The Marine Sponge *Diacarnus bismarckensis* as a Source of Peroxiterpene Inhibitors of *Trypanosoma brucei*, the Causative Agent of Sleeping Sickness

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Human African trypanosomiasis, also known as African sleeping sickness, is a neglected tropical disease with inadequate therapeutic options. We have launched a collaborative new lead discovery venture using our repository of extracts and natural product compounds as input into our growth inhibition primary screen against *Trypanosoma brucei*. Careful evaluation of the spectral data of the natural products and derivatives allowed for the elucidation of the absolute configuration (using the modified Mosher's method) of two new peroxiterpenes: (+)-muqubilone B (**1a**) and (−)-ent-muqubilone (**3a**). Five known compounds were also isolated: (+)-sigmosceptrellin A (**4a**), (+)-sigmosceptrellin A methyl ester (**4b**), (−)-sigmosceptrellin B (**5**), (+)-epi-muqubillin A (**6**), and (−)-epi-nuapapuin B methyl ester (**7**). The isolated peroxiterpenes demonstrated activities in the range $IC_{50} = 0.2-2$ μg/mL.

The search for chemotherapeutics to treat human African trypanosomiasis (HAT), also known as African sleeping sickness, has not been a major target of industry drug discovery campaigns, even though the currently available therapeutics are inadequate.[1] This is surprising, as there are 50 000 annual cases of infection, HAT is the world's third most devastating parasitic disease, and it remains a major threat to more than 60 million people.[2–4] Because it is a serious health problem for resource-poor regions of Africa, HAT is designated as a neglected tropical disease (NTD). The causative agent is a protozoan parasite, *Trypanosoma brucei,*[5] subdivided in two subgenera, *T. brucei gambiense* in West Africa and *T. brucei rhodesiense* in East Africa.[1] Most of the four current chemotherapeutics to combat these parasites at their different development stages are very antiquated. The drugs (by registration date for HAT treatment) consist of suramin[4] (1922), pentamidine[1] (1941), melarsoprol[3] (1949), and eflorithine[1] (1990). Another relevant point is that new strains of *T. brucei* are showing cross-resistance to some of these agents.[6] In recent years, only one compound, DB289[7] (a synthetic analogue of pentamidine), received serious clinical evaluation against HAT, but further development was discontinued in 2008.[3]

The quest to discover antiparasitic lead structures has rarely focused on the chemical diversity inherent in marine natural products. Alternatively, recent studies have used combinatorial chemistry libraries of purine-[2] and thiosemicarbazone[5]-derived structures to seek inhibitors of different *T. brucei* targets.[2,5] In the early 1990s the University of California Santa Cruz group participated in a collaborative effort with Syntex (with Dr. T. Matthews) to explore marine natural products for their potential to provide anthelminthic lead structures,[8] but unfortunately these efforts were prematurely suspended. In this context there is a rather attention-grabbing report showing that psammaplin A,[9] first isolated from the marine sponge *Psamaplysilla* in 1987 and found to possess anthelminthic activity (against *Nippostrongylus brasiliensis*),[8] was recently found to be active against *T. brucei* ($EC_{50} = 2.6$ μM).[10] Inspired by this recent development, we have launched a new collaborative lead discovery venture using our repository of extracts and natural product compounds as input into our growth inhibition primary screen against *T. brucei*. We were encouraged to continue this effort because of an assay hit from the sponge *Diacarnus bismarckensis*, which was available in large amounts. The current literature shows over two dozen peroxiterpenes[11] reported from *Diacarnus* taxon, and these have been primarily evaluated in anticancer screens.[12] Reported in this account are our efforts to isolate and define the total structures of the active principles, presumed to be peroxiterpenes, followed by further biological activity study.

Results and Discussion

Once the decision was made to select the *T. brucei* assay active crude extracts from *D. bismarckensis* (coll. no. 03512), the next step was to employ bioassay-guided fractionation. Although the methanol crude extract partition (XFM, 2.06 g, $IC_{50} = 5.0$ μg/mL) showed good activity, the hexanes (XFH, 8.80 g, $IC_{50} = 0.02$ μg/mL) and dichloromethane (XFD, 0.66 g, $IC_{50} = 0.3$ μg/mL) partitions were selected for further study due to their higher potencies. Analysis of their respective ^{13}C NMR spectra confirmed the presence of peroxiterpenes because of prominent resonances in the region δ_C 80−82. Orthogonal chromatography facilitated the isolation work, beginning with silica gel flash chromatography followed by reversed-phase HPLC, which eventually afforded seven peroxiterpene compounds. The XFH provided a mixture of **1a**, related to (+)-muqubilone/aikupikoxide A (**2**),[13,14] and (−)-ent-muqubilone (**3a**), an unreported enantiomer of **2**. Preparation of their respective methyl esters was required in the final purification of these compounds. Five additional compounds came from the XFH including (+)-sigmosceptrellin A (**4a**),[15,16] (+)-sigmosceptrellin A methyl ester (**4b**),[15,16] (−)-sigmosceptrellin B (**5**),[16] (+)-epi-muqubilin A (**6**),[12a] and (−)-epi-nuapapuin B methyl ester (**7**).[12a] The XFD was also a source of four pure compounds: **1a**, **4a**, **5**, and **6**. We obtained all compounds as free carboxylic acids, with the exception of minor quantities of methyl ester **4b** and **7**, which are most likely artifacts of isolation. The known compounds (**4**−**7**) have also been previously obtained from sponges classified as *Diacarnus* sp.[17]

