(3)°, V = 4664(4) Å^3, Z = 4, D_x = 1.275 g cm^{-3}, monochromatized radiation λ(Cu K_α) = 1.541 838 Å, μ =1.61 mm^{-1}, F(000) = 1888, T = 294 K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 71.39°. There are 4705 unique reflections out of 4779 measured, with 2380 observed at the $I \geq 2\sigma(I)$ level. The structure was solved by direct methods (SHELXS: Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467−473) and refined using full-matrix least-squares (SHELXL: Sheldrick, G. M. *J. Appl. Crystallogr.*, to be published) on F^2 using 543 parameters and all data (4705 reflections). All non-hydrogen atoms were refined with anisotropic thermal displacements, except for the oxygen of the water molecule, which was left as isotropic. Hydrogen atoms are included at their calculated positions and were allowed to "ride" during refinement. Final agreement statistics are R = 0.070, wR = 0.180, S = 1.08, $(\Delta/\sigma)_{max}$ = 0.32. The maximum peak height in a final difference Fourier map is 0.615 e Å^{-3}, and it has no chemical significance. Presumably as a result of poor crystal quality, the refinement results could not be used to unambiguously assign the absolute configuration. The authors have deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, UK.

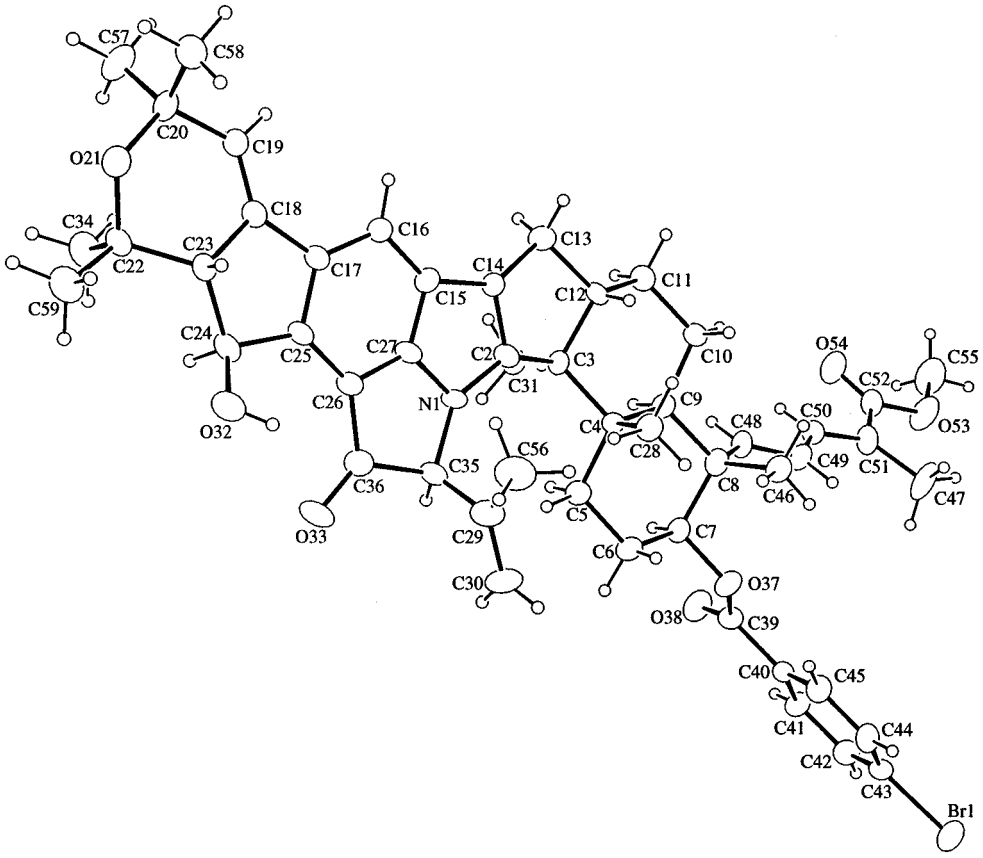


Figure 3. Perspective ORTEP drawing of X-ray structure of **1c**.

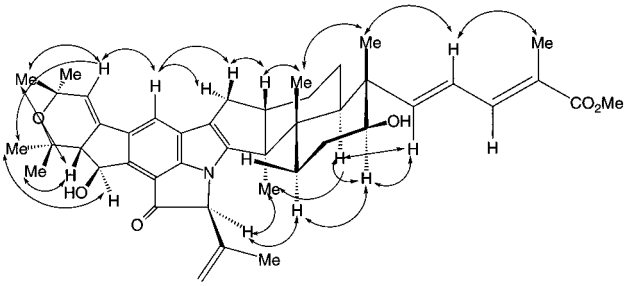


Figure 4. Selected ROESY/NOESY correlations in nodulisporic acid A (**1a**/**1b**).

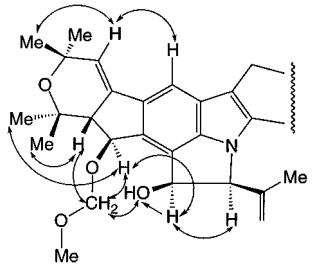


Figure 5. Selected NOESY/NOEDS correlations of western hemisphere of **5a**.

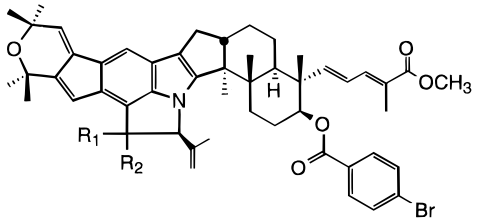
reospecific reduction of the ketone) should similarly exhibit NOEs to H24 of either a 24-*O*-methyl or MOM ether. The reduced β-ketodihydropyrrole ring system, therefore, became a stereochemical bridge between the two isolated zones.

The initial attempts at C24 *O*-methylation of selectively protected derivative **1c**, using several methods including boron trifluoride diethyl etherate/diazomethane and trimethyloxonium tetrafluoroborate, were unsuccessful. In fact, the reaction of **1c** with the latter reagent exclusively afforded the elimination product **4a**. On the other hand, reaction of **1c** with (MOM)Cl gave the desired ether, **1d**, in very good yield. The MOM group tended to eliminate during NMR analysis in $CDCl_3$ or CD_2Cl_2 to give **4a** but was found to be stable in CD_3CN. As would be predicted, $NaBH_4$ reduction of **1d** gave the β-hydroxy compound **5a** exclusively. The specificity for α-face reduction was diminished in the absence of the MOM group, as evidenced by the formation of a 9:1 mixture of epimeric alcohols **5b** and **5c** during the sodium borohydride reduction of **1c**.

