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Lipidyl Pseudopteranes A−F: Isolation, Biomimetic Synthesis, and PTP1B Inhibitory Activity of a New Class of Pseudopteranoids from the Gorgonian *Pseudopterogorgia acerosa*

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Novel lipidyl pseudopteranoids, lipidyl pseudopteranes A−F (**1**−**6**), have been isolated from the soft coral *Pseudopterogorgia acerosa* collected from the Bahamas. Structure elucidation of the six new compounds was based on 1D and 2D NMR data and mass spectrometry, and a biomimetic synthesis of **1** from pseudopterolide (**7**) was used to help establish its absolute configuration. These structures represent the first report of a pseudopterane diterpene with a fatty acid moiety. Lipidyl pseudopteranes A and D exhibited modest yet selective inhibitory activity against protein tyrosine phosphatase 1B, a promising drug target.

The marine gorgonian coral *Pseudopterogorgia acerosa* is known for its diversity of diterpenes. Pseudopterolide (**7**) was isolated by Fenical and co-workers[1] as the first pseudopterane diterpene from *P. acerosa* collected in the Florida Keys. In the 1990s, more than 22 new pseudopteranoid and cembranoid diterpenes were reported from specimens of *P. acerosa* collected from Puerto Rico and Tobago.[2−4] We have investigated the diterpene chemistry of samples of *P. acerosa* collected from The Bahamas and herein report the isolation of a new family of pseudopteranes, which were identified from a UHPLCMS analysis of a nonpolar fraction of the crude extract. The six most abundant members of this group are pseudopteranoids that we have named lipidyl pseudopteranes A−F (**1**−**6**). Characterization of the compounds was performed by 1D and 2D NMR and MS analysis.

A biomimetic synthesis of lipidyl pseudopterane A was completed by the reaction of palmitic acid with pseudopterolide in the presence of a catalytic amount of triethyl amine, suggesting that pseudopterolide is a likely precursor in the biosynthesis of this lipidyl pseudopterane. Four of the lipidyl pseudopteranes (**1**, **4**−**6**) possess a palmitic acid moiety, in keeping with the observation that this is the major fatty acid in *P. acerosa*.[5]

These compounds were evaluated for their selective inhibitory activity against protein tyrosine phosphatase 1B (PTP1B). PTP1B is well established as a critical modulator of metabolic signaling in mammals, and recent reviews have thoroughly described the PTP family and PTP1B in particular.[6−11] PTP1B null mice display enhanced sensitivity to insulin.[12] This correlates with enhanced tyrosine phosphorylation of the insulin receptor in muscle and liver. PTP1B is also involved in leptin signaling.[13−16] Ablation of PTP1B confers resistance to obesity induced by a high-fat diet,[12] and weight gain in leptin-deficient ob/ob mice lacking PTP1B is significantly decreased as compared to control littermates.[13] Moreover, recent findings also suggest that inhibiting PTP1B may have important applications in the treatment of breast cancers.[17] The well-established protective effect of PTP1B genetic ablation on the development of diabetes, obesity, and cancer prompted substantial commercial interest in PTP1B as a drug target.[18,19] Indeed, an antisense oligonucleotide therapy targeting PTP1B is currently in phase II clinical trials.[20] In the development of small-molecule PTP1B inhibitors, achieving sufficient target specificity has been an important challenge. Members of the PTP family share a well-conserved catalytic domain that can be broadly targeted by nonspecific inhibitors. However, the phenotypes of mouse models lacking a variety of individual PTPs indicate that inhibition of many