The structure elucidation of the new compound (+)-muqubilone B (**1a**), $C_{24}H_{40}O_6$, was initiated by using the characteristic ^{13}C NMR shifts at $\delta_C = 81.2$ (CH) and 80.0 (C) to indicate the presence of

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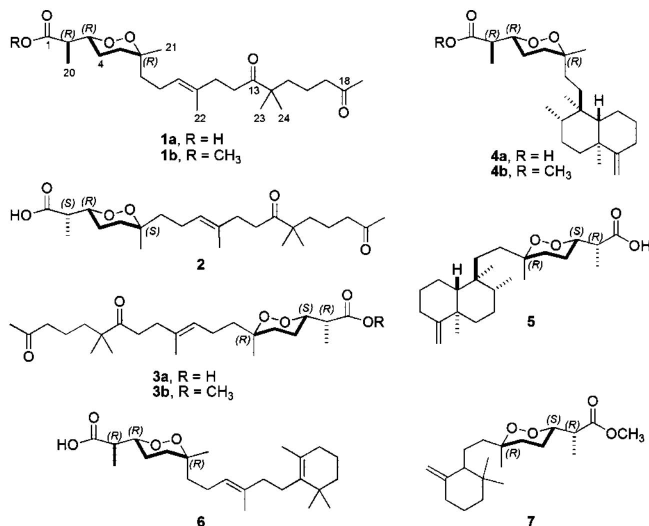


a peroxide functionality with trisubstitution (Table 1).[18] Distinct NMR signals for a carboxylic acid (δ_C = 178.6), an acetyl group (δ_C = 209.1, 29.8), an isolated ketone (δ_C = 215.4), and a trisubstituted double bond (δ_C = 124.6, 134.4), the molecular formula obtained from the HRESIMS, and dereplication insights all supported that **1** was a norsesterterpene containing an endoperoxide ring. Another diagnostic NMR signal was that of an isochronous gem-dimethyl functionality (δ_C = 24.3; δ_H = 1.13). At this point it was evident that the C_3-containing carboxylate and the C_{16} acyclic side chains were similar to that of **2**.[13,14] The side-by-side comparison of the similarities and differences in the NMR data between **1a** and **2** plus the 2D NMR data of the former (Figure 1) unequivocally confirmed that this pair had identical planar structures and must be diastereomers.

Establishing the complete absolute configuration of **1a**, while reasonably straightforward, required a multistep analysis. The *E* double-bond geometry was confirmed by the NOE data shown in Figure 1 (correlation from H-9 to H_2-11) and the characteristic C-22 shift.[19] We have previously used the empirical observations laid out by Capon and MacLeod[18] to set the relative configuration at each of the three chiral centers of the molecular fragment present in *Diacarnus*-derived peroxiterpenes and repeated this process here,[12a] but it first required the preparation of the methyl ester **1b** to obtain a data set consistent with the models.[20] The carbon substituent at C-3 was set as equatorial because H-3 exhibited vicinal axial coupling to H-4, $J_{3,4}$ = 8.0 Hz (J = 3−4 Hz is expected for equatorial). The methyl at C-6 (δ_C = 24.2) was next assigned as equatorial because of its diagnostic ^{13}C NMR shift ($\delta_{C,eq}$ = 23.5−24.0; $\delta_{C,ax}$ = 20.5−20.9). Finally the erythro (R*/R*) configuration at C-2/C-3 was designated based on the proton chemical shift position at H_3-20, δ_H 1.14 (standard values: $\delta_{H,erythro}$ = 1.13−1.14; $\delta_{H,threo}$ = 1.22−1.24).[18] The resulting all-R* configuration at each of the chiral centers confirmed that **1a** ([α] = +60) was an unreported diastereomer of **2**, whose relative configuration were previously independently reported as 2R*, 3S*, 6R* for both (+)-muqubilone ([α] = +48)[13] and aikupikoxide A ([α] = +81).[14]

