Neomangicols: Structures and Absolute Stereochemistries of Unprecedented Halogenated Sesterterpenes from a Marine Fungus of the Genus *Fusarium*

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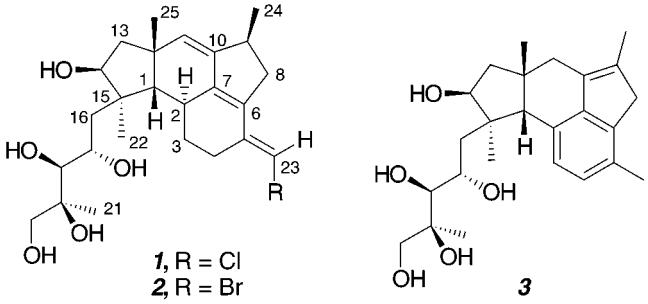
Three novel sesterterpenes, neomangicols A−C (**1**−**3**) were isolated from the mycelial extract of a marine fungus belonging within the genus *Fusarium*. The carbon skeleton of the neomangicols is undescribed and constitutes a new class of C_{25} rearranged sesterterpenes. The structures of the new metabolites were determined by 1D and 2D NMR methods, and the absolute configuration of **3** was determined by Mosher ester analysis of a diacetonide derivative. The configurations of the three stereocenters in the side-chain were assigned on the basis of molecular modeling and NOESY NMR correlations observed for several diacetonide derivatives of **3**. Neomangicols A and B are cytotoxic against HCT-116 human colon carcinoma in in vitro evaluation, while neomangicol B inhibits the growth of the Gram-positive bacterium *Bacillus subtilus* with a potency similar to that of the antibiotic gentamycin.

Introduction

Marine microorganisms, particularly marine fungi, represent an underdeveloped and potentially prolific source of structurally diverse secondary metabolites.[1] As part of our interest in the biomedical potential of marine microorganisms, we have focused considerable attention on marine fungi, which have been shown to produce novel metabolites with antibiotic[2] and antitumor[3] properties. In connection with our continuing interest in the development of new agents for the treatment of cancer, we investigated the secondary metabolites produced in culture by a marine fungus, strain CNC-477, isolated from the surface of driftwood collected in the Bahamas Islands. Under various saline culture conditions, this microorganism yielded three novel sesterterpenes, neomangicols A−C (**1**−**3**), which possess unprecedented and apparently rearranged C_{25} carbon skeletons. Neomangicols A and B were found to possess significant in vitro cytotoxicity toward HCT-116 human colon tumor cell line, while neomangicol B showed antibiotic properties toward the Gram-positive bacterium *Bacillus subtilus*. Neomangicol C (**3**) was inactive in our biological assays.

Results and Discussion

Fusarium sp. strain CNC-477 was isolated from a driftwood sample collected in a mangrove habitat at Sweetings Cay, Bahamas, in 1995.[4] The fungal isolate was cultured in a seawater-based medium, and the mycelium was separated by filtration and extracted. Bioassay-guided fractionation[5] of the crude mycelium extract involving C-18 flash chromatography,[6] Sephadex LH-20 chromatography, and reversed-phase C-18 HPLC afforded neomangicols A (**1**, 8 mg/L) and B (**2**, 1 mg/L). In a separate cultivation experiment in which the mycelium extract was chromatographed using silica gel, the aromatic compound neomangicol C (**3**) was the sole neomangicol obtained.



The molecular formula of neomangicol A (**1**) was assigned as $C_{25}H_{37}ClO_5$ on the basis of HRFABMS data and overall NMR information. The ^{1}H NMR spectrum in methanol-d_4 (Table 1) showed two noncoupled alkene protons, four methyl groups, and thirteen complex proton resonances between δ 1.4 and 2.9, suggestive of a polycyclic terpenoid structure. The chemical shifts of the remaining five signals (between δ 3.4 and 3.9) were indicative of protons attached to carbons bearing either hydroxyl or ether functionalities. Five exchangeable

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(3) (a) Numata, A.; Iritani, M.; Yamada, T.; Minoura, K.; Matsumura, E.; Yamori, T.; Tsuruo, T. *Tetrahedron Lett.* **1998**, *38*, 8215. (b) Takahashi, C.; Takai, Y.; Kimura, Y.; Numata, A.; Shigematsu, N.; Tanaka, H. *Phytochemistry* **1995**, *38*, 155. (c) Numata, A.; Takahashi, C.; Ito, Y.; Minoura, K.; Yamada, T.; Matsuda, C.; Nomoto, K. *J. Chem. Soc., Perkin Trans. 1* **1996**, 239. (d) Takahashi, C.; Numata, A.; Matsumura, E.; Minoura, K.; Eto, H.; Shingu, T.; Ito, T.; Hasegawa, T. *J. Antibiot.* **1994**, *47*, 1242. (e) Takahashi, C.; Numata, A.; Yamada, T.; Minoura, K.; Enomoto, S.; Konishi, K.; Nakai, M.; Matsuda, C.; Nomoto, K. *Tetrahedron Lett.* **1996**, *37*, 655.

(4) This fungus was identified as a strain of *Fusarium heterosporum* by David Porter (U. Georgia) based on morphological characteristics and as *Fusarium* sp. by FAME analysis (Microbial I.D., Inc., Newark, DE) with a similarity index of 0.965.

(5) Fractions were bioassayed against HCT-116, a human colon cancer cell line.

(6) Blunt, J. W.; Calder, V. L.; Fenwick, G. D.; Lake, R. J.; McCombs, J. D.; Munro, M. H. G.; Perry, N. G. *J. Nat. Prod.* **1987**, *50*, 290.

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Table 1. NMR Spectral Data for Neomangicol A (1) in Methanol-d_4

no.	^{1}H shift, δ (m, J Hz)	^{13}C shift, δ	COSY	HMBC
1	1.89 (d, 1)	55.4	H-2	C-2, C-3, C-7, C-11, C-12, C-15, C-16, C-22, C-25
2	2.33 (br d, 13)	32.0	H-1,3α	–
3α	2.00 (m)	34.5		overlapped
β	1.37 (dq, 4, 12.5)		H-3α,4α,4*β*	C-1, C-2, C-4, C-5, C-7
4α	2.29 (tt, 3.5, 13)	28.8	H-3*β*,4*β*	C-5
β	2.90 (br d, 17)		H-3*β*,4α	C-2, C-3, C-5, C-6, C-23
5	–	138.2		
6	–	133.0		
7	–	144.5		
8α	2.79 (ddd, 3, 8.5, 15)	39.8	H-8*β*	C-6, C-7, C-9, C-10
β	2.00 (m)			overlapped
9	2.84 (dq, 2, 5.5)	36.6	H-8*β*,Me-24	C-8, C-10, C-11, C-24
10	–	146.6		
11	5.30 (s)	128.2		C-1, C-7, C-9, C-12, C-13, C-25
12	–	43.9		
13α	2.10 (dd, 5.5, 15)	47.6	H-13*β*,14	C-1, C-11, C-12, C-14, C-25
β	1.66 (d, 15)		H-13α,14	C-1, C-11, C-12, C-14, C-15, C-25
14	3.88 (d, 5.5)	80.0	H-13α,13*β*	C-1, C-12, C-22
15	–	52.3		
16α	1.49 (dd, 6.5, 14)	43.0	H-16*β*,17	C-1, C-14, C-15, C-17, C-22
β	2.14 (dd, 1, 16)		H-16α	C-1, C-14, C-15, C-17, C-18, C-22
17	3.90 (ddd, 2, 7, 9)	71.0	H-16α,18	C-15, C-16, C-18
18	3.41 (d, 9)	75.9	H-17	C-16, C-17, C-19, C-20, C-21
19	–	76.8		
20	3.58 (d, 12)	69.3	H-20b	C-18, C-19, C-21
	3.46 (d, 12)		H-20a	C-18, C-19, C-21
21	1.22 (s, 3H)	19.5		C-18, C-19, C-20
22	0.85 (s, 3H)	21.5		C-1, C-14, C-15, C-16
23	5.94 (s)	112.6		C-4, C-5, C-6
24	1.17 (d, 3H, 6.5)	18.5	H-9	C-8, C-9, C-10
25	1.10 (s, 3H)	35.5		C-1, C-11, C-12, C-13

