Antitubercular Sesterterpenes from the Thai Sponge *Brachiaster* sp.

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Received May 11, 2004

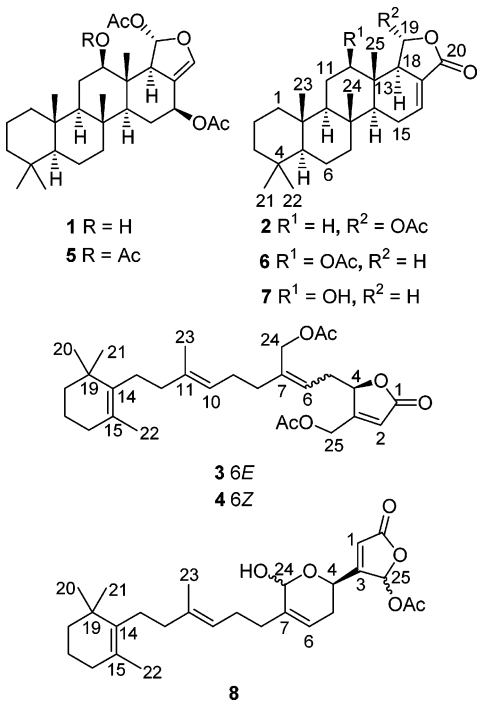
A new scalarane-type sesterterpene, 12-deacetoxyscalarin 19-acetate (**2**), and two naturally new derivatives of manoalide-type sesterterpenes, (*E*)- and (*Z*)-neomanoalide 24,25-diacetates (**3** and **4**), were isolated from the Thai sponge *Brachiaster* sp., along with five other known sesterterpenes: heteronemin (**1**), heteronemin acetate (**5**), 12-*epi*-19-deoxyscalarin (**6**), 12-deacetyl-12-*epi*-19-deoxyscalarin (**7**), and manoalide 25-acetate (**8**). The antitubercular and cytotoxic activities of all eight compounds were evaluated to reveal the potent activity of compounds **1**, **2**, **5**, and **8**. Among these, compound **2** showed an interesting bioactivity profile, in possessing potent antitubercular activity and being practically inactive in the cytotoxicity bioassay.

Tuberculosis is probably one of the deadliest bacterial infectious diseases ever known to have plagued mankind. Despite the availability of a vaccine (BCG) and various antibacterials and antibiotics specific for mycobacteria, the tuberculosis epidemic still occurs and is getting more virulent and widespread. This results partially from its association with AIDS as one of the most common opportunistic infections and also from the occurrence of multidrug-resistant (MDR) mycobacteria. In 1993, the World Health Organization proclaimed tuberculosis as a global emergency and launched several programs to attack the disease, including the search for new remedies and/or antibacterial agents to complement currently used agents and protocols, and particularly to extend available treatment toward MDR mycobacterial infections.[1]

Marine organisms have been reported in several reviews[2] as sources of potential antitubercular agents. Among these, terpenoid compounds and their derivatives are prominent, including the sesterterpene heteronemin (**1**),[3] which exhibited antimycobacterial activity against *M. tuberculosis* $H_{37}Rv$ with a MIC value of 6.25 μg/mL.[2b] Herein, we report the isolation of a series of antitubercular sesterterpenes of the scalarane and manoalide types, including three new derivatives, 12-deacetoxyscalarin 19-acetate (**2**) and (*E*)- and (*Z*)-neomanoalide 24,25-diacetates (**3** and **4**, respectively).

The Thai sponge *Brachiaster* sp. was collected from Koh-Tao, Surat-Thani, during two separate expeditions in April 2001 and in April 2002, and the specimens from each expedition were investigated separately. The 2001 specimen was freeze-dried, crushed, and extracted with MeOH, then partitioned sequentially with hexane, CH_2Cl_2, and *n*-BuOH. The active hexane- and CH_2Cl_2-soluble materials were separately subjected to a series of column chromatography steps to yield heteronemin (**1**) from the CH_2Cl_2 extract as major component and compounds **5** and **6**, which were identified as heteronemin acetate and 12-*epi*-19-deoxyscalarin, respectively, from the hexane extract.