We next concluded it was essential to push forward and ascertain the absolute configuration of **1a** with the expectation such findings could provide similar insights for **2**. Hydrogenation of **1b**[21] afforded diol **8**, which was further transformed into Mosher's esters **9a** and **9b** as shown in Scheme 1.[21,22] The circumstance that the Δ^9 double bond was also reduced during the hydrogenation did not affect the outcome in evaluating the $\Delta\delta$ values ($\delta_S - \delta_R$) to verify C-3 as R. Thus, the 2R, 3R, 6R final absolute configuration is now assigned for (+)-muqubilone B (**1a**). It is notable that (+)-2R,3R,6R-epi-muqubilin A[12a] (**6**) ([α] = +59) also isolated from this sponge represents an obvious biosynthetic precursor of (+)-**1a**, especially

Table 1. NMR Data for (+)-Muqubilone B (**1a**) in $CDCl_3$[a]

position	δ_C, type	δ_H, mult. (J in Hz)	HMBC (H to C)	COSY
1	178.6, C			
2	42.4, CH	2.55, quint. (7.2)	3, 20	3, 20
3	81.2, CH	4.25, ddd (8.4, 7.2, 4.2)		2, 4
4ax	23.8, CH_2	1.77, dtd (12.0, 8.4, 4.2)	21	5
4eq		1.70, dtd (12.0, 4.2, 3)	3	3
5	32.5, CH_2	1.66, m	6, 21	4
6	80.0, C			
7	34.7, CH_2	1.49, m	21	8
8a	22.0, CH_2	2.04, m		7, 9
8b		1.94, m		
9	124.6, CH	5.16, td (7.2, 1.2)		8
10	134.4, C			
11	33.4, CH_2	2.21, t (7.8)	9, 10, 12, 22	12
12	35.6, CH_2	2.56, t (7.8)	10, 11, 13	11
13	215.4, C			
14	47.5, C			
15a	39.1, CH_2	1.51, m	16	16
15b		1.46, m		
16	18.9, CH_2	1.42, m	15	15, 17
17	43.9, CH_2	2.42, t (7.2)	16, 18	16
18	209.1, C			
19	29.8, CH_3	2.14, s	17, 18	
20	12.7, CH_3	1.20, d (7.2)	1, 2, 3	2
21	22.6, CH_3	1.12, s	4, 5, 6, 8	
22	16.0, CH_3	1.62, d (1.2)	9, 10, 11	
23,24	24.3, CH_3	1.13, s	13, 14, 15	
OH		3.71, s	1	

[a] Measured at 600 MHz (^1H) and 150 MHz (^{13}C).

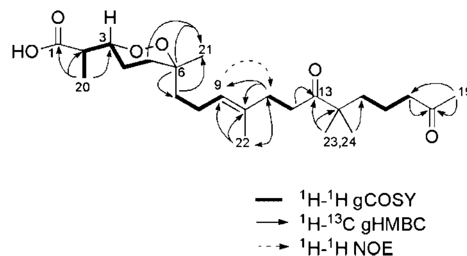
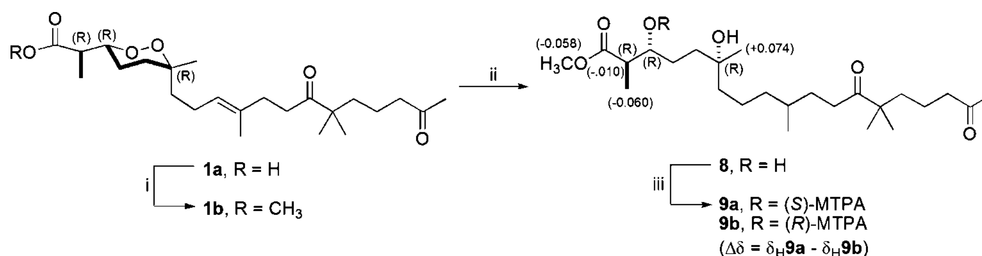


Figure 1. Key 2D NMR correlations for (+)-muqubilone B (**1a**).

in view of their identical chirality. Our further discussion on the absolute configuration of **2** shown here as 2S, 3R, 6S will accompany the characterization of **3**, which follows next.