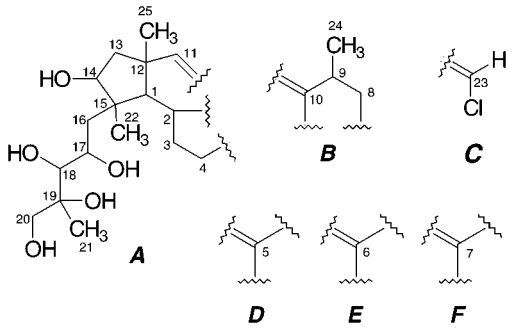


Figure 1. Substructures of neomangicol A (**1**).

hydroxyl protons were later assigned by their presence in a ^{1}H NMR spectrum of **1** recorded in acetone-d_6.[7]

The ^{13}C NMR spectrum of **1** showed signals characteristic of six alkene carbons (four quaternary), five oxygenated carbons (between δ 69.3 and 80.0), and 14 carbons between δ 18.5 and 55.4, while the DEPT spectrum indicated that four CH_3, six CH_2, eight CH, and seven quaternary carbons were present. The UV absorption at 284 nm, when considered with the lack of carbonyl carbons, indicated that the three alkenes were present as a conjugated triene. The remaining four degrees of unsaturation required that the molecule possess four carbocyclic rings.

Several substructures in neomangicol A (**1**) (Figure 1) were established using a combination of homo- and heteronuclear 2D NMR techniques. The proton NMR COSY spectrum (Table 1) showed correlations allowing connections between C-16 and C-18, between C-13 and C-14, and between C-1 and C-4 to be established. These fragments could be defined using data obtained from HMBC experiments. Correlations from methyl protons, in particular, led to the confident assignment of substructure A. The location of the ring junction, for example, was defined on the basis of an HMBC correlation between the C-25 methyl protons and C-1. This was confirmed by complementary HMBC correlations between H-1 and C-11, C-12, and C-25.

Substructure B was assigned by interpretation of proton COSY data which showed correlations between H-8α, H-8*β*, and H-9, and by analysis of HMBC data which showed correlations between the C-24 methyl protons and C-8, C-9, and C-10. What remained to be assigned were one alkene proton, four carbons, and one chlorine substituent. The unusually high field chemical shift of C-23 (δ 112.6), indicative of a vinyl chloride group, led to the definition of substructure C. The remaining three carbons were quaternary alkene carbons (substructures D–F). Substructures A–F were connected using a number of key HMBC NMR correlations. Substructures A and B, for example, were linked by correlations between H-9 and C-11 and between H-11 and C-9. Substructures A and C were connected by the observation of HMBC correlations between H-4α and C-23 and between H-23 and C-4. Finally, numerous HMBC correlations were used to assign the quaternary alkene carbons at C-5 (correlations with H-3*β*, H-4α, H-4*β*, and H-23), C-6 (correlations with H-4*β*, H-8α, and H-23), and C-7 (correlations with H-1, H-3*β*, H-8α, and H-11). Applying these combined NMR methods resulted in the unambiguous assignment of all protons and carbons as listed in Table 1 and allowed the complete planar structure for **1** to be assigned.

The ^{1}H and ^{13}C NMR data for neomangicol B (**2**) (Table 2) were very similar to those of neomangicol A. HR-FABMS data indicated the molecular formula $C_{25}H_{37}BrO_5$ for neomangicol B, suggesting that the only difference between **1** and **2** was the identity of the halogen sub-

(7) The ^{1}H NMR chemical shifts for these protons were δ 5.28, 4.70, 4.63, 4.53, and 3.89.

Table 2. NMR Spectral Data for Neomangicol B (2) in Methanol-d_4

no.	^{1}H shift, δ (m, J Hz)	^{13}C shift, δ	COSY	HMBC correlations
1	1.88 (d, 2.6)	55.3	H-2	C-2, C-3, C-7, C-11, C-12, C-15, C-22, C-25
2	2.29 (m)	32.1	H-1,3α	–
3α	2.00 (m)	34.7		overlapped
β	1.39 (dq, 4.1, 12.8)		H-3α,4α,4*β*	C-2, C-5, C-7
4α	2.25 (m)	31.4	H-3*β*,4*β*	C-5
β	2.90 (m)		H-3*β*,4α	C-2, C-3, C-5, C-6, C-23
5	–	140.9		
6	–	133.7		
7	–	144.5		
8α	2.79 (ddd, 3, 8.5, 15)	39.9	H-8*β*	C-6, C-7, C-24
β	2.00 (m)			overlapped
9	2.84 (m)	36.5	H-8*β*,Me-24	C-8, C-10, C-11, C-24
10	–	146.5		
11	5.33 (s)	128.6		C-1, C-7, C-9, C-12, C-13
12	–	43.9		
13α	2.09 (dd, 5, 14.5)	47.5	H-13*β*,14	C-1, C-11, C-12, C-14, C-25
β	1.66 (d, 14)		H-13α,14	C-1, C-11, C-12, C-14, C-15, C-25
14	3.87 (d, 5)	79.9	H-13α,13*β*	C-1, C-12, C-22
15	–	52.3		
16α	1.48 (dd, 6.9, 15.3)	42.9	H-16*β*,17	C-1, C-14, C-15, C-17, C-22
β	2.13 (d, 14)		H-16α	C-1, C-14, C-15, C-17, C-18, C-22
17	3.89 (ddd, 1.5,1.5, 7)	71.0	H-16α,18	C-15, C-18
18	3.41 (d, 8.7)	75.8	H-17	C-16
19	–	76.8		
20	3.57 (d, 11.2)	69.3	H-20b	C-18, C-21
	3.44 (d, 11.2)		H-20a	C-17, C-18, C-19, C-21
21	1.21 (s, 3H)	19.4		C-18, C-19, C-20
22	0.84 (s, 3H)	21.5		C-1, C-14, C-15, C-16
23	6.06 (s)	102.3		C-4, C-5, C-6
24	1.17 (d, 3H, 6.5)	18.5	H-9	C-8, C-9, C-10
25	1.10 (s, 3H)	35.5		C-1, C-11, C-12, C-13

Table 3. NMR Spectral Data for Neomangicol C (3) in Methanol-d_4

no.	^{1}H shift, δ (m, J Hz)	^{13}C shift, δ	COSY	HMBC correlations
1	3.04 (s)	54.3		C-3, C-7, C-11, C-12, C-15, C-16, C-22
2	–	128.2		
3	6.96 (d, 7.5)	127.4	H-4	C-1, C-4, C-5, C-6, C-7, C-10
4	6.81 (d, 7.5)	126.5	H-3	C-2, C-3, C-6, C-7, C-8, C-23
5	–	131.2		
6	–	140.3		
7	–	144.5		
8	3.19 (br s, 2H)	42.9		C-6, C-7, C-9, C-10
9	–	136.4		overlapped
10	–	134.3		
11	2.38 (br s, 2H)	34.4		C-1, C-7, C-9, C-10, C-12, C-25
12	–	40.5		
13α	2.08 (dd, 6.7, 12.5)	49.7	H-13α, 14	C-1, C-2, C-11, C-12, C-14, C-15
β	1.79 (dd, 9.6, 12.5)		H-13*β*,14	C-10, C-11, C-12, C-14, C-25
14	4.09 (dd)	81.8	H-13α,13*β*	C-1, C-12, C-13, C-16, C-22
15	–	48.9		
16α	2.16 (dd, 1.4, 15.2)	44.8	H-16α	C-1, C-14, C-15, C-17, C-18, C-22
β	1.75 (dd, 9, 15.2)		H-16*β*	C-1, C-14, C-15, C-17, C-18, C-22
17	4.21 (ddd, 1, 8.8, 8.8)	70.8	H-18	C-15, C-16, C-18, C-19
18	3.42 (d, 8.6)	75.8	H-17	C-16, C-17, C-19, C-20, C-21
19	–	77.0		
20	3.63 (d, 11)	69.4	H-20b	C-18, C-19, C-21
	3.47 (d, 11)		H-20a	C-18, C-19, C-21
21	1.26 (s, 3H)	19.4		C-18, C-19, C-20
22	0.60 (s, 3H)	28.4		C-1, C-15, C-16
23	2.28 (s, 3H)	18.4		C-4, C-5, C-6
24	2.04 (s, 3H)	13.7		C-8, C-9, C-10
25	0.92 (s, 3H)	29.1		C-1, C-11, C-12, C-13