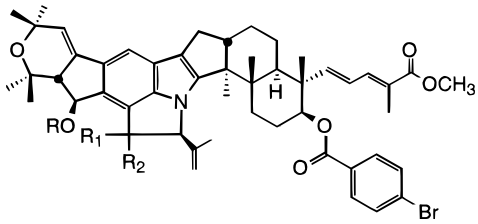
H1′ of both **5a** and **5b** gave strong NOESY correlations (Figure 5) to H2′ ($r_{H1',H2'}$, 2.4 Å), thus indicating their *cis* relationship. This was further substantiated by the $J_{vic} = 6.8$ Hz, which is consistent with the measured dihedral angle ($\phi = -8°$) in the minimized structure (ChemDraw 3D). The 2.8 Hz coupling constant between H1′ and H2′ of the epimeric compound **5c** is consistent with the measured ϕ of 113° and, together with the 3 Å interatomic distance, readily accounts for the weak NOE between them. A strong *syn* oxygen effect on the 5′-CH_3 group (downfield shift of 0.55 ppm) was observed for the 1′β hydroxy compounds **5a** and **5b** compared to the epimer **5c**, confirming the stereochemical assignments at C1′ and C2′.

Returning to **5a**, H24 gave a NOESY correlation to H1′, albeit weak ($r_{H1',H24}$, 3.3 Å), and to the MOM methylene group (Figure 5). Furthermore, the methylene protons also gave NOEs to the 1′-OH and H23. These observations are only possible with the 23β hydrogen, 24-β hydroxy, and 1′β hydroxy groups, thus establishing the relative configuration at these centers. Very weak NOESY correlations for both **5a** and **5b** were, once again, observed between the *anti* protons H23 and H24, consistent with the 149° dihedral angle and a 3.1 Å interatomic distance.

(c) Absolute Stereochemistry. The absolute stereochemistry of nodulisporic acid A was determined by application of the advanced Mosher method.[8] Both the *S*- and *R*-MTPA esters (**1e** and **1f**) at C7 of the methyl ester **1b** were prepared by



4a: R_1,R_2 = O
4b: R_1 = αH, R_2 = βOH



5a: R = MOM, R_1= αH, R_2 = βOH
5b: R = H, R_1= αH, R_2 = βOH
5c: R = H, R_1= βH, R_2 = αOH

reaction with the *R*- and *S*-MTPA chlorides, respectively, in methylene chloride. The ^{1}H NMR spectra of both compounds were carefully assigned using COSY, HMQC, and TOCSY experiments and the chemical shift differences, $\Delta\delta$ ($\delta_S - \delta_R$), calculated. These differences were profound and are shown in Figure 6. All protons to the right of the MTPA plane, which dissects the molecule through C7−C4−C3−C14, have positive $\Delta\delta$ increments, whereas negative values are observed for protons on the other side of the plane. The magnitude of the shift differences varies from 0.001 to 0.175 ppm, and significant long-range shielding effects are detected as far away as H19. Even though the 28-methyl appears to fall to the left of the MTPA plane in the drawing, examination of a molecular model shows it to reside just to the right of the plane, and this explains its small positive $\Delta\delta$ value. The clear division into positive and negative increment regions leaves no ambiguity in the assignment of the *S* configuration at C7, and, from this, the complete absolute stereochemistry of nodulisporic acid A methyl ester was inferred (Figure 6).

(d) Solution Conformation. The NMR methods helped in determination of the solution conformation of nodulisporic acid A methyl ester (**1b**), which was identical to that of the solid state conformation determined by X-ray crystallography of **1c**. The equatorial side chain at C8 stretches away from the core of the molecule. The isopropenyl group at C2′ and the hydroxy group at C24 tend to crowd the β-face of the β-ketodihydropyrrole ring. The conformation readily accounts for the exchange-broadened resonances of the isopropenyl group at C2′ as being due to restricted rotation about C2′−C3′. Supporting evidence came from the 2′-epimeric compound **2**, which was obtained by treatment of **1b** with DMAP in a mixture of $CH_3CN-CH_2Cl_2$. The resonances were no longer exchange-broadened in **2** as well as the oxidation products **3a** and **3b**, suggesting relief of steric hinderance between the side chain and groups on the β-face of the molecule, in particular H5β and the 29-methyl.

Biological Activity. Nodulisporic acid A has an LC_{50} of 0.5 ppm against *A. aegypti* and an LC_{50} of 0.3 ppm against *L. seracata*.[9] Nodulisporic acid A is more active than paraherquamide[10] (LC_{50} of 50 ppm in both assays) and less active than ivermectin[11] (LC_{50} of 0.02 and 0.045 ppb against *A. aegypti* and *L. seracata*, respectively). For the comparison of the biological activities, paxilline,[3] one of the known indole diterpenes, was tested in both assays and was found to be inactive at 25 ppm in *A. aegypti* and at 250 ppm in *L. seracata*.

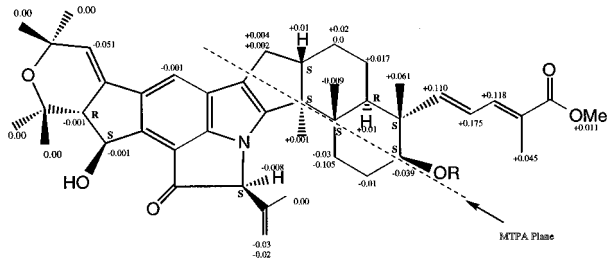


Figure 6. $\Delta\delta$ ($\delta_S - \delta_R$) values in ppm obtained for the *R*- and *S*-MTPA esters of nodulisporic acid A methyl ester (**1b**).

Conclusion

The structurally novel nodulisporic acid A is a potent insecticide. It is more potent than paraherquamide and less potent than the benchmark, ivermectin. The biological activity does not seem to be a general feature of indole diterpenes, as paxilline is inactive in the two assays tested. It bears some structural resemblance to a number of previously identified indole diterpenes, including janthitrem G,[12] the shearinines,[4] the lolitrems,[13] the penitrems,[14] and the paspalitrems.[15] All of these compounds, except nodulisporic acid A, possess a tertiary hydroxy group,[12−15] a feature implicated in tremorgenic properties. One of the more interesting structural/biogenetic features of nodulisporic acid A is the reversed ring fusion of the dihydropyran compared to those janthitrems[12] and shearinines[4] and cyclopentyl rings in the western hemisphere of the molecule; another is the unique, highly strained five-membered β-ketodihydropyrrole ring derived from isoprenylation of the indole moiety. The latter unit is unprecedented in the indole mycotoxins reported to date.

Experimental Section

Spectral Analysis. NMR spectra were determined on Varian Unity 500 and 400 spectrometers operating at 500 and 400 MHz for ^{1}H and at 125 and 100 MHz for ^{13}C, respectively. ^{1}H and ^{13}C chemical shifts are referenced to the solvent (CD_2Cl_2) signal at δ 5.32 (^{1}H) and 53.8 ppm (^{13}C). Homonuclear ^{1}H−^{1}H connectivities were determined by using 2D COSY, double-quantum-filtered COSY, and 1D decoupled experiments. Homonuclear ^{1}H NOEs were obtained by 1D difference NOE measurements and 2D NOESY/ROESY experiments. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined by 2D proton-detected HMQC experiments and long-range ^{1}H−^{13}C connectivities by

(8) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

(9) Oslind, D. A.; Felcetto, T.; Misura, A.; Ondeyka, J.; Smith, S.; Goetz, M.; Shoop, W.; Mickle, W. *Med. Vet. Entomol.*, submitted.