The specimen from the 2002 collection was macerated consecutively in a series of solvents from hexane to CH_2Cl_2 and to MeOH. From this, the major bioactive hexane extract was the only fraction chosen for further studies. Purification of an aliquot of the hexane extract led to the isolation of **1** as the most abundant component, along with three new compounds, **2**−**4**, and two other known substances, 12-deacetyl-12-*epi*-19-deoxyscalarin (**7**) and manoalide 25-acetate (**8**). The structures of all the known compounds, i.e., compounds **1** and **5**−**8**, were identified mainly by means of NMR spectral analysis and were confirmed by comparison with published data.[3−5] (See the Supporting Information for the complete spectral data of compounds **1** and **5**−**8**.)



Compound **2** was obtained as a white amorphous solid. Its molecular formula was deduced to be $C_{27}H_{40}O_4$ from the molecular peak at *m*/*z* 429 $[M + H]^+$ in the ESIMS. This was confirmed by the molecular mass at *m*/*z* 428.2910 (calcd for $C_{27}H_{40}O_4$, 428.2927) observed in the HREIMS. The molecular formula was consistent with a degree of unsaturation of 8, which was deduced to represent one olefinic double bond, two carbonyls, and five ring systems. From its IR spectrum, apart from the lactone and ester absorption bands at ν_{max} 1770 and 1730 cm^{-1}, no other prominent functionalities were observable. This agreed well

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10.1021/np0498354 CCC: $27.50
Published on Web 10/07/2004

Table 1. ^{1}H and ^{13}C Chemical Shifts of **2** (C_6D_6; 500 MHz for ^{1}H)

position	^{1}H[a] (mult)	^{13}C (mult)	HMBC correlations (C → H)
1-ax	0.64 (m)	40.0 (CH_2)	H-2ax; H-2eq; H-9;
1-eq	1.53 (m)		H-23
2-ax	1.36 (m)	18.9 (CH_2)	H-1ax; H-3ax;
2-eq	1.55 (m)		H-3eq; H-21
3-ax	1.14 (m)	42.4 (CH_2)	H-21; H-22
3-eq	1.37 (m)		
4		33.5 (C)	H-5; H-21; H-22
5	0.63 (m)	56.5 (CH)	H-21; H-22; H-23
6-ax	1.15 (m)	18.2 (CH_2)	H-5; H-7ax; H-7eq
6-eq	1.39 (m)		
7-ax	0.52 (m)	41.4 (CH_2)	H-5; H-24
7-eq	1.27 (m)		
8		37.4 (C)	H-9; H-14; H-24
9	0.45 (dd; J = 3.5, 9.6 Hz)	61.0 (CH)	H-5; H-14; H-23; H-24
10		37.6 (C)	H-5; H-9; H-24
11-ax	1.00 (m)	17.1 (CH_2)	H-9; H-12ax;H-12eq
11-eq	1.25 (m)		
12-ax	0.98 (m)	40.0 (CH_2)	H-9; H-25
12-eq	1.47 (m)		
13		33.7 (C)	H-11ax; H-11eq; H-12eq; H-14; H-18; H-25
14	0.78 (dd; J = 5.5, 10.7 Hz)	54.2 (CH)	H-24; H-25
15-ax	1.42 (m)	23.9 (CH_2)	H-14
15-eq	1.71 (br d; J = 17.5 Hz)		
16	6.61 (br ddd; J = 3.6, 4.0, 4.0 Hz)	136.6 (CH)	
17		126.6 (C)	
18	2.40 (br ddd; J = 3.6, 3.6, 5.5 Hz)	56.6 (CH)	H-14; H-16; H-25
19	6.60 (d; J = 5.5 Hz)	93.4 (CH)	OCOCH_3-19
20		165.7 (C)	H-16; H-19
21	0.81 (s; 3H)	21.4 (CH_3)	H-22
22	0.88 (s; 3H)	33.3 (CH_3)	H-5; H-21
23	0.70 (s; 3H)	16.5 (CH_3)	H-5; H-9
24	0.54 (s; 3H)	16.1 (CH_3)	H-9; H-14
25	0.35 (s; 3H)	14.5 (CH_3)	H-14
OCOCH_3-19		168.6 (C)	H-19; OCOCH_3-19
OCOCH_3-19	1.59 (s; 3H)	20.2 (CH_3)	