stituent at C-23. The only significant variations in the NMR data were the ^{1}H NMR chemical shift of H-23 (δ 6.06 versus 5.94) and the ^{13}C NMR chemical shift of C-23 (δ 102.3 versus 112.6), both of which were consistent with the presence of a bromine atom instead of a chlorine substituent. As in the case of **1**, comprehensive NMR data allowed all protons and carbons to be assigned, leading to the assignment of the planar structure, **2**, for neomangicol B.

Neomangicol C (**3**) was the only neomangicol isolated from one of our initial fermentations. The molecular formula of neomangicol C was assigned as $C_{25}H_{36}O_5$ on the basis of HRFABMS data. The ^{13}C NMR spectrum of **3** (Table 3) showed characteristic signals for eight aromatic carbons (five quaternary), five oxygenated carbons (between δ 81.8 and 69.4), and twelve carbons between δ 54.3 and 13.7, while the DEPT spectrum indicated five CH_3, five CH_2, six CH, and nine quaternary

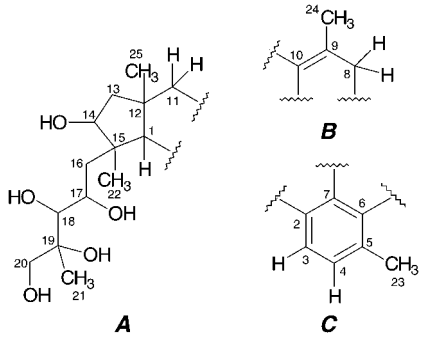
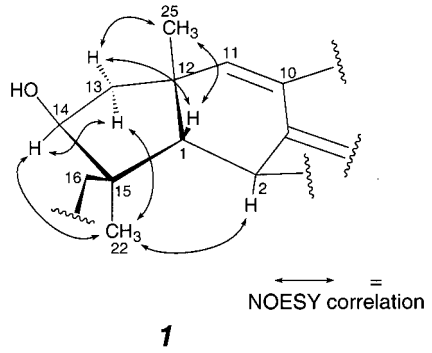


Figure 2. Substructures of neomangicol C (**3**).

carbons. The ^{1}H NMR spectrum of **3** in methanol-d_4 showed two aromatic protons, five methyl groups, and 16 proton resonances between δ 4.21 and 1.75. The ^{1}H NMR shift of five of these protons (between δ 4.21 and 3.42) indicated that they were likely attached to oxygenated carbons. The observation of an unusually deshielded, two-proton band (an AB doublet) at δ 3.19, generated by the presence of an adjacent olefin and aromatic ring, suggested the presence of an indene system. Five hydroxyl protons were again assigned by their observation in a ^{1}H NMR spectrum recorded in acetone-d_6.[8]

As in the case of **1** and **2**, substructures of **3** (Figure 2) were established using a combination of homo- and heteronuclear NMR spectroscopic techniques. Proton NMR COSY correlations enabled connections between C-16 and C-18 and between C-13 and C-14 to be established. Key HMBC correlations from methyl protons were again used to connect these fragments, leading to substructure A. Substructure B was established on the basis of HMBC correlations from the C-24 methyl protons to C-8, C-9, and C-10. This assignment was supported by HMBC correlations from H_2-8 to C-9 and C-10. The ^{1}H and ^{13}C NMR chemical shifts of the remaining protons and carbons were indicative of a trisubstituted aromatic ring. The coupling constant of 7.5 Hz observed between H-3 and H-4 (and the COSY correlation) indicated that these two protons were *ortho*-coupled. HMBC correlations from the C-23 methyl protons (to C-4, C-5, and C-6) led to the assignment of carbons 3−6 in substructure C.

Several important HMBC correlations and ^{1}H NMR chemical shift information led to the connection of the three substructures. For example, HMBC correlations from H-1 to both C-3 and C-7 required that C-1 be connected to C-2. This connection was supported by a complementary HMBC correlation from H-3 to C-1. The benzylic chemical shift of H-1 (δ 3.04) also confirmed this linking of substructures A and C. Substructures A and B were connected on the basis of HMBC correlations from H_2-11 to both C-9 and C-10. The connection of C-10 to C-11 was supported by the chemical shift of H_2-11 (an AB doublet at δ 2.38), consistent with that of an allylic methylene group. The HMBC correlation from H_2-11 to C-7 could only occur through C-10, requiring that C-7 and C-10 be connected. Finally, HMBC correlations from



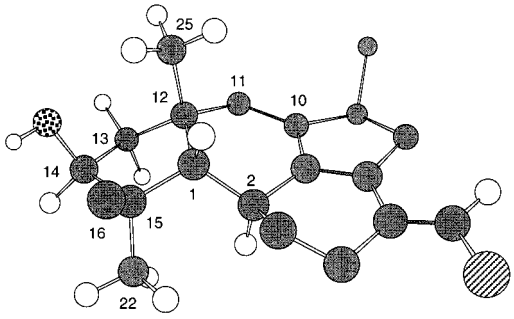


Figure 3. Chem3D representation of the global minimum conformation (Macromodel, MM2 force field, Monte Carlo conformation search) of neomangicol A (**1**).

H_2-8 to both C-6 and C-7 allowed connection of C-6 to C-8, thus completing the indene functionality suggested earlier and allowing the full planar structure of neomangicol C to be assigned.

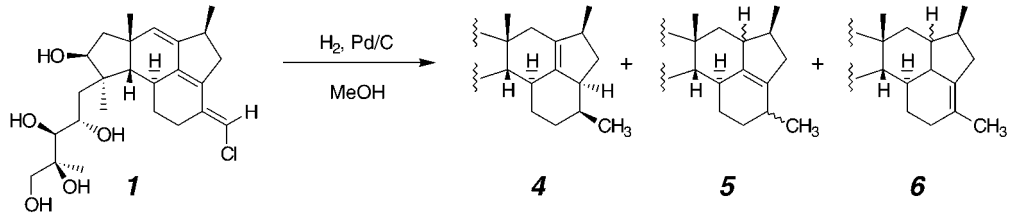
Relative and Absolute Configuration. The relative configurations for the stereocenters in the tetracyclic nuclei of the neomangicols (i.e., C-1, C-2, C-12, C-14, and C-15) were assigned primarily on the basis of NOESY NMR data acquired with neomangicol A (**1**). The C-1/C-12 ring fusion was assigned as cis based upon strong NOESY correlations between H-1, Me-25, and H-13β (Figure 3).[9] Similarly, NOESY correlations between Me-22, H-14, and H-13α established the spatial proximities of these protons on the bottom face of the molecule. A strong NOESY correlation between H-2 and Me-22 also allowed the assignment of H-2 to the bottom face of the molecule (i.e., cis to Me-22). The geometry of the exocyclic double bond was assigned as *E* on the basis of NOESY correlations between H-23 and both H-8α and H-8β.