(10) (a) Ondeyka, J. G.; Goegelman, R. T.; Schaeffer, J. M.; Keleman, L.; Zitano, L. *J. Antibiot.* **1990**, *43*, 1375. (b) Yamazaki, M. E.; Okuyama, E.; Kobayashi, M.; Inoue, H. *Tetrahedron Lett.* **1981**, *22*, 135.

(11) Chabala, J. C.; Mrozik, H.; Tolman, R. L.; Eskola, P.; Lusi, A.; Peterson, L. H.; Woods, M. F.; Fisher, M. H.; Campbell, W. C.; Egerton, J. R.; Ostlind, D. A. *J. Med. Chem.* **1980**, *23*, 1134.

(12) de Jesus, A. E.; Steyn, P. S.; Van Heerden, F. R.; Vleggaar, R. *J. Chem. Soc. Perkin Trans. I* **1984**, 697.

(13) (a) Gallagher, R. T.; Hawkes, A. D.; Steyn, P. S.; Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1984**, 614. (b) Ede, R. M.; Miles, C. O.; Meagher, L. P.; Munday, S. C.; Wilkins, A. L. *J. Agric. Food. Chem.* **1994**, *42*, 231 and references cited therein.

(14) (a) de Jesus, A. E.; Steyn, P. S.; Van Heerden, F. R.; Vleggaar, R.; Wessels, P. L. *J. Chem. Soc., Perkin Trans. I* **1983**, 1857 and references cited therein.

(15) Dorner, J. W.; Cole, R. J.; Cox, R. H.; Cunfer, B. M. *J. Agric. Food. Chem.* **1984**, *32*, 1069 and references cited therein.

2D proton-detected HMBC experiments. Nalorac 3 mm triple-resonance and dedicated ^{13}C microprobes were used. Natural abundance INADEQUATE spectra were obtained on ~26 mg of **1a** in experiments optimized for J_{CC} values of 40 and 60 Hz using a 3 mm dedicated ^{13}C microprobe, using the method of Bax and Mareci.[16]

Mass spectra, including high-resolution mass measurements, were determined in the EI mode using a Ultramark 1960 standard. FAB-MS (fast atom bombardment mass spectra) were recorded on a JEOL HX110 mass spectrometer. UV spectra were measured in MeOH at 20 °C on a Beckman DU-20. Optical rotations were measured in ($CHCl_3$) at 22 °C at the sodium D line on a Perkin Elmer 241 instrument.

Isolation and Fermentation. *Nodulisporium* sp. MF5954 was isolated as an endophytic fungus from an unidentified woody plant tissue. The woody tissue was surface sterilized by sequential immersion in dilute NaOCl solution followed by 95% ethanol. Branched conidiophores are nodulose, which distinguishes the genus from other similar fungi, such as *Geniculosporium*, *Xylocladium*, and anamorphs of Xylariales (Ascomycotina) such as *Hypoxylon*, *Xylaria*, *Camillea*, and *Biscogniauxia*. These fungi often dwell inside woody plants. It might be of interest to know that indole mycotoxins are unknown among fungi of the Xylariales.[17]

The *Nodulisporium* sp. was cultured at 25 °C in shake flasks from vegetative mycelial growths on a rotary shaker for 21−28 days in a nutrient medium. The nutrient medium consisted of glycerol (75 g/L), glucose (10 g/L), ardamine pH (5 g/L), $(NH_4)_2SO_4$ (2 g/L), soybean meal (5 g/L), tomato paste (5 g/L), sodium citrate (2 g/L), and distilled water at pH 7.0.

Methyl ethyl ketone (MEK) extracts from 6 L of 24 day liquid shake flask fermentations were filtered, combined, and evaporated to dryness under vacuum to a weight of 8 g. This material was dissolved in CH_2Cl_2 (20 mL) and applied to a silica gel column (E. Merck silica gel 60, 4.5 cm × 100 cm, flow rate 6.5 mL/min) equilibrated in CH_2Cl_2−MeOH (95:5). The column was washed with 9:1 CH_2Cl_2−MeOH followed by 3:1 to elute **1a**. The solvents were evaporated under reduced pressure and crude **1a** (200 mg) was charged to a Sephadex LH20 column (Pharmacia, 2.5 cm × 200 cm, flow rate 5 mL/min) in MeOH. Fractions containing **1a** were combined (530−650 mL, 90 mg), dried under vacuum, and chromatographed further on an Eka Nobel C-18 HPLC column (9.6 mm × 250 mm) using 70% CH_3CN in H_2O containing 0.1% TFA to yield pure **1a** (4.8 mg). The compound had a R_f value of 0.47 on TLC plates (EM silica gel 60_{F254}) with 9:1 CH_2Cl_2−MeOH. A similar process was used to purify material from several further fermentation batches ranging in volumes from 1 to 20 L.

Nodulisporic Acid A (1a): mp 250−255 °C; $[\alpha]^{22}_D$ +13° (*c* 0.4, $CHCl_3$); UV (MeOH) λ_{max} 241 (ϵ = 43 900), 265 (ϵ = 49 800), 385 (ϵ = 7360) nm; IR (ZnSe) ν_{max} 2980, 2364, 1704 (br), 1378, 1228, 1034 cm^{-1}; HRFAB-MS *m/z* 680.3891 (M + H; calcd for $C_{43}H_{53}NO_6$ + H, 680.3951), 662.3790 (M + H − H_2O; calcd for $C_{43}H_{51}NO_5$ + H, 662.3845); ^1H NMR (500 MHz, CD_2Cl_2), see Table 1; ^{13}C NMR (125 MHz, CD_2Cl_2), see Table 1.