[a] Unless stated otherwise, each proton signal is integrated as one proton.

with the ^{1}H and ^{13}C NMR spectra (Table 1), in which most signals were found in the high-field region, suggesting the core structure of a mainly aliphatic framework. The structure elucidation of this compound therefore relied heavily on the analysis of the COSY and HMBC spectra (see Table 1), from which the large cluster of aliphatic proton signals resonating at 0.4−1.8 ppm was correlated with a tetra-carbocyclic sesterterpene skeleton. Only two major functionalities were observed. One was the trisubstituted olefin composed of two carbons at δ 136.6 (C-16) and 126.6 (C-17) and one proton at δ 6.61 (br ddd, J = 3.6, 4.0, 4.0 Hz; H-16). The other was a γ-lactone ring with an acetylated acetal γ-carbon. The carbonyl of the lactone was found at δ 165.7 (C-20), whereas the acetal functionality was deduced from the characteristic signal of H-19 at δ 6.60 (d, J = 5.5 Hz) and C-19 at δ 93.4. The acetate substituent on C-19 was found at δ 168.6 and 20.2 (O*C*OCH$_3$-19 and OCO*C*H$_3$-19, respectively). Linking all the above functional groups to the tetracarbocyclic sesterterpene skeleton thus yielded the gross structure of **2** as 12-deacetoxyscalarin 19-acetate, a new 12-deacetoxy analogue of the scalarane-type sesterterpenes.

The relative configuration of the tetracarbocyclic moiety of **2** was determined primarily according to the orientation of the ring junction methyl groups. The carbon chemical shifts of the three methyl groups (C-23, C-24, C-25) indicated that all are in the axial orientation; that is, they were found at higher field below 20 ppm (δ 16.5, 16.1, 14.5, respectively) due to a 1,3-diaxial effect, as compared to the equatorial methyls that are normally found at lower field above 20 ppm.[3b] This indicated the all-*trans* configuration of the tetracarbocyclic moiety. The lactone ring, on the other hand, was proposed as *cis* to the main skeleton, as rationalized from the orientation of H-18 and H-19. The pseudoaxial orientation of H-18 was rationalized from the strong homoallylic coupling between H-18 and H-15a (^5J = 3.6 Hz), whereas the dipolar coupling between H-25 and H-19, observed from a NOEDS experiment, suggested the proximity between these two groups.

To determine the absolute configuration of **2**, the CD spectrum was analyzed. According to the octant rule, the first positive Cotton effect at λ 251.5 nm (θ +2177) indicated the *S* and *R* configurations of C-13 and C-19, respectively. This first positive Cotton effect also agreed well with the *M* conformation of the α,β-enone moiety (C-20−C-17−C-16), which was twisted due to the *cis* conformation between the lactone ring and the main skeleton.[6] The configurations of the remaining asymmetric carbons were elucidated accordingly, and the structure of **2** was proposed as shown.

Compounds **3** and **4** were both obtained as clear colorless viscous liquid. Both compounds shared the same molecular formula of $C_{29}H_{42}O_6$, as deduced from the $[M + Na]^+$ peak at *m*/*z* 509 in their ESIMS. The proposed molecular formula agreed well with the HRESIMS (*m*/*z* 509.2898 for **3**, *m*/*z* 509.2794 for **4**; calcd for $C_{29}H_{42}O_6Na$, 509.2868). The other spectroscopic data of **3** and **4**, including the UV and IR spectra, were also almost identical (λ_{max} 224 nm, log ϵ 3.72 for UV; major absorption band ν_{max} 1755 cm^{-1} for IR). Compared with the published spectral data of other known sesterterpenes, the spectra of **3** and **4** and of the neomanoalides[7] suggested that all shared the same skeleton. The major difference between the neomanoalides and compounds **3** and **4** was the presence of two acetate groups at C-24 and C-25. Thus, the acetate groups of **3** resonated at δ 169.8 (O*C*OCH$_3$-24), 20.4 (OCO*C*H$_3$-24), 169.1 (O*C*OCH$_3$-25), and 19.8 (OCO*C*H$_3$-25), whereas those of **4** were observed at δ 170.1 (O*C*OCH$_3$-24), 20.4 (OCO*C*H$_3$-24), 169.1 (O*C*OCH$_3$-25), and 19.8 (OCO*C*H$_3$-25). Hence, the compounds **3** and **4** were proposed as 24,25-diacetate derivatives of the neomanoalides.