The stereocenter at C-9, isolated from the remainder of the molecule by the planar triene system, was impossible to define by NOESY or other NMR methods. We predicted, however, that complete or partial hydrogenation of the triene would lead to derivatives for which the configuration at C-9 could be related to that at C-12. Catalytic hydrogenation of **1** using Pd/C in methanol (Scheme 1) resulted in a mixture of tetrahydro-derivatives assigned as neomangicols D−F (**4**−**6**). Data from the NOESY NMR spectrum of **4** allowed unambiguous assignment of the relative configuration of C-9 (Figure 4).[9] An observed NOESY correlation between Me-24 and H-11β (identified by a NOESY interaction to Me-25) showed that Me-24 was on the top face of the molecule (i.e., cis to Me-25). A complementary NOESY correlation was observed between H-9 and H-11α. Neomangicols E and F, obtained as an inseparable mixture (~2:1 E:F),

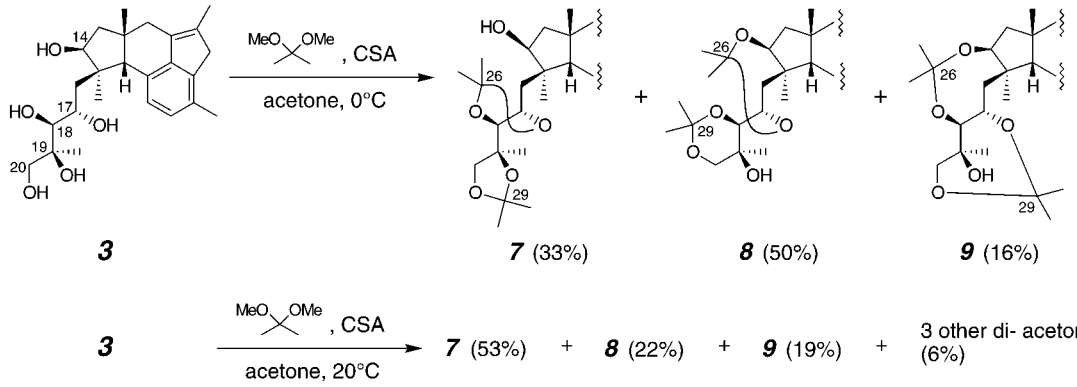
(8) The ^{1}H NMR chemical shifts for these protons were δ 6.19, 5.43, 4.90, 4.56, and 4.20.

(9) The Chem3D structures shown in this and following figures are low-energy conformers identified by a Monte Carlo conformation search performed in Macromodel 6.0 using the MM2 force field. No competing low energy conformers within the range of 2 kcal/mol were defined. Some hydrogens have been hidden for clarity. For this conformation search, the side chain was simulated as a methyl group.

Scheme 1

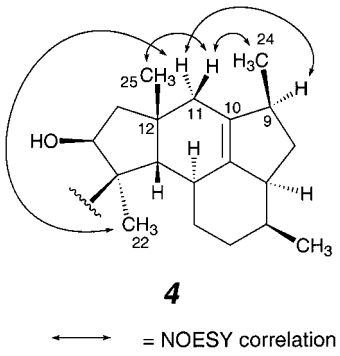


Scheme 2



were identified through analysis of the NMR and mass spectral data of the mixture.

Assignment of the remaining stereocenters in the side-chain, isolated from the rest of the molecule by a methylene group, represented a significant challenge. To approach this difficult problem, we attempted to introduce structural rigidity by preparing several cyclic acetonide derivatives. Neomangicols A and B were found to experience acid instability, thus we chose neomangicol C (**3**) for preparation of the acetonides. Treatment of neomangicol C with 2,2-dimethoxypropane, acetone, and catalytic camphorsulfonic acid at room temperature afforded a complex mixture of products (Scheme 2). Six products, all of which appeared to be diacetonides by NMR and MS analysis, were obtained in high overall yield. Repeating the reaction at 0 °C resulted in a less complicated mixture of products. From the complex mixture, diacetonides **7**−**9** were then purified in facilitating quantities.



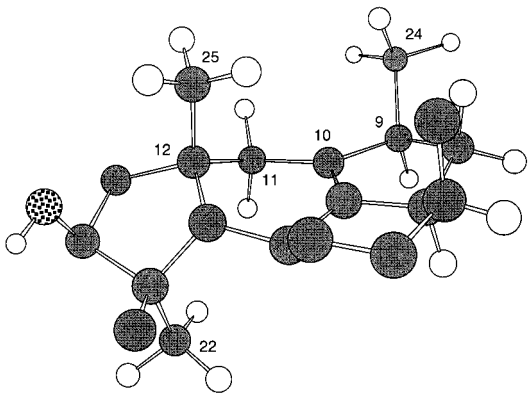


Figure 4. Chem3D representation of the global minimum conformation (Macromodel, MM2 force field, Monte Carlo conformation search) of neomangicol D (**4**).

The nontrivial task of identifying diacetonides **7**−**9** (out of the 15 possible diacetonides) was accomplished using a vareity of 1D and 2D NMR spectral methods. For diacetonide **7**, the ^{1}H NMR signals (in chloroform-*d*) for H-14 (ddd, J = 7, 11, and 11 Hz) and the underivatized hydroxyl (d, J = 11 Hz) indicated that the C-14 hydroxyl was intact. This observation was consistent with three diacetonides: one composed of two five-membered rings (connection of the C-17 hydroxyl to the C-18 hydroxyl and the C-19 hydroxyl to the C-20 hydroxyl), one possessing two six-membered rings (C-17 to C-19 and C-18 to C-20), and another acetonide possessing one five- and one seven-membered ring (C-17 to C-20 and C-18 to C-19). HMBC correlations were observed between H-18 and the acetal carbon at δ 107.7 (C-26) and between both C-20 protons and the acetal carbon at δ 110.2 (C-29), indicating that **7** was not the 6,6-diacetonide isomer. No HMBC correlation was observed between H-17 and an acetal carbon, thus we could not differentiate the 5,5- and 5,7-diacetonide systems based on the HMBC data obtained. Buchanan and Edgar have shown, however, that the ^{13}C NMR chemical shifts of acetonide acetal carbons are related to the size of the acetonide ring. Acetal carbons for five-membered acetonides typically resonate between δ 108 and 111, while those for seven-membered acetonides resonate between δ 101 and 102.[10] The downfield ^{13}C NMR shifts of the two acetal carbons in **7** (δ

(10) Buchanan, J. G.; Edgar, A. R.; Rawson, D. I.; Shahidi, P.; Wightman, R. H., *Carbohydr. Res.* **1982**, *100*, 75.

107.7 and 110.2) were consistent with two five-membered rings; hence, we have assigned **7** as the 5,5-diacetonide.

The ^{1}H NMR signal for the underivatized hydroxyl proton in diacetonide **8** was observed as a singlet at δ 3.70, indicating that the C-19 hydroxyl was underivatized. This was consistent with three possible diacetonide isomers: one with six- and seven-membered rings, one possessing seven- and eight-membered rings, and one with five- and ten-membered rings. Proton H-18 and both C-20 protons showed HMBC correlations with the acetal carbon at C-29. Proton H-14, on the other hand, showed an HMBC correlation with the other acetal carbon at C-26. Once again H-17 showed no HMBC correlation to an acetal carbon, but the aforementioned correlations were sufficient to establish the identity of diacetonide **8**.

The NMR data for diacetonide **9** also showed that the C-19 hydroxyl was underivatized. Therefore, **9** was either the 7,8-diacetonide or the 5,10-membered diacetonide. The C-20 protons showed strong HMBC correlations with the acetal carbon at C-29, while H-14 showed a weak HMBC correlation with C-26. In addition, the acetonide methyls all showed correlations with their corresponding acetal carbons, and H-14 showed a significant NOESY correlation with one of the methyls that correlated to C-26. In the 5,10-system, it would have been impossible for H-14 to interact with either of the acetonide methyls on the five-membered ring; hence, the identity of **9** was established as the 7,8-diacetonide.