Noted above was that the XFH provided a mixture of **1a** and an additional new compound, (−)-ent-muqubilone (**3a**). The separation of these compounds was accomplished by first subjecting the mixture to methylation using TMS−diazomethane followed by isocratic HPLC purification to cleanly afford **1b** ([α] = +58) and **3b** ([α] = −47) in a 2:1 ratio. The absolute stereostructure assigned for **3a** was based on data obtained for **3b** using the process parallel to that described above for **1**. Both **1b** and **3b** possessed identical molecular formulas and afforded nearly identical NMR shifts with **2**.[13] The following observations of H-3, J_{ax} = 8.0 Hz; CH_3-21, $\delta_{C,ax}$ = 20.9; CH_3-20, $\delta_{H,threo}$ = 1.25 indicated that the relative configurations reported for **3b** were equivalent with that of **2**, but these compounds possessed opposite rotation data (see above) and must be enantiomers. A review of the current literature (see Table S1, Supporting Information)[16,18,23] affirms that the variations in the absolute configurations at C-2 and C-6 within the peroxide-containing substructure comprised of the chain from C-1 to C-6 do not influence the rotation sign. Additionally, the sign of optical rotation does not change upon substitution of the free carboxylate. Thus, the absolute configuration at C-3 can be used as an empirical anchor point. For example, when H-3 is in the axial orientation, a (−) $[\alpha]_D$ is observed for the 3S configuration and the (+) rotation is observed for 3R (in $CHCl_3$). Consequently, the compound (−)-**3b** can be concluded to have the stereostructure of 2R, 3S, 6R.

Scheme 1. Modified Mosher's Method Analysis of (+)-Muqubilone B (**1a**)[a]



[a] (i) TMS−CHN_2 (2 M in hexanes), CH_3OH, rt, 30 min; (ii) H_2 (1 atm), Pd/C, EtOH, rt, 1 h; (iii) (+)- or (−)-MTPA-Cl, pyr., rt, 18 h.

Furthermore (+)-muqubilone/aikupikoxide A[13,14] (**2**), whose rotation data were discussed above, can be assigned as 2*S*, 3*R*, 6*S*.

The peroxiterpenes isolated in this work and evaluated against *T. brucei* can be grouped into four structural types based simply on the side chain attached at C-6 of the 1,2-dioxane ring. The first three types each have the C_{16} carbon side chain that is either acyclic (**1**−**3**), monocyclic (**6**), or bicyclic (**4**, **5**). The final type (**7**) has a C_{11} monocyclic carbon side chain. The muqubilones (**1a**, **1b**, **3b**) showed similar bioactivities independent of the nature of the carboxylate: free acid (**1a**, IC_{50} = 2 μg/mL) or methyl ester **1b** (IC_{50} = 5 μg/mL) and **3b** (IC_{50} = 3 μg/mL). Consistent with the supposition that bioactivity hinges on the presence of the 1,2-dioxane ring was that diol **8** (IC_{50} > 25 μg/mL) prepared from **1b** was inactive. A change in the side chain to that containing either a monocyclic or bicyclic ring imparts a barely observable change, as shown by the data for sigmosceptrellin A (**4a**, IC_{50} = 1 μg/mL) and its methyl ester (**4b**, IC_{50} = 2 μg/mL), sigmosceptrellin B (**5**, IC_{50} = 0.2 μg/mL), and epi-muqubilin A (**6**, IC_{50} = 0.9 μg/mL). Finally, the length of the side chain does not greatly influence the activity, as shown by epi-nuapapuin B methyl ester (**7**, IC_{50} = 2 μg/mL).

It is relevant to overlay our observations with those from recent studies involving 1,2-dioxane ring containing terpenoids or polyketides and somewhat analogous peroxide ring compounds studied by others. An investigation of *Diacarnus megaspinorhabdosa* afforded a series of peroxiterpenes and one diol product with structures parallel to those reported here.[12] There were varying EC_{50} cytotoxicity effects observed from the evaluation of these compounds against three cancer cell lines, and those possessing a C_{16} side chain at C-6 were generally more cytotoxic than those with a C_{11} appendage. Curiously, the one natural product evaluated where the peroxide ring was not present, but replaced by a diol, displayed mild activity against one cell line and inactivity against the other two cell types. In another study, the cytotoxic and antiparasitic properties were compared for 1,2-dioxane and furan ketides isolated from the sponge *Plakortis angulospiculatus*.[24] These results showed that both heterocyclic ring types displayed modest cytotoxicity effects, while one of the 1,2-dioxane structures potently inhibited *Leishmania chagasi* and *Trypanosoma cruzi*. These preceding literature findings, and those not discussed here showing that other polyketide 1,2-dioxanes have antileishmanial activity,[25] alongside our results together suggest that the polyalkylated 1,2-dioxane frameworks are of promise for further development as lead structures. In addition, the therapeutic index of antiparasitic to cytotoxicity action for these structures is in the correct direction.