Nodulisporic Acid A Methyl Ester (1b). To a cooled (−78 °C) solution of nodulisporic acid A (**1a**, 80 mg) in CH_2Cl_2 (1 mL) was added an excess of an ethereal solution of CH_2N_2. Progress of the reaction was monitored by TLC (CH_2Cl_2−MeOH, 9:1). The reaction was complete within 5 min. Excess of CH_2N_2 was destroyed by addition of HOAc, and solvents were removed to give clean methyl ester (**1b**) as a yellow powder in quantitative yield. An analytical sample of the methyl ester was prepared by chromatography on a small silica gel column, eluting with 20−30% EtOAc in hexane. Crystallization from CH_2Cl_2−MeOH gave yellow granules: mp 270−72 °C; $[\alpha]^{22}_D$ + 19° (*c* 0.5, $CHCl_3$); IR (ZnSe) ν_{max} 3505, 2976, 1708, 1634, 1589, 1452, 1378, 1290, 1244, 1230, 1096, 1076, 1023, 1008, 980, cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) δ 0.97 (s, 28-H_3), 1.06 (s, 30-H_3), 1.13 (s, 33-H_3), 1.15 (s, 29-H_3), 1.32 (s, 32-H_3), 1.34 (s, 31-H_3), 1.43 (s, 34-H_3), 1.48 (br s, 5′-H_3), 1.48, 1.55 (m, 11-H_2), 1.55 (m, 10-H_2), 1.65 (m, 9-H), 1.76, 1.93 (m, 5-H_2), 1.74, 1.78 (m, 6-H_2), 1.96 (d, *J* = 1.2 Hz, 6″-H_3), 2.33 (dd, *J* = 13.6, 10.8 Hz, 13-Hα), 2.75 (dd, *J* = 13.6, 6.4 Hz, 13-Hβ), 2.82 (dd, *J* = 6.4, 2.8 Hz, 23-H), 2.85 (m, 12-H), 3.10 (br s, 24-OH), 3.40 (m, 7-H), 3.74 (s, 5″-OCH_3), 4.97 (br s, 4′-Hb), 5.10 (s, 2′-H), 5.21 (br s, 4′-Ha), 5.22 (d, *J* = 6.4 Hz, 24-H), 5.89 (d, *J* = 15.2 Hz, 1″-H), 6.07 (d, *J* = 3.2 Hz, 19-H), 6.40 (dd, *J* = 15.2, 11.2 Hz, 2″-H), 7.22 (d, *J* = 11.2 Hz, 3″-H), 7.71 (s, 16-H); HRFAB-MS (*m/z*) 694.4172 (M + H; calcd for $C_{44}H_{56}NO_6$, 694.4107).

7-(*p*-Bromobenzoyl)nodulisporic Acid A Methyl Ester (1c). *p*-Bromobenzoyl chloride (16 mg, 0.07 mmol) was added to a cooled (0 °C) solution of methyl ester **1b** (34 mg, 0.05 mmol), diisopropylethylamine (0.1 mL), and 4-(dimethylamino)pyridine (DMAP, 10 mg) in CH_2Cl_2 (0.5 mL). The solution was stirred at 0 °C for 10 min, followed by stirring overnight at room temperature. Ice was added after completion (TLC, hexane−EtOAc, 7:4) of the reaction, the whole mixture was poured onto 50 mL of EtOAc, and the layers were separated. The EtOAc layer was washed sequentially with 20 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and water, dried, evaporated under reduced pressure, and chromatographed by preparative TLC on silica gel to give pure benzoate **1c** (38 mg, 88.4%) as a yellow powder. Crystallization from CH_2Cl_2−MeOH gave thick yellow needles: mp 273−75 °C dec; $[\alpha]^{22}_D$ +82.5° (*c* 0.6, $CHCl_3$); IR (ZnSe) ν_{max} 3527, 2976, 1713, 1636, 1591, 1455, 1378, 1271, 1246, 1230, 1098, 1068, 1013, 919, 846, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 28-H_3), 1.14 (s, 33-H_3), 1.19 (s, 30- or 29-H_3), 1.27 (s, 29- or 30-H_3), 1.34 (s, 32-H_3), 1.35 (s, 31-H_3), 1.44 (br s, 5′-H_3), 1.48 (s, 34-H_3), 1.51, 1.56, 1.75, 1.89−2.03 (m, 5-H_2, 6-H_2, 9H, 10-H_2, 11-H_2), 1.80 (d, *J* = 1.2 Hz, 6″-H_3), 2.32 (dd, *J* = 14, 10.8 Hz, 13-Hα), 2.74 (dd, *J* = 14, 6.4 Hz, 13-Hβ), 2.85 (m, 12-H), 2.88 (dd, *J* = 6.0, 2.8 Hz, 23-H), 3.10 (br s, 24-OH), 3.70 (s, 5″-OCH_3), 4.92 (m, 7-H), 4.97 (br s, 4′-Hb), 5.06 (s, 2′-H), 5.17 (br s, 4′-Ha), 5.24 (d, *J* = 6.4 Hz, 24-H), 5.86 (d, *J* = 15.6 Hz, 1″-H), 6.05 (d, *J* = 3.2 Hz, 19-H), 6.26 (dd, *J* = 15.6, 11.2 Hz, 2″-H), 7.11 (d, *J* = 11.2 Hz, 3″-H), 7.55 (d, *J* = 8.4 Hz, Ar-H_2), 7.70 (s, 16-H), 7.80 (d, *J* = 8.4 Hz, Ar-H_2); HRFAB-MS (*m/z*) 876.3439 (M + H; calcd for $C_{51}H_{58}NO_7$-Br^{79} + H, 876.3475).

7-(*p*-Bromobenzoyl)-24-(methoxymethyl)nodulisporic Acid A Methyl Ester (1d). To a cooled (−30 °C) solution of **1c** (40 mg, 0.045 mmol) in CH_2Cl_2 (0.5 mL) was added diisopropylethylamine (0.5 mL) and methoxymethyl chloride (0.3 mL), and the solution was stirred at that temperature for 30 min, followed by overnight reaction at room temperature. After completion of the reaction (large excess of reagents needed), volatile reagents were removed under reduced pressure. The product was purified directly on silica gel plates (hexane−EtOAc, 7:3), and the yellow band was eluted with EtOAc. Evaporation of solvent gave pure MOM ether **1d** (30 mg) as an amorphous powder: IR (ZnSe) ν_{max} 2975, 1718, 1591, 1455, 1366, 1268, 1231, 1098, 1034, 756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 28-H_3), 1.06 (s, 33-H_3), 1.20 (s, 30- or 29-H_3), 1.27 (s, 29- or 30-H_3), 1.33 (s, 32-H_3), 1.35 (s, 31-H_3), 1.48 (br s, 5′-H_3), 1.55 (s, 34-H_3), 1.51, 1.58, 1.75, 1.89 −2.03 (m, 5-H_2, 6-H_2, 9H, 10-H_2, 11-H_2), 1.80 (d, *J* = 1.2 Hz, 6″-H_3), 2.32 (dd, *J* = 14, 10.8 Hz, 13-Hα), 2.75 (dd, *J* = 14, 6.4 Hz, 13-Hβ), 2.86 (m, 12-H), 3.00 (dd, *J* = 4.4, 2.4 Hz, 23-H), 3.46 (s, (MOM)−OCH_3), 3.71 (s, 5″-OCH_3), 4.86 (d, *J* = 7.6 Hz, (MOM)−OCH_b−O), 4.92 (m, 7-H), 4.95 (br s, 4′-Hb), 4.97 (s, 2′-H), 5.14 (br s, 4′-Ha), 5.15 (d, *J* = 4.4 Hz, 24-H), 5.32 (d, *J* = 7.6 Hz, (MOM)−OCH_a−O), 5.86 (d, *J* = 15.6 Hz, 1″-H), 6.05 (d, *J* = 2.8 Hz, 19-H), 6.26 (dd, *J* = 15.6, 11.2 Hz, 2″-H), 7.11 (d, *J* = 10.8 Hz, 3″-H), 7.55 (d, *J* = 8.4 Hz, Ar-H_2), 7.73 (s, 16-H), 7.80 (d, *J* = 8.4 Hz, Ar-H_2); HRFAB-MS (*m/z*) 920.3712 (M + H; calcd for $C_{53}H_{63}NO_8Br^{79}$, 920.3737).