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Table 1. NMR Data for Lipidyl Pseudopterane A (**1**) in $CDCl_3$

position	^{13}C (mult.)[a]	^{1}H mult. (*J* Hz)	$^{1}H-^{1}H$ COSY	HMBC ($^{1}H-^{13}C$)	$^{1}H-^{1}H$ NOESY
1	43.4 (CH)	3.33 dd (13.1, 3.7)	H2a, H2b		H2b
2	32.2 (CH_2)	3.61 dd (15.7, 13.1)	H2b, 1	C-1	H2b, 12, 14b
		2.88 dd, (15.7, 3.7)	H2a, 1	C-12, C-3	H2a, 1
3	160.5 (C)				
4	116.5 (C)				
5	110.4 (CH)	6.38 s		C-4, C-6, C-3	7
6	150.7 (C)				
7	48.6 (CH)	3.82 d (4.3)[b]	8		8, 19, 5, 9
8	80.1 (CH)	5.41 dd (4.1, 0.99)[b]	7, 9	C-10, C-20	7, 9, 19
9	148.2 (CH)	6.89 br s	8	C-11, C-8, C-10, C-20	8, 7, 11[b], 12
10	132.0 (C)				
11	75.5 (CH)	5.41 d (2.6)[b]	12	C-1, C-12, C-10, C-20	12[b], 9[b]
12	75.4 (CH)	3.17 br s	11,12(OH)		11, 2a
12(OH)		2.43 nr[e]	12		
13	144.4 (C)				
14	117.2 (CH_2)	5.30 s	14b, 15	C-15, C-1	14b, 15
		5.17 s	14a	C-15, C-1	14a, 2a, 1
15	24.2 (CH_3)	2.07 s	14a	C-1, C-14, C-13	1, 14a
16	164.0 (C)				
17	141.2 (C)				
18	115.6 (CH_2)	5.05 s	19	C-19, C-7	18b, 19
		4.83 s		C-19, C-7	18a
19	21.8 (CH_3)	1.96 s	18a	C-7, C-18, C-17	7, 18a, 8
20	170.6 (C)				
21	51.8 (CH_3)	3.82 s		C-16	
FA 1′[c]	174.0 (C)				
2′	34.0 (CH_2)	2.43 m	3′	C-1′, FAC	2′b, 3′
		2.36 m		C-1′, FAC	2′a, FAC
3′	24.8(CH_2)	1.63 m	2′a	FAC	2′a, FAC
14′[d]	32.1 (CH_2)	1.26 m			
15′[d]	22.9 (CH_2)	1.26 m			
16′	14.3 (CH_3)	0.89 t (6.7)	15′	FAC	FAC

[a] ^{13}C multiplicities were based on the DEPTQ and HMQC experiments. [b] CD_3OD was used as a solvent for NMR experiments. [c] Signals for the fatty acid chain other than mentioned in Table 1: ^{1}H NMR δ 1.26 (H-4′-H-13′); ^{13}C NMR δ 29.1−29.7 (C-4′−13′). [d] Signals were assigned on the basis of the HMBC spectrum. Fatty acid chain signals were represented by FAC. [e] Not resolved.

of these enzymes is not desirable. Thus, numerous groups have attempted to modify known, general PTP inhibitors to attain specificity for PTP1B, but no such compounds have succeeded in late-stage clinical trials. Moreover, the highly charged phosphotyrosine substrates of the PTPs have important consequences, and most of the small-molecule inhibitors identified to date are themselves highly hydrophilic. Hence, few have been identified with good membrane diffusible properties. In this report, we have identified lipidyl pseudopteranes A and D as a new class of PTP inhibitors that shows modest but selective activity against PTP1B. We suggest that their lipidyl moiety could aid in their ability to diffuse into mammalian cells and inhibit the intracellular PTPs such as PTP1B.

Results and Discussion

P. acerosa, collected at Sweetings Cay, Bahamas, was sun-dried and extracted with ethyl acetate and dichloromethane. The crude extract was subjected to silica gel flash column chromatography with a step gradient of solvents, hexanes to ethyl acetate, resulting in 11 fractions. The 50% hexane fraction was further purified by semipreparative HPLC using phenyl hexyl as a stationary phase, which led to the isolation of lipidyl pseudopteranes A−C (**1**−**3**). Lipidyl pseudopteranes D−F (**4**−**6**) were isolated from the 60% hexane fraction using C18 flash chromatography followed by semipreparative HPLC.