The responses of *T. brucei rhodesiense* have been evaluated for two other sets of peroxide-containing structures as follows. The wormwood tree peroxisesquiterpene, artemisinin, exhibits nM action against *Plasmodium falciparum*.[26] This compound (*T. b. rhodesiense* IC_{50} = 6[27] or 25[26] μg/mL) and its close analogue deoxyartemisinin (*T. b. rhodesiense* IC_{50} = 34[26]) possess similar mild activity against *T. b. rhodesiense*, and the latter, devoid of the cyclic peroxide functionality, is inactive versus *P. falciparum*. A similar SAR activity pattern is shown for another matched set. This includes the 1,2,4-trioxolane-containing synthetic polycyclic OZ277 (*T. b. rhodesiense* IC_{50} = 0.6 μg/mL)[26] versus the 1,3-dioxolane-containing synthetic polycyclic carbaOZ277 (*T. b. rhodesiense* IC_{50} = 0.9 μg/mL),[26] which are equipotent against *T. b. rhodesiense* (and have not been tested versus *P. falciparum*). Overall, it would appear that structural features of **5** (*T. brucei* IC_{50} = 0.2 μg/mL) would be an excellent starting point for further SAR modifications, as it showed similar potencies on par with a current HAT therapeutic, pentamidine (*T. b. rhodesiense* IC_{50} = 0.4 μg/mL).[27]

Conclusions

This study has revealed new dimensions to the structural and biological activity properties of peroxiterpenes isolated from *Diacarnus* sponges. First, careful evaluation of the spectral data of the natural products and derivatives allowed for the elucidation of the absolute configuration of compounds we isolated including **1a** and **3a** plus a proposal for the absolute configuration of **2**. Second, the observation of enantiomeric structures (**2** versus **3**) from the same genus, *Diacarnus*, but different species from different oceans (**2** from a Red Sea specimen, *D. erythaeanus*, and **3** from the Indo-Pacific specimen *D. bismarckensis*) is a stunning and rare observation in marine natural products chemistry. Third, we have further demonstrated the value in vigorously considering peroxiterpenes, especially the known compound (−)-sigmosceptrellin B (**5**), as a template for the development of therapeutic leads against *T. brucei*.

Experimental Section

General Experimental Procedures. All NMR experiments were run on Varian UNITY 500 (500 and 125 MHz for ^{1}H and ^{13}C, respectively) and Varian INOVA 600 spectrometers (600 and 150 MHz for ^{1}H and ^{13}C, respectively) using standard pulse sequences with residual solvent protons and carbons as references ($CDCl_3$: 7.27 and 77.23 ppm for ^{1}H and ^{13}C, respectively). Mass measurements were obtained on a benchtop Mariner ESI-TOF-MS. Crude extractions were obtained using an accelerated solvent extractor (1500 psi, 100 °C, 25 min). Flash chromatography was done on a CombiFlash, using prepacked 40 g silica columns. HPLC was performed with Phenomenex Synergi RP-Max preparative (10 μm, C_{18}, 21.2 mm × 250 mm) and Luna semipreparative (5 μm, C_{18}, 10 mm × 250 mm) columns.

Collection and Identification. The sponge materials were collected in December 2003 by scuba at a depth range of 31−40 ft near Sanaroa, Papua New Guinea (GPS = 9°37.214′ S: 150°57.332′ E). The sponge samples were ramose in shape with very small oscules throughout and had a purple-colored endosome with a gray-colored ectosome. The collection was identified as *Diacarnus bismarckensis* (Kelly-Borges & Vacelet, 1995) by Dr. R. W. M. van Soest. Voucher samples have been deposited at UCSC and the Zoological Museum of Amsterdam (UCSC coll. no. 03512 = ZMAPOR 18576). Photographs are also available from the Crews laboratory.

Extraction and Isolation of 03512. Following standard Crews laboratory field protocol, the sponge was soaked for over 24 h in an ethanol/seawater (1:1) preservative solution, which was discarded in the field. The samples were then packed in bottles and shipped to UCSC, where the sponge was cut into 1.5 in. segments and dried under air for 2 days. A 73.4 g amount of dried sponge was extracted using an