7-(*S*)-α-Methoxy-α-[(trifluoromethyl)phenylacetoyl]nodulisporic Acid A Methyl Ester (1e). A solution of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (*R*-MTPACl, 25 mg) in 0.1 mL of CH_2Cl_2 was added to a solution of **1b** (10 mg), pyridine (0.2 mL), and DMAP (25 mg) in 0.1 mL of CH_2Cl_2. The solution was stirred at room temperature overnight. The progress of the reaction was monitored by silica gel TLC (hexane−EtOAc, 3:2). Reaction was extremely slow and was quenched with ice before disappearance of all starting material. Fifty milliliters of EtOAc was added, and the organic layer was washed with water, 10% aqueous citric acid, water, and 10% aqueous $NaHCO_3$, dried, and evaporated under reduced pressure. Purification by preparative silica gel TLC gave pure (*S*)-MTPA ester **1e** (3 mg) as an amorphous powder and unreacted **1b**.

1e: IR (ZnSe) ν_{max} 3539, 2976, 1746, 1709, 1452, 1379, 1247, 1185, 1112, 1065, 1020, 722 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) (assignment

(16) Bax, A.; Mareci, T. H. *J. Magn. Reson.* **1983**, 53, 360.

(17) Whalley, A. J. S.; Edwards, R. L.*Can. J. Bot.* **1993**, *73* (Suppl. 1), S802.

is based on COSY, HMQC spectrum) δ 0.999 (s, 28-H_3), 1.135 (s, 33-H_3), 1.154 (s, 30-H_3), 1.176 (s, 29-H_3), 1.318 (s, 32-H_3), 1.346 (s, 31-H_3), 1.43 (s, 34-H_3), 1.47 (br s, 5′-H_3), 1.49 (m, 10-H_2), 1.55, 1.77 (m, 11-H_2), 1.746 (m, 9-H), 1.895, 1.989 (m, 5-H_2), 1.90 (d, J = 1.2 Hz, 6″-H_3), 1.93 (m, 6-H_2), 2.34 (dd, J = 14, 10.8 Hz, 13-Hα), 2.769 (dd, J = 14, 6.8 Hz, 13-Hβ), 2.82 (dd, J = 6.4, 3.2 Hz, 23-H), 2.88 (m, 12-H), 3.47 (d, J = 1.2 Hz, OCH_3), 3.77 (s, 5″-OCH_3), 4.97 (br s, 4′-Hb), 4.98 (m, 7-H), 5.09 (s, 2′-H), 5.20 (br s, 4′-Ha), 5.229 (d, J = 6.4 Hz, 24-H), 5.931 (d, J = 15.6 Hz, 1″-H), 6.075 (d, J = 3.2 Hz, 19-H), 6.3535 (dd, J = 15.2, 11.2 Hz, 2″-H), 7.24 (d, J = 11.6 Hz, 3″-H), 7.30−7.42 (m, Ar-H_5), 7.722 (s, 16-H); HRFAB-MS (*m/z*) 910 (M + H; high-resolution data could not be measured due to low intensity), 892.4335 [(M + H − H_2O)$^+$; calcd for $C_{54}H_{61}NO_7F_3$, 892.4399].

7-(*R*)-α-Methoxy-α-[(trifluoromethyl)phenylacetoyl]nodulisporic Acid A Methyl Ester (1f). Prepared as described above from (*S*)-(+)-MTPACl, to give a yellow amorphous powder: IR (ZnSe) ν_{max} 3512, 2976, 1742, 1709, 1452, 1378, 1248, 1169, 1111, 1021, 721 cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2) (assignment is based on COSY, HMQC spectrum) δ 0.998 (s, 28-H_3), 1.093 (s, 30-H_3), 1.136 (s, 33-H_3), 1.185 (s, 29-H_3), 1.318 (s, 32-H_3), 1.346 (s, 31-H_3), 1.43 (s, 34-H_3), 1.47 (br s, 5′-H_3), 1.473 (m, 10-H_2), 1.55, 1.75 (m, 11-H_2), 1.736 (m, 9-H), 1.855 (d, J = 1.2 Hz, 6″-H_3), 1.91, 2.019 (m, 5-H_2), 1.94 (m, 6-H_2), 2.338 (dd, J = 14, 10.8 Hz, 13-Hα), 2.7645 (dd, J = 14, 6.8 Hz, 13-Hβ), 2.8185 (dd, J = 6.4, 3.2 Hz, 23-H), 2.87 (m, 12-H), 3.48(d, J = 1.2 Hz, OCH_3), 3.76 (s, 5″-OCH_3), 4.99 (br s, 4′-Hb), 5.0185 (m, 7-H), 5.098 (s, 2′-H), 5.22 (br s, 4′-Ha), 5.23 (d, J = 6.4 Hz, 24-H), 5.821 (d, J = 15.2 Hz, 1″-H), 6.0755 (d, J = 2.8 Hz, 19-H), 6.177 (dd, J = 15.2, 11.2 Hz, 2″-H), 7.1215 (d, J = 11.6 Hz, 3″-H), 7.30−7.45 (m, Ar-H_5), 7.723 (s, 16-H), ^{13}C NMR (100 MHz, CD_2Cl_2) δ 12.18 (C30), 12.80 (C6″), 15.16 (C28), 19.49 (C29), 17.79 (C5′), 23.68 (C6), 24.25 (C10), 23.47 (C33), 25.55 (C11), 27.76 (C13), 29.91 (C31), 30.10 (C34), 31.97 (C32), 32.21 (C5), 39.02 (C4), 45.68 (C9), 46.01 (C8), 48.00 (C12), 51.99 (5″-OCH_3), 55.77 ((MTPA)−OCH_3), 55.99 (C3), 58.22 (C23), 58.80 ((MTPA)−CF_3), 71.44 ((MTPA)−C2), 72.59 (C20), 73.87 (C22), 75.34 (C24), 76.24 (C5′), 81.43 (C7), 113.5 (C26), 116.74 (C16), 117.57 (C4′), 121.73 (C15), 122.08 (C19), 122.83 (C14), 125.98 (C2″), 126.69 (C4″), 127.56 (2C, (MTPA)−Ar), 128.58 (2C, (MTPA)−Ar), 129.83 ((MTPA)−Ar), 134.11 (C17), 135.90 (C18), 138.43 (C3″), 138.50 (C25), 140.14 (C3′), 150.91 (C1″), 154.26 (C2), 161.97 (C27), 165.94 ((MTPA)-C1), 168.97 (C5″), 197.84 (C1′); HRFAB-MS (*m/z*) 910.4394 (low intensity, M + H; calcd for $C_{54}H_{63}NO_8F_3$, 910.4505), 892.4331 (M + H − H_2O; calcd for $C_{54}H_{61}NO_7F_3$, 892.4399).