The difference between **3** and **4** was the configuration of the C-6−C-7 double bond. The ^{13}C NMR spectrum of **3** showed that C-8 resonated at a comparatively higher field (δ 28.8) and C-24 at a lower field (δ 67.4), whereas those of compound **4** were different, with C-8 at δ 35.6 and C-24 at δ 61.4. This classic electronic repulsion effect, similar to that observed in the neomanoalides,[7] indicated that **3** is the 6*E* isomer and **4** the 6*Z* isomer. The dipolar couplings between H-24 and H-6 for compound **3** and between H-24 and H-5 for **4**, observed from the NOEDS, also confirmed the proposed structures. The CD spectra of compounds **3** and **4** showed the first negative Cotton effect ($[\theta]_{218}$ −5504 for **3**; $[\theta]_{214.5}$ −10 485 for **4**), indicating the 4*R* configuration for both compounds, which was supported by similar observations made for the neomanoalides.[7] Compounds **3** and **4** were first obtained as derivatives by de Silva and Scheuer[7] as part of the structure determination of the neomanoalides; however, no spectral data nor biological activities were reported.

All the isolated compounds were evaluated for their antitubercular and cytotoxic activities. With the exception of compound **1**, their biological activities (Table 2) are

Table 2. Antitubercular and Cytotoxic Activities of Compounds **1**−**8** (in μM)

	anti-TB (MIC)	cytotoxicity (IC_{50} ± SEM)			
		MCF-7	HT-29	HeLa	KB
1	3	0.29 ± 0.08	0.39 ± 0.002	0.45 ± 0.03	0.37 ± 0.01
2[a]	4	−	−	−	−
3[a]	51	9.34 ± 0.03	−	−	−
4[a]	26	5.99 ± 0.12	−	−	−
5[a]	6	6.45 ± 0.30	−	−	−
6	117	5.21 ± 0.08	2.34 ± 0.27	5.49 ± 0.24	3.01 ± 1.59
7	16	0.32 ± 0.01	0.91 ± 0.15	1.99 ± 0.21	0.75 ± 0.13
8	7	0.26 ± 0.01	0.76 ± 0.13	1.68 ± 0.39	0.63 ± 0.11

[a] The negative results for these samples denoted that the samples were virtually inactive within the ranges of the concentrations used, i.e., exhibiting 80−100% viability of targeted cancer cells at the highest concentration tested (10 μM), so the determinations of their IC_{50} values were not performed.

reported herein for the first time. It was not surprising that within the manoalide series compounds that were cytotoxic exhibited antitubercular activity as well. Compounds **3** and **4** were only slightly active in the antitubercular assay and practically inactive as cytotoxic agents, whereas compound **8** was strongly active in both types of assay. Although the biological activities of manoalide 25-acetate (**8**) have never been reported, its better known prototype, manoalide, is an established inhibitor of phospholipase A2. Consequently, the compound has served as a useful biochemical probe for inflammation research.[8]

For the compounds in the scalarane series, it was suggested originally by Crews and Bescansa[3b] that the oxygenation pattern at C-19 of the scalarane-type sesterterpenes might assert a strong influence on their resultant biological activities. Such an influence was evident among the five scalaranes isolated. Compounds **1**, **2**, and **5**, which possess an acetate group at C-19, were strongly active in the antitubercular assay, whereas the activities of **6** and **7** were much weaker (Table 2). For all practical purposes, compounds **1**, **2**, and **5** were similar in potency in the antitubercular assay. However, unlike compounds **1** and **5**, compound **2** was insignificantly cytotoxic (Table 2). The limited number of analogues tested in the present study did not permit any definitive conclusions to be made regarding the pharmacophore that might contribute to the dissimilar biological profiles of the isolated compounds. Additional studies into this class of compounds would include the synthesis of analogues aimed at improving the potency and selectivity of the scalarane-type sesterterpenes against mycobacterial cells.