accelerated solvent extractor (100 °C, 1500 psi, 25 min) to yield hexanes (XFH, 8.80 g), CH_2Cl_2 (XFD, 0.6615 g), and CH_3OH (XFM, 2.06 g) crude extracts. A bioassay-guided fraction then followed on the XFH and XFD fractions. The large quantity of XFH crude warranted a further Kupchan-like solvent partition to yield hexanes (XFHFH, 4.08 g), CH_2Cl_2 (XFHFD, 4.08 g), and CH_3OH (XFHFM, 0.518 g) fractions. The XFHFH crude was subjected to normal-phase automated flash chromatography using a linear gradient on silica gel (CH_2Cl_2 to CH_3OH, 40 min), yielding six fractions (XFHFHC1−FHFHC6). An orthogonal chromatographic approach was used on the C3 (1.10 g) and C4 (942 mg) fractions with reversed-phase preparative HPLC (80% aq to 100% CH_3CN linear gradient, 0.1% formic acid, 20 min, ELSD) followed by semipreparative HPLC (70% aq CH_3CN isocratic, 0.1% formic acid, UV = 200 nm). From these fractions, (−)-epi-nuapapuin B methyl ester[12a] (**7**, 2.9 mg), (+)-sigmosceptrellin A[15,16] (**4a**, 68.6 mg), (−)-sigmosceptrellin B[16] (**5**, 33.8 mg), (+)-epi-muqubillin A[12a] (**6**, 23.9 mg), and (+)-sigmosceptrellin A methyl ester[15,16] (**4b**, 8.2 mg) were isolated. The XFHFD was treated in a similar manner to yield a 2:1 mixture (21.4 mg) of (+)-muqubilone B (**1a**) and (−)-ent-muqubilone (**3a**), which were separable after methyl esterification and HPLC (isocratic 85% aq CH_3CN, 0.1% formic acid, UV = 200 nm). The XFD crude fraction was subjected to orthogonal chromatography, as previously described. These fractions yielded (+)-muqubilone B (**1a**, 14.3 mg), (+)-sigmosceptrellin A[15,16] (**4a**, 34.3 mg), (−)-sigmosceptrellin B[12a] (**5**, 6.1 mg), and (+)-epi-muqubillin A[12a] (**6**, 6.9 mg).

***Trypanosoma brucei brucei* Assay.** *T. brucei brucei* strain 221 was grown in complete HMI-9 medium containing 10% FBS, 10% Serum Plus medium (Sigma Inc., St. Louis, MO) and 1X penicillin/streptomycin. The trypanosomes were diluted to 1×10^5 per mL in complete HMI-9 medium. Then 95 μL per well of the diluted trypanosomes was added to sterile Greiner 96-well flat white opaque culture plates that contained 5 μL of test samples (in 10% DMSO). Control wells contained 95 μL of the diluted trypanosomes and 5 μL of 10% DMSO, while control wells for 100% inhibition contained 95 μL of the diluted trypanosomes and 5 μL of 1 mM thimerosal (in 10% DMSO). Trypanosomes were incubated with test samples for 48 h at 37 °C with 5% CO_2 before monitoring viability. Trypanosomes were then lysed in the wells by adding 50 μL of CellTiter-Glo (Promega Inc., Madison, WI). Lysed trypanosomes were placed on an orbital shaker at room temperature for 2 min. The resulting ATP-bioluminescence of the trypanosomes in the 96-well plates was measured at room temperature using an Analyst HT plate reader (Molecular Devices, Sunnyvale, CA). All IC_{50} curve fittings were performed with Prism 4 software (GraphPad, San Diego, CA).

(+)-Muqubilone B (1a): clear oil; $[\alpha]^{28}_D$ +59.5 (*c* 0.046, $CHCl_3$); NMR in Table 1; HRESIMS 425.2934 $[M + H]^+$ (calcd for $C_{24}H_{41}O_6$, 425.2898).

(+)-Sigmosceptrellin A (4a): physical data in accordance with published data.[15,18]

(+)-Sigmosceptrillin A methyl ester (4b): physical data in accordance with published data.[15,18]

(−)-Sigmosceptrellin B (5): physical data in accordance with published data.[16,18]

(+)-Epi-muqubilin A (6): physical data in accordance with published data.[12a]

(−)-Epi-nuapapuin B methyl ester (7): physical data in accordance with published data.[12a]