C2′-(*R*)-Nodulisporic Acid A Methyl Ester (2). To a solution of methyl ester **1b** (20 mg) in CH_3CN (4 mL) and CH_2Cl_2 (1 mL) was added excess DMAP (80 mg). The solution was stirred at room temperature for 48 h. TLC examination of the reaction mixture indicated the formation of a less polar major product. Solvent was removed from the reaction mixture, and the product was purified on a preparative silica gel plate (hexane−EtOAc, 3:2). Two bands were eluted with EtOAc. The more polar band gave 5.5 mg of unreacted **1b**, and the less polar band afforded 9.9 mg of epimeric compound **2** as a yellow amorphous powder: $[\alpha]^{22}_D$ −57° (*c* 0.99, $CHCl_3$); IR (ZnSe) ν_{max} 3511, 2974, 1707, 1635, 1587, 1459, 1378, 1289, 1244, 1229, 1077, 1023, 980, cm^{-1}; ^{1}H NMR (400 MHz, CD_2Cl_2) (assignments based on COSY, HMQC, and HMBC experiments) δ 1.06 (s, 30-H_3), 1.09 (s, 29-H_3), 1.148 (s, 28-H_3), 1.154 (s, 33-H_3), 1.32 (s, 32-H_3), 1.35 (s, 31-H_3), 1.43 (s, 34-H_3), 1.47 (m, 10-H_2), 1.57, 1.78 (m, 5-H_2), 1.74 (m, 9-H), 1.76, 1.78 (m, 6-H_2), 1.60, 1.76 (m, 11-H_2), 1.79 (s, 5′-H_3), 1.96 (d, J = 1.2 Hz, 6″-H_3), 2.43 (dd, J = 13.6, 10.8 Hz, 13-Hα), 2.71 (dd, J = 13.6, 6.8 Hz, 13-Hβ), 2.78 (dd, J = 6.4, 2.8 Hz, 23-H), 2.80 (m, 12-H), 3.38 (m, 7-H), 3.74 (s, 5″-OCH_3), 4.88 (s, 4′-Hb), 5.03 (s, 2′-H), 5.17 (apparent t, 4′-Ha), 5.25 (d, J = 6.0 Hz, 24-H), 5.88 (d, J = 15.6 Hz, 1″-H), 6.05 (d, J = 2.8 Hz, 19-H), 6.40 (dd, J = 15.6, 11.2 Hz, 2″-H), 7.22 (d, J = 11.2 Hz, 3″-H), 7.71 (s, 16-H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 11.15 (C30), 12.95 (C6″), 14.12 (C28), 19.28 (C5′), 19.74 (C29), 23.55 (C33), 24.96 (C10), 25.71 (C11), 26.14 (C6), 27.64 (C13), 29.91 (C31), 30.13 (C34), 31.98 (C32), 33.97 (C5), 39.24 (C4), 44.92 (C9), 47.68 (C8), 49.93 (C12), 51.98 (5″OCH$_3$), 54.73 (C3), 58.19 (C23), 72.58 (C20), 73.86 (C22), 75.38 (C24), 75.64 (C2), 76.62 (C7), 112.96 (C26), 115.25 (C4′), 116.74 (C16), 121.41 (C15), 121.76 (C14), 122.07 (C19), 126.02 (C2″), 126.15 (C4″), 134.46 (C17), 136.21 (C18), 138.33 (C25), 138.53 (C3″), 139.81 (C3′), 153.17 (C1″), 154.72 (C2), 160.75 (C27), 169.05 (C5″), 198.06 (C1′); HRFAB-MS (*m/z*) 694.4106 (M + H, calcd for $C_{44}H_{56}NO_6$, 694.4107).

Oxidation Product 3a of Nodulisporic Acid A: ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.08 (s, 30-H_3), 1.13 (s, 34-H_3), 1.15 (s, 28-H_3), 1.30 (s, 32-H_3), 1.34 (s, 31-H_3), 1.41 (s, 33-H_3), 1.45 (s, 29-H_3), 1.90 (s, 5′-H_3), 1.94 (d, J = 1.2 Hz, 6″-H_3), 2.47 (dd, J = 3.7, 11.8 Hz, 13-Hα), 2.81 (dd, J = 3.0, 6.6 Hz, 23-H), 2.96 (m, 12-H), 3.31 (dd, J = 4.6, 11.2 Hz, 7-H), 3.56 (dd, J = 5.7, 11.8 Hz, 13-Hβ), 5.03 (br s, 4′-Hb), 5.07 (br s, 4′-Ha), 5.14 (s, 2′-H), 5.20 (d, J = 6.6 Hz, 24-H), 5.86 (d, J = 15.4 Hz, 1″-H), 6.20 (d, J = 3.0 Hz, 19-H), 6.38 (dd, J = 11.3, 15.4 Hz, 2″-H), 7.28 (br d, J = 11.3 Hz, 3″-H), 8.28 (s, 16-H); ^{13}C NMR (125 MHz, CD_2Cl_2) δ 11.6 (C30), 12.6 (C6″), 15.6 (C28), 19.1 (C29), 19.5 (C5′), 22.9 (C10), 23.3 (C34), 26.4 (C6), 27.5 (C11), 29.5 (C31), 29.9 (C33), 30.7 (C5), 31.5 (C32), 42.0 (C12), 44.2 (C9), 45.2 (C4), 45.4 (C13), 47.5 (C8), 56.0 (C3), 57.4 (C23), 72.7 (C20), 74.0 (C22), 75.5 (C2′), 75.5 (C24), 76.8 (C7), 112.5 (C4′), 123.3 (C26), 125.2 (C4″), 126.3 (C2″), 126.3 (C19), 128.7 (C16), 129.1 (C15), 133.9 (C17), 136.1 (C18), 139.9 (C3′), 140.7 (C3″), 151.1 (C25), 154.2 (C1″), 153.4 (C27), 172.4 (C5″), 174.5 (C2), 197.8 (C14), 198.6 (C1′).

Oxidation Product 3b of Nodulisporic Acid A: ^{1}H NMR (500 MHz, CD_2Cl_2) δ 1.04 (s, 30-H_3), 1.13 (s, 34-H_3), 1.15 (s, 28-H_3), 1.30 (s, 32-H_3), 1.34 (s, 31-H_3), 1.41 (s, 33-H_3), 1.44 (s, 29-H_3), 1.90 (s, 5′-H_3), 1.97 (d, J = 1.2 Hz, 6″-H_3), 2.47 (dd, J = 3.7, 11.8 Hz, 13-Hα), 2.81 (dd, J = 3.0, 6.6 Hz, 23-H), 2.96 (m, 12-H), 3.27 (dd, J = 4.6, 11.2 Hz, 7-H), 3.56 (dd, J = 5.7, 11.8 Hz, 13-Hβ), 5.03 (br s, 4′-Hb), 5.07 (br s, 4′-Ha), 5.14 (s, 2′-H), 5.20 (d, J = 6.6 Hz, 24-H), 5.66 (d, J = 15.4 Hz, 1″-H), 6.20 (d, J = 3.0 Hz, 19-H), 7.16 (dd, J = 11.2, 15.4 Hz, 2″-H), 6.56 (br d, J = 11.2 Hz, 3″-H), 8.28 (s, 16-H).