Experimental Section

General Experimental Procedures. The optical rotations (at the sodium D-line wavelength) and CD spectra were obtained on a JASCO J-810 polarimeter. UV spectra were recorded on a Spectronic Genesys 5 spectrometer. IR spectra were obtained on a JASCO IR-810 spectrometer. The NMR experiments were performed on an FT-NMR variant Unity Inova 500 spectrometer, using the solvent signals (C_6D_6 or $CDCl_3$) as references. Low-resolution mass spectra were obtained on a Micromass LCT mass spectrometer, whereas high-resolution mass spectra were run on an HP 5890 GC series 2 plus-HP 5972 mass selective detector.

Animal Materials. The sponge specimens used in this study were collected in the vicinity of Koh-Tao, Surat-Thani Province, Thailand, at a depth of 18−24 m by scuba diving in April 2001 and in April 2002. Underwater, the specimen appeared lumpy and irregular shaped with a tannish-gray color externally. On the surface, the external color was dark gray, and tannish-yellow internally. The texture was prickly, tough, incompressible, and very resistant to cutting. The specimens from both expeditions were later identified to be a member of the genus *Brachiaster* (family Pachastrellidae) by Dr. Somchai Bussarawit of the Phuket Marine and Biology Research Center, Phuket, Thailand (after Hooper and van Soest; and Hooper).[9] Voucher specimens (designated no. AP01-008-03) were deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Prince of Songkla University, and at the Phuket Marine and Biology Research Center, Phuket, Thailand.

Extraction and Isolation. The specimen from the 2001 expedition (158 g) was lyophilized and macerated exhaustively in MeOH before being subjected to a solvent-extraction process, to yield the hexane, CH_2Cl_2, and *n*-BuOH extracts. The active CH_2Cl_2 fraction was chromatographed over Sephadex LH-20 (MeOH), then silica gel (3% EtOAc in CH_2Cl_2), to afford compound **1** as the major component (99 mg). The hexane extract, which was also active, was fractionated over a silica gel column (5% EtOAc in CH_2Cl_2), then purified by HPLC (ODS, 5 μm, 250 × 4.6 mm, 87% aqueous MeCN), and compounds **5** and **6** were obtained (10 and 2 mg, respectively).

The lyophilized specimen from the 2002 collection (212 g) was crushed and macerated consecutively in a series of solvents, from which hexane-, CH_2Cl_2-, and MeOH-soluble materials were obtained. An aliquot of the active hexane extract (4.3 g) was subjected to passage over a silica gel column and eluted with EtOAc−acetone−hexane (20:5:75) to afford four major fractions. The first one was recrystallized to yield the major component, compound **1** (300 mg). The second fraction was further separated by means of HPLC (silica gel, 10 μm, 250 × 7.0 mm, 5% *i*-PrOH in hexane; then PRP-C_{18}, 10 μm, 305 × 7.0 mm, 75% aqueous MeCN), and compounds **3** (4 mg), **4** (7 mg), and **7** (2 mg) were obtained. The third fraction was also further fractionated using HPLC (ODS, 5 μm, 250 × 4.6 mm, 85% aqueous MeCN) to yield compound **2** (3 mg). The last fraction was separated by HPLC (silica gel, 10 μm, 250 × 7.0 mm; 2% *i*-PrOH in hexane) to afford compound **8** (7 mg).

12-Deacetoxyscalarin 19-acetate (2): white amorphous solid; $[\alpha]_D$ −24.3° (*c* 0.014, MeOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.78) nm; CD (*c* 0.014, MeOH) θ (nm) 0 (275), +2177 (251.5), 0 (240); IR (thin film) ν_{max} 1770, 1730 cm^{-1}; ^1H and ^{13}C NMR (C_6D_6, 500 MHz for ^1H), see Table 1; ESIMS *m*/*z* 429 $[MH]^+$ (29), 407 (29), 369 (100), 358 (29), 336 (29); HREIMS *m*/*z* 428.2910 (calcd for $C_{27}H_{40}O_4$, 428.2927).