Methylation of (+)-Muqubilone B (1a). A 6.5 mg sample of (+)-muqubilone B (**1a**) was dissolved into 1 mL of CH_3OH to which 2 mL of trimethylsilyldiazomethane (2 M in hexanes) was added, and the mixture was stirred at room temperature for 30 min. The solvent was evaporated under nitrogen, and the reaction mixture was purified by HPLC (C_{18} column, 85% aq CH_3CN isocratic, UV = 200 nm) to yield 6.2 mg of (+)-muqubilone B methyl ester (**1b**). **1b**: $[\alpha]^{28}_D$ +57.6 (*c* 0.011, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 1.11 (3H, s, H_3-21), 1.12 (6H, s, H_3-23, H_3-24), 1.14 (3H, d, J = 7.5 Hz, H_3-20), 1.41 (2H, m, H_2-7), 1.47 (2H, m, H_2-16), 1.55 (2H, m, H_2-15), 1.62 (3H, d, J = 1 Hz, H_3-22), 1.63 (2H, m, H_2-5), 1.76 (1H, dtd, J = 13, 4.5, 2.5 Hz, H_{eq}-4), 1.85 (1H, dtd, J = 13, 8, 4.5 Hz, H_{ax}-4), 1.94 (1H, m, H_b-8), 2.04 (1H, m, H_a-8), 2.13 (3H, s, H_3-19), 2.21 (2H, t, J = 8 Hz, H_2-11), 2.41 (2H, t, J = 7 Hz, H_2-17), 2.55 (2H, t, J = 8 Hz, H_2-12), 2.58 (1H, quint., J = 7.5 Hz, H-2), 3.70 (3H, s, OCH_3), 4.24 (1H, ddd, J = 8.0, 7.5, 4.5 Hz, H-3), 5.16 (1H, tq, J = 6.5 Hz, 1, H-9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 13.1 (CH_3-20), 16.3 (CH_3-22), 19.2 (CH_2-16), 22.3 (CH_2-8), 22.9 (CH_2-4), 24.2 (CH_3-21), 24.6 (CH_3-23), 24.6 (CH_3-24), 30.2 (CH_2-7), 32.7 (CH_3-11), 33.7 (CH_2-19), 35.0 (CH_2-12), 35.9 (CH_2-5), 39.4 (CH_2-15), 42.9 (CH-2), 44.2 (CH_2-17), 44.2 (C-14), 52.2 (O-CH_3), 80.1 (C-6), 81.5 (CH-3), 124.8 (CH-9), 134.6 (C-10), 174.6 (C-1), 208.8 (C-18), 215.4 (C-13); HRESIMS *m/z* 461.2906 $[M + Na]^+$ (calcd for $C_{25}H_{42}O_6Na$, 461.2874).

Hydrogenation of (+)-Muqubilone B Methyl Ester (1b). A 4 mg amount of (+)-muqubilone B methyl ester (**1b**) was dissolved into 2 mL of ethanol. Then 10 mg of palladium on carbon was added, and the slurry was stirred under 1 atm of H_2 for 1 h at room temperature. The reaction mixture was quenched with water, and the palladium catalyst was filtered through Celite to afford 4 mg of (+)-muqubilone B diol (**8**). **8**: $[\alpha]^{28}_D$ +1.7 (*c* 0.017, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.87 (3H, d, J = 6.5 Hz, H_3-22), 1.11 (6H, s, H_3-23, H_3-24), 1.16 (3H, s, H_3-21), 1.21 (3H, d, J = 7 Hz), 1.30 (2H, m, H_2-8), 1.37 (1H, m, H_b-5), 1.40 (2H, m, H_2-11), 1.43 (1H, m, H_b-15), 1.44 (2H, m, H_2-7), 1.45 (2H, m, H_2-16), 1.46 (1H, m, H_a-15), 1.50 (1H, m, H_a-5), 1.59 (1H, m, H_b-9), 1.60 (1H, m, H_a-10), 1.66 (1H, m, H_a-9), 1.67 (1H, m, H_a-4), 2.13 (3H, s, H_3-19), 2.41 (2H, t, J = 6.5 Hz, H_2-17), 2.46 (2H, t, J = 8.5 Hz, H_2-12), 2.56 (H, quint., J = 7 Hz, H-2), 3.70 (H, ddd, J = 8, 6, 2 Hz, H-3), 3.72 (3H, s, O-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 14.5 (CH_3-20), 19.2 (CH_3-22), 19.8 (CH_2-16), 21.5 (CH_2-8), 24.6 (CH_3-23), 24.6 (CH_3-24), 27.1 (CH_3-21), 29.0 (CH_2-4), 30.1 (CH_3-19), 30.9 (CH-10), 32.6 (CH_2-11), 34.7 (CH_2-12), 37.6 (CH_2-9), 39.5 (CH_2-15), 42.3 (CH_2-7), 42.6 (CH_2-5), 44.1 (CH_2-17), 45.5 (CH-2), 47.7 (C-14), 52.0 (O-CH_3), 72.6 (C-6), 74.0 (CH-3), 176.7 (C-1), 208.9 (C-18), 216.1 (C-13); HRESIMS *m/z* 465.3224 $[M + Na]^+$ (calcd for $C_{25}H_{46}O_6Na$, 465.3192).