7-(*p*-Bromobenzoyl)-24-deoxy-23,24-dehydronodulisporic Acid A Methyl Ester (4a). To a solution of **1c** (28 mg, 0.032 mmol) in CH_2Cl_2 (0.7 mL) were added 1,8-bis(dimethylamino)naphthalene, *N*,*N*,*N*′,*N*′-tetramethyl-1,8-naphthalenediamine (Proton Sponge, 30 mg, 0.14 mmol), and trimethyloxonium tetrafluoroborate (20 mg, 0.14 mmol), and the solution was stirred overnight. A single, dark yellow, less polar product was formed (TLC, hexane−EtOAc, 7:4). The crude reaction mixture was directly charged onto silica gel preparative plates and developed in the same solvent. The main band was eluted with EtOAc to give 24 mg (87.5%) of pure **4a** as a yellow powder: IR (ZnSe) ν_{max} 2977, 1713, 1636, 1590, 1438, 1374, 1266, 1230, 1174, 1099, 1067, 1013, 850, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) (only distinct shifts have been listed) δ 0.97 (s, 28-H_3), 1.20 (s, 3H), 1.28 (s, 3H), 1.46 (br s, 3H), 1.49 (s, 6H), 1.56 (s, 3H), 1.57 (s, 3H), 1.80 (d, J = 1.2 Hz, 6″-H_3), 2.31 (dd, J = 14, 10.4 Hz, 13-Hα), 2.74 (dd, J = 14, 6.4 Hz, 13-Hβ), 2.78 (m, 12-H), 3.71 (s, 5″-OCH_3), 4.92 (m, 7-H), 4.93 (br s, 4′-Hb), 4.97 (s, 2′-H), 5.12 (br s, 4′-Ha), 5.87 (d, J = 15.6 Hz, 1″-H), 6.26 (dd, J = 15.6, 11.2 Hz, 2″-H), 6.76 (d, J = 2.0 Hz, 19-H), 6.85 (dd, J = 1.6, 0.4 Hz, 24-H), 7.11 (d, J = 11.2 Hz, 3″-H), 7.55 (d, J = 8.4 Hz, Ar-H_2), 7.78 (s, 16-H), 7.81 (d, J = 8.4 Hz, Ar-H_2), HRFAB-MS (*m/z*) 858.3398 (M + H, calcd for $C_{51}H_{57}NO_6Br$, 858.3369).

7-(*p*-Bromobenzoyl)-24-(methoxymethyl)-1′β-hydroxynodulisporic Acid A (5a). Sodium borohydride (10 mg) was added to a solution of **1d** (14 mg) in a mixture of THF (0.4 mL) and MeOH (0.4 mL) and stirred at room temperature. The reaction mixture almost instantaneously became colorless, and the reaction was complete within 10 min. A single product was formed in the reaction (TLC, hexane−EtOAc, 7:3). Acetone was added to consume excess reducing agent. Solvents were removed under a stream of N_2. The product was purified on preparative silica gel plates (hexane−EtOAc, 7:3), and the band was eluted with EtOAc, which gave **5a** (10 mg) as a light yellow powder: IR (ZnSe) ν_{max} 3485, 2975, 1714, 1636, 1590, 1438, 1365, 1268, 1246, 1100, 1013, 756 cm^{-1}; ^{1}H NMR (400 MHz, CD_3CN) δ 0.97 (s, 28-H_3), 1.06 (s, 33-H_3), 1.16 (br s, 29-H_3), 1.27 (s, 30-H_3), 1.28 (s, 31-H_3, 32-H_3), 1.42 (s, 34-H_3), 1.44, 1.51 (m, 10-H_2), 1.53, 1.74 (m, 11-H_2), 1.73 (d, J = 1.2 Hz, 6″-H_3), 1.86 (m, 9H), 1.92 (m, 6-H_2), 1.92 (br s, 5′-H_3), 1.93, 2.10 (m, 5-H_2), 2.23 (dd, J = 14, 11 Hz, 13-Hα), 2.65 (dd, J = 14, 6.4 Hz, 13-Hβ), 2.77 (m, 12-H), 2.88 (dd, J = 6.0, 3.0 Hz, 23-H), 3.39 (s, (MOM)−OCH_3), 3.65 (s, 5″-OCH_3), 4.20 (br s, 1′-OH), 4.86 (s, (MOM)−OCH_2−O), 4.92 (br s, 4′-Hb), 4.95 (m, 7-H), 5.06 (br s, 4′-Ha), 5.13 (br d, J = 5.4 Hz, 24-H), 5.32 (very broad s, 2′-H), 5.99 (d, J = 15.4 Hz, 1″-H), 6.04 (d, J

= 2.9 Hz, 19-H), 6.20 (br t, J = 7.0 Hz, 1′-H, collapsed to a doublet, J_{23-24H}= 6.8 Hz, after decoupling at δ 4.20, and a broad doublet after decoupling at 5.33 ppm), 6.33 (dd, J = 15.64, 11.2 Hz, 2″-H), 7.02 (d, J = 11.1 Hz, 3″-H), 7.37 (s, 16-H), 7.64 (d, J = 8.8 Hz, Ar-H_2), 7.84 (d, J = 8.8 Hz, Ar-H_2); HRFAB-MS (*m/z*) 922.3890 (M + H; calcd for $C_{53}H_{65}NO_8Br$, 922.3893).

7-(*p*-Bromobenzoyl)-1′*β*- and -1′α-hydroxynodulisporic Acid A (5b and 5c). Sodium borohydride reduction of **1c** (14 mg) in a manner similar to that described above, followed by purification by preparative TLC, gave 11 mg of **5b** and 1.1 mg of **5c**, both as amorphous powders.