The relative configurations of the three side-chain stereocenters were assigned using a combination of NMR methods (NOESY and ^{1}H−^{1}H NMR coupling constants) coupled with molecular modeling techniques. The eight possible stereoisomers for acetonide **7** (*RRR*, *RRS*, etc.)[11] were modeled both by hand and by Monte Carlo conformation searching[12] using the MM2 force field[13] in Macromodel.[14,15] Comparison of these models with NMR data allowed elimination of inconsistent possibilities. All four of the models for which C-17 and C-18 have the opposite configuration (i.e., *RSR*, *RSS*, *SRR*, and *SRS*) had H-17 and H-18 in a trans orientation. The NOESY spectrum of **7** showed a significant correlation between H-17 and H-18, inconsistent with a trans orientation. In addition, the observed ^{1}H−^{1}H NMR coupling constant between these two protons was only 6.9 Hz, more consistent with a cis orientation for these protons (Figure 5). Thus, four of the eight possible stereoisomers were eliminated. The possibility of rotation about the C-15,16; C-16,17; and C-18,19 bonds made further stereochemical information from this compound unreliable.

Rotation about C-16 was restricted in diacetonide **9**, however, making it possible to relate the side-chain relative stereochemistry to that of the chiral centers in the tetracyclic portion of the molecule (Figure 6). Most informative was a pronounced NOESY correlation between H-18 and Me-22, requiring that the configuration of C-18 be *S*[11] rather than *R*. This model was supported by NOESY interactions between H-17 and H-16β and between H-16β and H-1, consistent with the *S* configuration at C-17. Both C-17 and C-18 having the *S* configuration is supported by the large 9.6 Hz coupling constant observed between H-17 and H-18, consistent with the trans orientation shown in the model. The configuration at C-19 was also addressed using NMR data derived from acetonide **9**. The C-19 hydroxyl proton showed a NOESY correlation with the pro-*R* acetonide methyl of the eight-membered ring acetonide, which was itself identified by a NOESY interaction with H-14. Similarly, Me-21 showed a NOESY correlation to the pro-*S* acetonide methyl group as well as H-17. Consequently, we have assigned the configuration of C-19 as *S**.

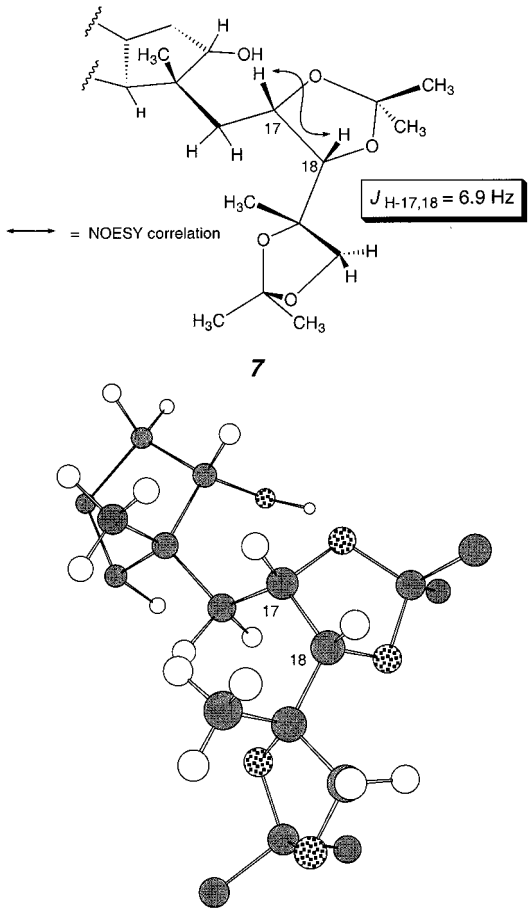


Figure 5. Chem3D representation of the global minimum conformation (Macromodel, MM2 force field, Monte Carlo conformation search) of diacetonide **7**.

Fortuitously, the free secondary hydroxyl group at C-14 in diacetonide **7** allowed the application of Mosher's NMR method to determine the absolute configuration at that center. Because the relative configuration at C-14 had been already related to the chiral centers in the tetracyclic nucleus and side-chain, this allowed the absolute stereochemistry of all chiral centers to be assigned. The observed differential NMR shielding pattern for the *R*- and *S*-MTPA esters was consistent with an *S* configuration at C-14 (Figure 7).[15] Thus, the overall absolute stereochemistry for **3** was assigned as 1*S*, 12*R*, 14*S*, 15*R*, 17*S*, 18*S*, 19*S*. By analogy, the absolute configurations

(11) Stereochemical descriptors for C-17, C-18, and C-19 were based on an arbitrary assignment of C-14 as *S*. For ease of reading, we have abbreviated 17*R*, 18*R*, 19*R* as *RRR* (etc.) in the discussion of the side chain stereochemistry.

(12) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

(13) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127.

(14) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(15) These conformation searches were performed on the entire molecules described. Much of the polycyclic ring structure, as well as some hydrogens, is hidden for clarity.

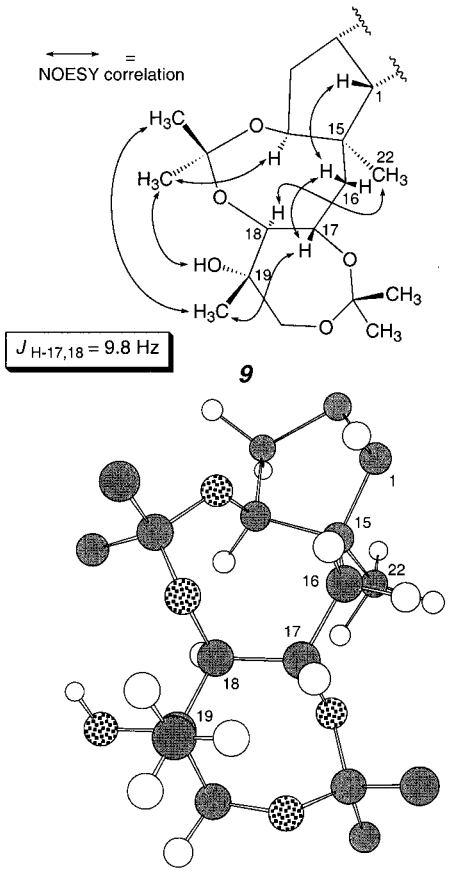


Figure 6. Chem3D representation of the global minimum conformation (Macromodel, MM2 force field, Monte Carlo conformation search) of diacetonide **9**.

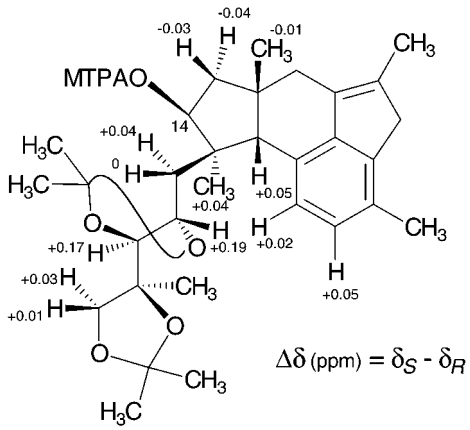


Figure 7. Mosher ester analysis of diacetonide **7**.

of **1** and **2** are assigned as 1*S*, 2*S*, 9*S*, 12*R*, 14*S*, 15*R*, 17*S*, 18*S*, 19*S*.