(*R*)- and (*S*)-MTPA Esterification of (+)-Muqubilone B Diol (8). A 2 mg sample of (+)-muqubilone B diol (**8**) was dissolved in 200 μL of dry pyridine to which 15 μL of (−)-(*R*)-MTPA-Cl was added. This was stirred at room temperature, under nitrogen, overnight. After the solvent was evaporated under nitrogen, the reaction mixture was purified by HPLC (C_{18} column, 85% aq CH_3CN isocratic, UV = 254 nm) to yield 1.8 mg of (*S*)-MTPA ester (**9a**). Similarly, 1.8 mg of the diol (**8**) was derivatized with (+)-(*S*)-MTPA-Cl to afford 1.7 mg of the (*R*)-MTPA ester (**9b**). **9a**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.863 (3H, d, J = 6 Hz, H_3-22), 1.110 (3H, s, H_3-21), 1.117 (6H, s, H_3-23, H_3-24), 1.126 (3H, d, J = 6 Hz, H_3-20), 2.123 (3H, s, H_3-19), 2.410 (2H, t, J = 6.5 Hz, H_2-17), 2.855 (2H, t, J = 6.5 Hz, H_2-12), 2.855 (H, quint., J = 7 Hz, H-2), 3.522 (3H, s, MTPA-OCH_3), 3.586 (3H, s, O-CH_3), 5.394 (H, td, J = 7.5, 4 Hz, H-3), 7.425 (4H, m, MTPA-Ar), 7.561 (1H, m, MTPA-Ar); ESIMS *m/z* 681 $[M + Na]^+$. **9b**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.873 (3H, d, J = 6.5 Hz, H_3-22), 1.036 (3H, s, H_3-21), 1.121 (6H, s, H_3-23, H_3-24), 1.186 (3H, d, J = 7.5 Hz, H_3-20), 2.125 (3H, s, H_3-19), 2.413 (2H, t, J = 6.5 Hz, H_2-17), 2.467 (2H, t, J = 6.5 Hz, H_2-12), 2.865 (1H, quint., J = 7.5 Hz, H-2), 3.540 (3H, s, MTPA-O-CH_3), 3.644 (3H, s, O-CH_3), 5.382 (1H, td, J = 7.5, 4 Hz, H-3), 7.428 (4H, m, MTPA-Ar), 7.560 (1H, m, MTPA-Ar); ESIMS *m/z* 681 $[M + Na]^+$.

Methylation of (−)-Ent-muqubilone (3a). A 5 mg amount of an inseparable mixture of (+)-muqubilone B (**1a**) and (−)-ent-muqubilone (**3a**) (2:1) was dissolved into 1 mL of CH_3OH to which 2 mL of trimethylsilyldiazomethane (2 M in hexanes) was added, and the mixture was stirred at room temperature for 30 min. The solvent was evaporated under nitrogen, and the reaction mixture was purified by HPLC (C_{18} column, 85% aq CH_3CN isocratic, UV = 200 nm) to yield 3.2 mg of (+)-muqubilone B methyl ester (**1b**) and 1.6 mg of (−)-ent-muqubilone methyl ester (**3b**). **3b**: $[\alpha]^{28}_D$ = −47 (*c* 0.004, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 1.11 (6H, s, H_3-23, H_3-24), 1.25 (3H, d, J = 7.0 Hz, H_3-20), 1.29 (3H, s, H_3-21), 1.41 (2H, m, H_2-16), 1.44 (2H, m, H_2-7), 1.47 (2H, m, H_2-15), 1.60 (3H, s, H_3-22), 1.63 (2H, m, H_2-5), 1.71 (2H, m, H_2-4), 2.00 (2H, m, H_2-8), 2.13 (3H, s, H_3-19), 2.20 (2H, t, J = 8.0 Hz, H_2-11), 2.41 (2H, t, J = 7.0 Hz, H_2-17), 2.54 (2H, t, J = 8.0 Hz, H_2-12), 2.66 (1H, q, J = 7.5 Hz, H-2), 3.70 (3H, s, O-CH_3), 4.12 (1H, ddd, J = 8.0, 7.5, 4.5 Hz, H-3), 5.10 (1H, t, J = 7.0 Hz, H-9); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 13.8 (CH_3-20), 16.3 (CH_3-22), 19.2 (CH_2-16), 20.9 (CH_3-21), 21.9 (CH_2-8), 23.7 (CH_2-4), 24.5 (CH_3-23), 24.5 (CH_3-24), 30.1 (CH_3-19), 32.2 (CH_2-5), 33.7 (CH_2-11), 35.8 (CH_2-12), 39.4 (CH_2-15), 39.8 (CH_2-7), 43.2 (CH-2), 44.1 (CH_2-17), 47.7 (C-14), 52.7 (O-CH_3), 80.3 (C-6), 81.6 (CH-3), 124.6 (CH-9), 134.8 (C-10), 174.5 (C-1), 208.8 (C-18), 215.3 (C-13); HRESIMS *m/z* 461.2898 $[M + Na]^+$ (calcd for $C_{25}H_{42}O_6Na$, 461.2874).

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Supporting Information Available: NMR and MS spectra, sponge photographs, isolation scheme, and table of optical rotations and absolute configurations of known norterpene peroxides. This material is available free of charge via the Internet at http://pubs.acs.org.

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