5b: IR (ZnSe) ν_{max} 3448, 2975, 1713, 1591, 1437, 1365, 1269, 1247, 1099, 1067, 1013, 993, 756 cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.00 (s, 28-H_3), 1.11 (s, 33-H_3), 1.13 (br s, 29-H_3), 1.26 (s, 31-H_3), 1.28 (s, 30-H_3), 1.30 (s, 32-H_3), 1.37 (s, 34-H_3), 1.45, 1.50 (m, 10-H_2), 1.53, 1.72 (m, 11-H_2), 1.73 (d, J = 1.2 Hz, 6″-H_3), 1.86 (m, 9H), 1.93 (m, 6-H_2), 1.93 (br s, 5′-H_3), 1.93, 2.10 (m, 5-H_2), 2.23 (dd, J = 14, 10.8 Hz, 13-Hα), 2.639 (dd, J = 5.6, 2.8 Hz, 23-H), 2.643 (dd, J = 13.6, 10.8 Hz, 13-H*β*), 2.75 (m, 12-H), 3.65 (s, 5″-OCH_3), 4.00 (br s, 24-OH), 4.50 (br s, 1′-OH), 4.94 (m, 7-H), 4.95 (br s, 4′-Hb), 4.97 (apparent t, J = 1.6 Hz, 4′-Ha), 5.13 (dd, J = 5.6, 2.8 Hz, 24-H), 5.34 (very broad s, 2′-H, sharpened after decoupling δ 6.29 ppm), 5.99 (d, J = 15.6 Hz, 1″-H), 6.04 (d, J = 3.2 Hz, 19-H), 6.29 (br dd, J = 6.8, 4.4 Hz, 1′-H, collapsed to a doublet, J_{23-24H} = 6.4 Hz, after decoupling at δ 4.50 ppm and a broad doublet after decoupling at δ 5.33 ppm), 6.33 (dd, J = 15.6, 11.2 Hz, 2″-H), 7.02 (d, J = 11.6 Hz, 3″-H), 7.32 (s, 16-H), 7.64 (d, J = 8.8 Hz, Ar-H_2), 7.84 (d, J = 8.8 Hz, Ar-H_2); ^{13}C NMR (400 MHz, CD_3CN) δ 12.71 (C6″), 12.79 (C30), 15.39 (C28), 19.74 (C29), 23.53 (C33), 24.15 (C6), 24.18 (very weak in broad-band and DEPT spectrum due to exchange broadening, assigned by HMQC, C5′), 24.88 (C10), 26.09 (C11), 28.41 (C13), 30.19 (C32), 30.42 (C34), 32.21 (C31), 32.69 (C5), 39.55 (C4), 46.14 (C8), 46.93 (C9), 47.94 (C12), 52.19 (5″-OCH_3), 56.2 (C3), 59.52 (C23), 72.95 (C20), 74.28 (C22), 75.44 (C24), 76.45 (C2′ by HMQC only), 79.05 (C1′ by HMQC only), 80.10 (C7), 109.65 (C16), 112.32 (C26), 117.63 (C4″ by HMQC only), 120.81 (C19), 125.60 (C2″), 126.39 (C4″), 128.30, 130.30, 131.92, 132.73 (all benzoate), 134.31 (C17), 137.57 (C25), 139.03 (C3″), 152.64 (C2), 153.17 (C1″), 160.77 (C27), 165.70 (benzoate), 169.25 (C5″) (signals for C14, C15, and C18 were not detected, probably due to exchange broadening); HRFAB-MS (*m/z*) 878.3635 (M + H, calcd for $C_{51}H_{61}NO_7Br$, 878.3631).

5c: IR (ZnSe) ν_{max} 3448, 2972, 1713, 1636, 1591, 1438, 1375, 1268, 1247, 1101, 1068, 1012, 846, 756 cm^{-1}; ^1H NMR (400 MHz, CD_3CN) δ 1.09 (s, 28-H_3), 1.14 (s, 33-H_3), 1.17 (br s, 29-H_3), 1.26 (s, 31-H_3), 1.29 (s, 30-H_3), 1.30 (s, 34-H_3, 32-H_3), 1.38 (s, (br s, 5′-H_3),), 1.45, 1.50 (m, 10-H_2), 1.55, 1.75 (m, 11-H_2), 1.65 (m, 9H), 1.73 (d, J = 1.2 Hz, 6″-H_3), 1.93 (m, 6-H_2), 1.95−2.05 (m, 5-H_2), 2.38 (dd, J = 13.6, 11.2 Hz, 13-Hb), 2.59 (dd, J = 13.6, 6.8 Hz, 13-Ha), 2.64 (dd, J = 5.6, 2.8 Hz, 23-H), 2.75 (m, 12-H), 3.33 (d, J = 7 Hz, 24-OH), 3.65 (s, 5″-OCH_3), 4.66 (broad hump, 4′-Hb), 4.91 (dd, J = 12, 4.4 Hz, 7-H), 5.04 (t, J = 6.8 Hz, 24-H), 5.08 (br s, 4′-Ha), 5.29 (broad hump, 2′-H, sharpened after decoupling δ 6.25 ppm), 5.98 (d, J = 15.6 Hz, 1″-H), 5.99 (d, J = 2.8 Hz, 19-H), 6.25 (d, J = 2.8 Hz, 1′-H), 6.34 (dd, J = 15.6, 11.2 Hz, 2″-H), 7.02 (d, J = 11.2 Hz, 3″-H), 7.30 (s, 16-H), 7.64 (d, J = 8.8 Hz, Ar-H_2), 7.84 (d, J = 8.8 Hz, Ar-H_2); HRFAB-MS (*m/z*) 878.3582 (M + H, calcd for $C_{51}H_{61}NO_7Br$, 878.3631).

7-(*p*-Bromobenzoyl)-23,24-dehydro-24-deoxy-1′*β*-hydroxynodulisporic Acid A (4b). When dissolved in $CDCl_3$ (and even, on occasion, in CD_2Cl_2), **5a** tended to give the elimination product **4b** as a yellow powder: IR (ZnSe) ν_{max} 3488, 2976, 1713, 1636, 1591, 1435, 1373, 1265, 1230, 1173, 1099, 1067, 1012, 993, 847, 756 cm^{-1}; ^1H NMR (400 MHz, CD_2Cl_2) (only distinct shifts listed) δ 0.99 (br s, 3H), 1.17 (br s, 3H), 1.29 (s, 3H), 1.45 (br s, 6H), 1.53 (s, 9H), 1.79 (d, J = 1.6 Hz, 6″-H_3), 2.25 (dd, J = 13.2, 11.6 Hz, 13-Hb), 2.69 (dd, J = 13.6, 6.4 Hz, 13-Ha), 2.77 (m, 12-H), 3.68 (s, 5″-OCH_3), 4.02 (broad hump, 1′-OH), 4.95 (m, 7-H), 5.10 (br s, 4′-Hb), 5.27 (broad hump, 2′-H), 5.91 (d, J = 15.2 Hz, 1″-H), 6.30 (dd, J = 15.6, 11.2 Hz, 2″-H), 6.55 (d, J = 1.6 Hz, 19-H), 6.58 (dd, J = 1.6 Hz, 24-H), 7.10 (d, J = 11.2 Hz, 3″-H), 7.47 (s, 16-H), 7.59 (d, J = 8.8 Hz, Ar-H_2), 7.84 (d, J = 8.8 Hz, Ar-H_2); HRFAB-MS (*m/z*) 860.3472 (M + H, calcd for $C_{51}H_{59}NO_6Br$, 860.3526).

Acknowledgment. We thank Gabe Dezeny for fermentation support and Kevin Byrne for furnishing ^{13}C-labeled nodulisporic acid A.

JA971664K