Given the acid instabilities of neomangicol A and B, and that neomangicol C formally represents the loss of HX from **1** and **2**, we undertook careful experiments to determine whether neomangicol C was an artifact of the isolation process. The use of silica gel chromatography was avoided, and fractions from chromatography were concentrated as soon as possible, stored in amber vials under argon, and kept in a freezer until NMR analyses could be undertaken. Under these conditions, only neomangicols A and B (**1** and **2**) were obtained. Neomangicols A and B were found to be moderately unstable, although we were unable to determine exactly what conditions triggered decomposition. We observed decomposition most frequently in test tubes after chromatography, and particularly when the top layer of solvent was allowed to evaporate. Decomposition was typically signaled by a dramatic color change, first to blue-green (minutes) and eventually yellow (days). The first step in the process appeared to be autocatalytic, leading to significant conversion of **1** or **2** to neomangicol C.[16] Our attempts to reproduce the decomposition of neomangicol A under more controlled conditions, however, were largely unsuccessful. For example, bubbling air through solutions or exposure to UV, direct sunlight, acetic acid, and 10% sodium hydroxide did not induce decomposition. Heating at 60 °C overnight or treatment with 20% HCl resulted in significant decomposition, although neither of these conditions could account for the spontaneous decomposition we sometimes observed. The yield of neomangicol C from the decomposition of neomangicol A was generally between 40 and 70%. Unfortunately, scarcity of material precluded more extensive probing of this transformation. That pure neomangicol A could be converted to neomangicol C (among other intractible products), and that neomangicol C could not be detected in crude extracts purified by mild methods, led us to the conclusion that neomangicol C is most likely a product of complex dehydrohalogenation.

Conclusions

The neomangicols represent a unique new class of cytotoxic sesterterpenes. Although not yet proven by isotope incorporation experiments, the tetracyclic ring system appears to be produced by atypical terpenoid pathways resulting in irregular "head to tail" isoprene configurations. While sesterterpenes (C_{25}) are the rarest of the terpenoid classes of natural products, several unusual sesterterpenes have been isolated from terrestrial fungi (although none from marine fungi), including fusaproliferin,[17] variecolin,[18] and retigeranic acid,[19] among others.[20] A CAS Online substructure search of the cyclopent[*e*]acenaphthylene ring system (neomangicol skeleton without alkyl substituents) revealed no natural products composed of this specific tetracyclic ring architecture. In addition, while halogenated terpenoid compounds are well-known marine natural products,[21] neomangicols A and B appear to be the first examples of halogenated sesterterpenoid natural products.[22]

The producing organism, CNC-477, has been tentatively identified as *Fusarium* sp. based on biochemical

(16) We had observed this color change during purification of the initial fermentations. As the change in TLC behavior between 1 and 3 is almost imperceptible, we originally attributed the color change to decomposition of a minor component.

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(21) Gribble, G. W. *Prog. Chem. Org. Nat. Prod.* **1996**, *68*, 24.

(22) Three halogenated norsesterterpenes have been reported from *Ircinia* sponges. (a) De Giulio, A.; De Rosa, S.; Di Vincenzo, G.; Strazzullo, G.; Zavodnik, N. *J. Nat. Prod.* **1990**, *53*, 1503. (b) N′Diaye, I.; Guella, G.; Mancini, I.; Kornprobst, J.-M.; Pietra, F. *J. Chem. Soc, Chem. Commun.* **1991**, 97.

analysis and as *Fusarium heterosporum* based on overall morphological features. *F. heterosporum* is well-known from terrestrial environments and has been isolated from a variety of agricultural sources, including cereal grains[23] and melons.[24] A terrestrial isolate produces a series of trichothecene mycotoxins,[23] but we saw no indications of these compounds in our own extract and no reports of compounds related to the neomangicols from any terrestrial strains. The issue of the true identy of this marine isolate must await the application of modern genetic methods such as 18S rRNA sequence analysis before its true taxonomic position can be confirmed. That both chlorinated and brominated terpenes are produced by *Fusarium*, strain CNC477, is a clear indicator that halogenating enzymes are present in this fungal species. The observation of the capacity of bromination, a feature prominent in the biosynthesis of marine metabolites, is indicative that this strain is adapted to the marine environment.

Neomangicols A and B were found to be active against a variety of cancer cell lines. Neomangicol A was most active against MCF-7 (human breast carcinoma) and CACO-2 (human colon carcinoma) cell lines, displaying IC_{50} values of 4.9 and 5.7 μM, respectively. Neomangicol B was less active, having a mean IC_{50} value of 27 μM across the entire cell panel (versus 10 μM for neomangicol A). Neomangicol B, however, displayed antibacterial activity similar to that of the known antibiotic, gentamycin, against the Gram-positive bacterium *Bacillus subtilus*.[25]

Experimental Section

General. Proton NMR spectra were recorded at 600, 500, 400, or 300 MHz, while ^{13}C NMR spectra were recorded at 100 or 75 MHz. All spectra were recorded in methanol-d_4 or chloroform-*d*, and chemical shifts were referenced to either the corresponding solvent signal or tetramethylsilane: δ 3.31/δ 49.0, δ 0.0/δ 77.0. The numbers of attached protons on carbon atoms were determined through DEPT experiments, and all carbon assignments made were consistent with the DEPT results. 2D HMBC and HMQC experiments were optimized for $^{n}J_{CH}$ = 8.0 Hz and $^{1}J_{CH}$ = 150.0 Hz, respectively. HPLC separations were accomplished using a Rainin DYNAMAX-60 Å ODS column (250 × 10 mm) at a flow rate of 2.5 mL/min or a Rainin DYNAMAX-60 Å SiO_2 column (250 × 10 mm) at a flow rate of 3.5 mL/min with refractive index detection.

Cultivation, Extraction, and Isolation. The fungus was cultured for 21 days in a total of 20 L of a medium composed of sterile seawater, yeast extract (0.5%), peptone (0.5%), glucose (1%), and crab meal (0.2%). The mycelium and broth were separated by filtration and the mycelium freeze-dried and extracted with CH_2Cl_2:MeOH (1:1). The neomangicols were found only in the mycelial extract, which, when concentrated, afforded 15.6 g of brown oil. The oil was first fractionated by C-18 reversed-phase flash chromatography, and the portion eluting between 75:25 MeOH:H_2O and 90:10 MeOH:H_2O (1.53 g) was further fractionated by Sephadex LH-20 chromatography (3:1:1 hexane:toluene:methanol, 0.5 mL/min flow rate). A portion of one of the fractions (60 mg of 237 mg) was purified by C-18 reversed-phase HPLC (90:10 MeOH:H_2O) to afford neomangicols A (**1**, 41.1 mg, t_R = 17.3 min) and B (**2**, 7.3 mg, t_R = 19.5 min).

In an earlier fermentation, the crude extract obtained from an identical fermentation was subjected to C-18 reversed-phase flash chromatography followed by silica flash chromatography and C-18 reversed-phase HPLC. Under these conditions the only neomangicol isomer obtained was neomangicol C (**3**, ca. 5 mg/L).

Neomangicol A (1): white noncrystalline solid; $[\alpha]_D = -96°$ (*c* 0.39, MeOH); UV λ_{max} (MeOH): 284 nm (ϵ 12200), 295 nm (ϵ 10100), 274 nm (ϵ 9400); IR (film): 3352, 2940, 2925, 1472, 1031, 765 cm^{-1}; HRFABMS: $[M]^+$ *m/z* obsd 453.2417, calcd for $C_{25}H_{38}{}^{35}ClO_5$ 453.2408. For ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC data, see Table 1.

Neomangicol B (2): white noncrystalline solid; $[\alpha]_D = -106°$ (*c* 0.20, MeOH); UV λ_{max} (MeOH): 286 nm (ϵ 10200), 298 nm (ϵ 8900), 276 nm (ϵ 8100), 194 nm (ϵ 5000); IR (film): 3355, 2941, 2925, 1470, and 1033 cm^{-1}; HRFABMS: $[M]^+$ *m/z* obsd 497.1920, calcd for $C_{25}H_{38}{}^{79}BrO_5$ 497.1903. For ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC data, see Table 2.