(*E*)-Neomanoalide 24,25-diacetate (3): clear colorless viscous liquid; $[\alpha]_D$ −32.9° (*c* 0.023, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.72) nm; CD (*c* 0.023, MeOH) θ (nm) −5504.7 (218); IR (thin film) ν_{max} 1755, 1225 cm^{-1}; ^1H NMR (C_6D_6, 500 MH) δ 1.07 (6H, s, H-20 and H-21), 1.44 (2H, m, H-18), 1.53 (3H, s, OCOC*H*$_3$-25), 1.56 (2H, m, H-17), 1.63 (3H, s, H-22), 1.67 (3H, s, H-23), 1.71 (3H, s, OCOC*H*$_3$-24), 1.88 (2H, dd, J = 6.2, 6.4 Hz, H-16), 1.93 (1H, ddd, J = 6.5, 7.3, 15.3 Hz, H-5a), 2.05 (2H, m, H-13), 2.06 (1H, m, H-8a), 2.17 (1H, m, H-8b), 2.19 (2H, m, H-12), 2.23 (2H, m, H-9), 2.26 (1H, ddd, J = 5.0, 7.3, 15.3 Hz, H-5b), 4.15 (1H, dd, J = 1.6, 16.2 Hz, H-25a), 4.25 (1H, dd, J = 1.6, 16.2 Hz, H-25b), 4.26 (1H, m, H-4), 4.43 (1H, d, J = 12.4 Hz, H-24a), 4.49 (1H, d, J = 12.4 Hz, H-24b), 5.23 (1H, t, J = 7.3 Hz, H-6), 5.26 (1H, dd, J = 6.2, 6.9 Hz, H-10), 5.62 (1H, ddd, J = 1.6, 1.6, 3.4 Hz, H-2); ^{13}C NMR (C_6D_6, 125 MHz) δ 16.2 (CH_3, C-23), 19.8 (CH_3, OCO*C*H$_3$-25), 19.9 (CH_2, C-17), 20.0 (CH_3, C-22), 20.4 (CH_3, OCO*C*H$_3$-25), 26.9 (CH_2, C-13), 28.3 (CH_2, C-9), 28.8 (CH_2, C-8), 28.8 (2CH_3, C-20 and C-21), 30.7 (CH_2, C-5), 33.0 (CH_2, C-16), 35.2 (C, C-19), 40.2 (CH_2, C-18), 40.8 (CH_2, C-12), 58.9 (CH_2, C-25), 67.4 (CH_2, C-24), 80.5 (CH, C-4), 117.2 (CH, C-2), 121.1 (CH, C-6), 123.2 (CH, C-10), 127.3 (C, C-15), 137.2 (C, C-11), 137.2 (C, C-14), 139.2 (C, C-7), 164.6 (C, C-3), 169.1 (C, O*C*OCH$_3$-25), 169.8 (C, O*C*OCH$_3$-24), 170.6 (C, C-1); ESIMS *m*/*z* 509 $[M + Na]^+$ (100), 172 (24); HRESIMS *m*/*z* 509.2898 (calcd for $C_{29}H_{42}O_6$-Na, 509.2868).

(*Z*)-Neomanoalide 24,25-diacetate (4): clear colorless viscous liquid; $[\alpha]_D$ −23.3° (*c* 0.015, MeOH); UV (MeOH) λ_{max} (log ϵ) 224 (3.72) nm; CD (*c* 0.015, MeOH) θ (nm) −10485 (214); IR (thin film) ν_{max} 1755, 1225 cm^{-1}; ^1H NMR (C_6D_6, 500 MH) δ 1.07 (6H, s, H-20 and H-21), 1.44 (2H, m, H-18), 1.56 (3H, s,

OCOCH_3-25), 1.57 (2H, m, H-17), 1.65 (3H, s, H-22), 1.65 (3H, s, H-23), 1.69 (3H, s, OCOCH_3-24), 1.88 (2H, dd, J = 6.2, 6.4 Hz, H-16), 1.94 (1H, ddd, J = 6.9, 7.3, 15.1 Hz, H-5a), 2.09 (1H, m, H-8a), 2.10 (2H, m, H-13), 2.13 (1H, m, H-8b), 2.19 (2H, m, H-9), 2.20 (2H, m, H-12), 2.41 (1H, ddd, J = 4.4, 7.3, 15.1 Hz, H-5b), 4.21 (1H, ddd, J = 0.6, 1.8, 16.2 Hz, H-25a), 4.33 (1H, dd, J = 1.6, 16.2 Hz, H-25b), 4.33 (1H, m, H-4), 4.52 (2H, s, H-24), 5.21 (1H, t, J = 7.3 Hz, H-6), 5.24 (1H, ddd, J = 1.1, 6.2, 7.1 Hz, H-10), 5.62 (1H, ddd, J = 1.6, 1.8, 3.4 Hz, H-2); ^{13}C NMR (C_6D_6, 125 MHz) δ 16.2 (CH_3, C-23), 19.8 (CH_3, OCO*C*H_3-25), 20.0 (CH_2, C-17), 20.0 (CH_3, C-22), 20.4 (CH_3, OCO*C*H_3-25), 27.0 (CH_2, C-13), 28.4 (CH_2, C-9), 28.8 (2CH_3, C-20 and C-21), 30.5 (CH_2, C-5), 33.0 (CH_2, C-16), 35.2 (C, C-19), 35.6 (CH_2, C-8), 40.2 (CH_2, C-18), 40.8 (CH_2, C-12), 59.0 (CH_2, C-25), 61.4 (CH_2, C-24), 80.7 (CH, C-4), 117.7 (CH, C-2), 122.4 (CH, C-6), 123.4 (CH, C-10), 127.3 (C, C-15), 136.8 (C, C-11), 137.3 (C, C-14), 138.9 (C, C-7), 164.9 (C, C-3), 169.1 (C, O*C*OCH$_3$-25), 170.1 (C, O*C*OCH$_3$-24), 170.7 (C, C-1); ESIMS m/z 509 $[M + Na]^+$ (100), 172 (24); HRESIMS m/z 509.2794 (calcd for $C_{29}H_{42}O_6Na$, 509.2868).

Bioactivity Determinations. The antitubercular activity was assessed against a nonvirulent strain of *Mycobacterium tuberculosis* (H_{37}Ra) according to the protocol described by Collins and Franzblau,[10a] using the microtiter plate alamar blue technique. Isoniazid and kanamycin sulfate were used as positive controls and exhibited MICs in the ranges 0.29−0.66 and 3.5−8.5 μM, respectively.

Cytotoxicity was determined according to the sulforhodamine B procedure described by Skehan et al.[10b] The target cell line panel consisted of MCF-7 (human breast adenocarcinoma), HeLa (human cervical carcinoma), KB (human oral epidermoid carcinoma), and HT-29 (colorectal carcinoma). Cell viability (% survival) after exposure to test samples was determined colorimetrically at 515 nm. Dose−response evaluations yielded concentration mediating 50% cytotoxic response (IC_{50}). Camptothecin was used as the standard reference. The compound demonstrated IC_{50} values in the range (0.6−6) × 10^{-6} μM.

Acknowledgment. We thank Dr. Palangpol Kongsaeree, Faculty of Science, Mahidol University, for his assistance in the access to the CD spectrometer-polarimeter. The determination of antitubercular activity was carried out by the Bioassay Research Facility, the National Center of Genetic Engineering and Biotechnology (BIOTEC), Thailand, in collaboration with Dr. Prasat Kittakoop. Financial support throughout this project was provided by the International Foundation for Science (F/3270-1) and by the Biodiversity Research and Training Program (BRT 643003 and BRT T-645002).

Supporting Information Available: 1-D ^{1}H and ^{13}C NMR and CD spectra of 12-deacetoxyscalarin 19-acetate (**2**); 1-D ^{1}H and ^{13}C NMR and CD spectra and tabulated extensive HMBC correlations of (*E*)- and (*Z*)-neomanoalide 24,25-diacetates (**3** and **4**); and the spectral and physical data of compounds **1** and **5**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP0498354