Neomangicol C (3): clear oil; $[\alpha]_D = +57°$ (*c* 0.40, MeOH); UV λ_{max} (MeOH): 216 nm (ϵ 26000), 260 nm (ϵ 15000); IR (film): 3331, 2924, 1453, 1377, 1048 cm^{-1}; HRFABMS $[M + Na]^+$ *m/z* obsd 439.2469, calcd for $C_{25}H_{36}O_5Na$ 439.2460. For ^{1}H NMR, ^{13}C NMR, HMQC, and HMBC data, see Table 3.

Hydrogenation of Neomangicol A (1) (Hydrogenation Products 4−6). Neomangicol A (6.5 mg, 0.014 mmol) was dissolved in MeOH (2.5 mL). Palladium on carbon (10%, 2 mg) was added to the stirred solution, and the headspace of the flask was flushed with hydrogen from a balloon. The reaction mixture was stirred at room temperature for 1 h, filtered, and concentrated. The resulting oil was purified by reversed-phase HPLC (90:10 MeOH:H_2O), affording **4** (1.5 mg, t_R = 20.0 min) and an inseparable mixture of **5** and **6** (2.0 mg, t_R = 21.5 min). **4**: ^{1}H NMR (500 MHz, CD_3OD): δ 3.93−3.89 (m, 2H, H-14 and H-17), 3.57 (d, 1H, *J* = 11.5 Hz, H-20), 3.43 (d, 1H, *J* = 11.5 Hz, H-20), 3.38 (d, 1H, *J* = 8.5 Hz, H-18), 2.73 (m, 1H, H-6), 2.56 (m, 1H, H-9), 2.19 (ddd, 1H, *J* = 9.5, 9.5, 14 Hz, H-8), 2.00 (dd, 1H, *J* = 6, 13.5 Hz, H-13), 1.97 (dd, 1H, *J* = 1.5, 15 Hz, H-16), 1.94−1.88 (m, 2H, H-2 and H-5), 1.90 (d, 1H, *J* = 16 Hz, H-11β), 1.78 (br d, 1H, *J* = 17 Hz, H-11α), 1.70 (ddddd, 1H, *J* = 4, 4, 13.5, 13.5, 13.5 Hz, H-4), 1.65−1.60 (m, 3H, H-1, H-4, H-13), 1.53 (dd, 1H, *J* = 7.5, 15 Hz, H-16), 1.47 (m, 1H, H-3), 1.40 (ddd, 1H, *J* = 3.5, 13, 13, 13 Hz, H-3), 1.19 (s, 3H, Me-21), 1.17 (ddd, 1H, *J* = 5.5, 5.5, 13.5 Hz, H-8), 1.11 (s, 3H, Me-25), 0.99 (d, 3H, *J* = 7 Hz, Me-24), 0.84 (s, 3H, Me-22), 0.81 (d, 3H, *J* = 7 Hz, Me-23); ^{13}C NMR (100 MHz, CD_3OD): δ 136.4, 136.3, 80.5, 76.9, 75.8, 71.0, 69.3, 55.8, 50.3, 50.0, 48.4, 43.7, 41.5, 40.7, 37.6, 35.3, 35.1, 34.9, 32.9, 32.8, 30.3, 23.4, 20.1, 19.3, 14.5; HRFABMS: $[M + Na]^+$ *m/z* obsd 445.2944, calcd for $C_{25}H_{42}O_5Na$ 445.2930. For the mixture of **5**/**6**: ^{1}H NMR (500 MHz, CD_3OD): δ 3.90−3.77 (m, 4H), 3.56 (d, 2H, *J* = 11.5 Hz), 3.42 (d, 2H, *J* = 11.5 Hz), 3.37 (d, 2H, *J* = 11.5 Hz), 2.64 (m, 1H), 2.44−2.30 (m, 3H), 2.23−1.82 (m, 12H), 1.79−1.36 (m, 16 H), 1.29−1.23 (m, 2H), 1.22 (s, 3H), 1.19 (s, 9H), 1.10 (s, 3H), 1.03 (s, 3H), 1.02 (s, 3H), 0.98 (d, 3H, *J* = 7 Hz), 0.95 (d, 3H, *J* = 7 Hz), 0.94 (d, 3H, *J* = 7 Hz); HRFABMS: $[M + Na]^+$ *m/z* obsd 445.2943, calcd for $C_{25}H_{42}O_5Na$ 445.2930.

Preparation of Acetonides from Neomangicol C (3) (7−9). Neomangicol C (23 mg, 0.055 mmol) was dissolved in acetone (200 μL) under argon and cooled to 0 °C. To the stirred solution were added 2,2-dimethoxypropane (DMP, 400 μL) and camphorsulfonic acid (1 mg), and the reaction mixture was stirred at 0 °C for 1 h. The reaction was quenched with 5 μL of triethylamine, concentrated under a stream of argon, dissolved in EtOAc, and purified by silica gel chromatography to give a yellow oil (26 mg, 95%). Separation by HPLC (80:20 isooctane:EtOAc) afforded acetonides **7** (6.7 mg), **8** (10.2 mg), and **9** (3.3 mg). **For diacetonide 7:** ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.88 (d, 1H, *J* = 7.8 Hz, H-3), 6.85 (d, 1H, *J* = 7.8 Hz, H-4), 4.77 (ddd, 1H, *J* = 1.8, 6.9, 6.9 Hz, H-17), 4.18 (d, 1H, *J* = 6.9 Hz, H-18), 4.11 (d, 1H, *J* = 8.7 Hz, H-20a), 4.03 (ddd, 1H, *J* = 6.9, 10.8, 10.8 Hz, H-14), 3.74 (d, 1H, *J* = 9.0 Hz, H-20β), 3.70 (d, 1H, *J* = 11.1 Hz, OH-14), 3.22 (br s, 2H, H-7), 2.86 (s, 1H, H-1), 2.36 (br s, 2H, H-11), 2.31 (s, 3H, Me-23), 2.30 (dd, 1H, *J* = 1.8, 15 Hz, H-16β), 2.13 (dd, 1H, *J* =

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(25) Antibacterial activity was determined using the standard disc-diffusion assay. Neomangicol B (50 μg) caused a 10 mm zone of inhibition during a 24-h incubation period, compared to the standard gentamycin (10 μg), which also resulted in a 10 mm zone of inhibition.

6.3, 12.3 Hz, H-13β), 2.04 (s, 3H, Me-24), 2.03 (dd, 1H, J = 9.3, 15 Hz, H-16α), 1.62 (dd, 1H, J = 10.8, 12.6 Hz, H-13α), 1.51 (s, 3H, Me-27), 1.47 (s, 3H, Me-30), 1.45 (s, 3H, Me-21), 1.44 (s, 3H, Me-31), 1.39 (s, 3H, Me-28), 0.94 (s, 3H, Me-25), 0.68 (s, 3H, Me-22); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.3, 139.3, 135.4, 132.8, 130.1, 126.7, 125.8, 125.2, 110.2, 107.7, 81.7, 81.2, 81.0, 75.4, 74.6, 53.6, 49.4, 47.4, 42.1, 39.0, 37.8, 33.0, 28.8, 27.5 (2), 26.8 (2), 24.7, 21.0, 18.3, 13.8; HRFABMS: $[M]^+$ m/z obsd 496.3178, calcd for $C_{31}H_{44}O_5$ 496.3189. **For diacetonide 8:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (d, 1H, J = 7.8 Hz, H-3), 6.87 (d, 1H, J = 7.8 Hz, H-4), 4.19 (dd, 1H, J = 7.5, 7.5 Hz, H-14), 4.01 (d, 1H, J = 8.4 Hz, H-20a), 3.89 (ddd, 1H, J = 2.1, 7.8, 7.8 Hz, H-17), 3.70 (d, 1H, J = 8.1 Hz, H-20b), 3.55 (d, 1H, J = 8.1 Hz, H-18), 3.21 (br s, 2H, H-7), 2.73 (d, 1H, J = 1.5 Hz, H-1), 2.60 (s, 1H, OH-19), 2.37 (br s, 2H, H-11), 2.31 (m, 1H, H-16β), 2.31 (s, 3H, Me-23), 2.13 (dd, 1H, J = 7.2, 12.9 Hz, H-16α), 2.09 (dd, 1H, J = 2.7, 14.4 Hz, H-13β), 2.03 (s, 3H, Me-24), 1.74 (dd, 1H, J = 7.8, 13.2 Hz, H-13α), 1.45 (s, 3H, Me-27), 1.40 (s, 3H, Me-30), 1.36 (s, 3H, Me-28), 1.35 (s, 3H, Me-31), 1.32 (s, 3H, Me-21), 0.89 (s, 3H, Me-25), 0.72 (s, 3H, Me-22); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.4, 139.0, 135.4, 133.0, 130.1, 127.0, 126.1, 125.2, 109.1, 100.6, 83.8, 80.8, 75.6, 70.4, 69.2, 58.6, 48.6, 47.4, 42.1, 40.8, 40.4, 33.6, 28.6, 27.9, 26.2, 25.2, 24.6 (2), 24.4, 18.3, 13.7; HRFABMS: $[M]^+$ m/z obsd 496.3182, calcd for $C_{31}H_{44}O_5$ 496.3189. **For diacetonide 9:** ^{1}H NMR (300 MHz, $CDCl_3$): δ δ 6.90 (d, 1H, J = 7.8 Hz, H-3), 6.85 (d, 1H, J = 7.8 Hz, H-4), 4.21 (dd, 1H, J = 7.8, 7.8 Hz, H-14), 4.11 (ddd, 1H, J = 1.8, 6.8, 9.0 Hz, H-17), 3.71 (s, 1H, OH-19), 3.67 (d, 1H, J = 9.6 Hz, H-18), 3.58 (d, 1H, J = 11.1 Hz, H-20α), 3.42 (d, 1H, J = 10.8 Hz, H-20β), 3.21 (br s, 2H, H-7), 2.57 (s, 1H, H-1), 2.37 (br s, 2H, H-11), 2.32 (s, 3H, Me-23), 2.25 (dd, 1H, J = 7.8, 14.1 Hz, H-16β), 2.15 (dd, 1H, J = 7.5, 13.2 Hz, H-13β), 2.04 (s, 3H, Me-24), 1.91 (dd, 1H, J = 1.8, 14.1 Hz, H-16α), 1.75 (dd, 1H, J = 8.4, 13.2 Hz, H-13α), 1.52 (s, 3H, Me-27), 1.45 (s, 3H, Me-30), 1.41 (s, 3H, Me-28), 1.35 (s, 3H, Me-31), 1.32 (s, 3H, Me-21), 0.89 (s, 3H, Me-25), 0.76 (s, 3H, Me-22); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.4, 139.2, 135.7, 132.9, 130.2, 126.8, 125.6, 125.1, 101.5, 99.3, 81.1, 75.0, 71.1, 70.3, 68.3, 58.8, 48.7, 47.3, 42.1, 40.8, 38.9, 33.4, 29.0, 28.6, 25.2, 24.8, 24.7, 19.8, 19.2, 18.4, 13.8; HRFABMS: $[M]^+$ m/z obsd 496.3177, calcd for $C_{31}H_{44}O_5$ 496.3189.

Preparation of Mosher Ester 10. Diacetonide **7** (2.5 mg, 0.0050 mmol) was dissolved in CH_2Cl_2 (150 μL) under argon. Added to the stirred solution were DMAP (1 mg), pyridine (10 μL), and (*S*)-MTPA-Cl (5 μL, 0.027 mmol, 5.3 equiv). The reaction mixture was stirred at room temperature for 72 h, concentrated, and quenched with 1 M NH_4Cl (500 μL) for 25 min. The mixture was extracted with CH_2Cl_2 (3 × 1 mL), dried over $MgSO_4$, and concentrated to give a yellow oil (12.1 mg). Purification by silica gel HPLC (80:20 isooctane:EtOAc) afforded ester **10** (0.8 mg, 22%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60−7.54 (m, 2H), 7.42−7.39 (m, 3H), 7.07 (d, 1H, J = 8.1 Hz, H-3), 6.87 (d, 1H, J = 7.8 Hz, H-4), 5.32 (m, 1H, H-14), 4.09 (ddd, 1H, J = 1.8, 6.3, 8.7 Hz, H-17), 3.90 (d, 1H, J = 9 Hz, H-20a), 3.66 (d, 1H, J = 8.7 Hz, H-20β), 3.59 (s, 3H, OMe), 3.46 (d, 1H, J = 6.3 Hz, H-18), 3.21 (br s, 2H, H-7), 2.95 (s, 1H, H-1), 2.56 (br d, 1H, J = 16.8 Hz, H-16β), 2.46 (m, 2H, H-11), 2.42 (dd, 1H, J = 5.7, 15 Hz, H-13β), 2.32 (s, 3H, Me-23), 2.06 (dd, 1H, J = 10.8, 14.7 Hz, H-16α), 2.04 (s, 3H, Me-24), 1.90 (dd, 1H, J = 3.9, 15.3 Hz, H-13α), 1.42 (s, 3H), 1.35 (s, 6H), 1.26 (s, 3H), 1.21 (s, 3H), 0.89 (s, 3H, Me-25), 0.80 (s, 3H, Me-22); HRFABMS M^+ m/z obsd 712.3554, calcd for $C_{41}H_{51}F_3O_7$ 712.3587.

Preparation of Mosher Ester 11. Ester **11** was prepared from acetonide **7** (3.2 mg) and (*R*)-MTPA-Cl (7 μL, 0.037 mmol, 5.8 equiv) using a procedure analogous to the preparation of **10** from **7** (1.5 mg, 33%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.57−7.52 (m, 2H), 7.45−7.42 (m, 3H), 7.09 (d, 1H, J = 7.8 Hz, H-3), 6.87 (d, 1H, J = 7.2 Hz, H-4), 5.31 (m, 1H, H-14), 4.28 (ddd, 1H, J = 1.8, 6.9, 8.7 Hz, H-17), 3.93 (d, 1H, J = 8.1 Hz, H-20α), 3.67 (d, 1H, J = 8.7 Hz, H-20β), 3.63 (d, 1H, J = 6.3 Hz, H-18), 3.51 (s, 3H, OMe), 3.21 (br s, 2H, H-7), 3.00 (s, 1H, H-1), 2.56 (d, 1H, J = 16.5 Hz, H-16β), 2.46 (m, 2H, H-11), 2.38 (dd, 1H, J = 5.4, 14.4 Hz, H-13β), 2.32 (s, 3H, Me-23), 2.10 (dd, 1H, J = 10.8, 15 Hz, H-16α), 2.03 (s, 3H, Me-24), 1.87 (dd, 1H, J = 3.3, 14.4 Hz, H-13α), 1.41 (s, 3H), 1.38 (s, 3H), 1.30 (s, 3H), 1.29 (s, 3H), 1.28 (s, 3H), 0.88 (s, 3H, Me-25), 0.84 (s, 3H, Me-22); HRFABMS: $[M]^+$ m/z obsd 712.3619, calcd for $C_{41}H_{51}F_3O_7$ 712.3587.

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Supporting Information Available: ^{1}H NMR spectra for **1**−**10**; ^{13}C NMR spectra for **1**−**4** and **6**−**8**; HMBC spectra for **1**, **3**, and **6**−**8**; NOESY spectra for **1**, **3**, **4**, **6**, and **8**; DEPT and HMQC spectra for **1** and **3**; and a COSY spectrum for **1** (37 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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