Lipidyl pseudopterane A (**1**) was obtained as a colorless oil, and HRMS analysis (observed $[M + Na]^+ = 649.3715$, calculated $[M + Na]^+ = 649.3716$) revealed the molecular formula $C_{37}H_{54}O_8$ with 11 degrees of unsaturation. IR absorptions at 3529, 3087, 1737, and 1720 cm^{-1} suggested the presence of a hydroxyl group, an alkene, an unsaturated γ-lactone, and a conjugated ester, which are characteristic of the pseudopteranoids.[3] The ^{1}H signals at δ 6.38 (1H, s) and 3.82 (3H, s) with the ^{13}C signals at δ 160.5(C), 116.5(C), 110.4(CH), 150.7(C), 164.0(C), and 51.8 (CH_3) suggested the presence of an α,α'-disubstituted β-carboxymethoxyfuran moiety. This was confirmed by HMBC cross-peaks of H-5 with C-4, C-6, and C-3 and H-21 with C-16 (Table 1). The proton signal at 6.89 showed cross-peaks with ^{13}C at δ 80.1 (CH), 132.0 (C), and 170.6 (C) in an HMBC experiment. These HMBC cross-peaks along with a comparison of the ^{1}H and ^{13}C signals with those of gorgiacerodiol[21] (**8**) revealed the presence of an α,γ-disubstituted α,β-unsaturated γ-lactone. The presence of two isopropenyl groups in **1** was evident from the ^{1}H and ^{13}C NMR data. The ^{1}H signals at δ_H 5.30 (br s, 1H), 5.17 (br s, 1H), and 2.07 (s, 3H) and the ^{13}C signals at δ_C 117.2 (CH_2), 144.4 (C), and 24.2 (CH_3) were assigned to one isopropenyl group, and the ^{1}H signals at δ_H 5.05 (br s, 1H), 4.83 (br s, 1H), and 1.96 (s, 3H) and the ^{13}C signals at δ_C 115.6 (CH_2) and 141.2 (C), 21.8 (CH_3) were assigned to a second such group. This analysis, together with $^{1}H-^{1}H$ COSY correlations (Table 1), clearly demonstrates the presence of a pseudopterane skeleton in **1**.

The mass spectrometry analysis indicated that the molecular weight of **1** was 238 mass units greater than gorgiacerodiol (**8**). The negative mode ionization of **1** followed by MS/MS fragmentation of the $[M - H]^-$ ion indicated that the peaks at *m/z* 387 and 255 corresponded to $C_{21}H_{21}O_6$ and $C_{16}H_{31}O_2$, respectively, indicating the presence of a palmityl group. The ^{1}H NMR signals at δ 0.89 (t), 1.26 (br s) 1.63 (m), and 2.43 (m) confirmed the presence of a fatty acid unit. To confirm the identity of this fatty acid moiety, base hydrolysis of the fraction enriched with lipidyl pseudopterane A was carried out followed by methyl esterification. This led to the formation of fatty acid methyl esters that were analyzed by GC-MS. The MS of the major peak was identical to that of methyl palmitate in the NIST library.

The location of the palmityl group on the pseudopterane ring was assigned on the basis of the chemical shift of H-11 (5.41 ppm)

and the comparison of the ^{1}H spectrum of **1** and of gorgiacerodiol (**8**).[21] All ^{1}H NMR signals in **1** and **8** were within ±0.12 ppm with the exception of H-11, which was at 4.65 ppm in gorgiacerodiol and at 5.41 ppm (Δ0.75) in **1**. Somewhat surprisingly, the three-bond HMBC correlation was not observed between H-11 (5.41 ppm) and C-1′ (174.0 ppm).

The relative configuration of **1** was established on the basis of 2D NOESY cross-peaks as well as ^{1}H−^{1}H coupling constants. H-5 showed a cross-peak with H-7, which in turn showed a cross-peak with H-8 in the NOESY spectrum, suggesting a *cis* orientation of these hydrogens. This was supported by the observed coupling constant (4.3 Hz) between H-7 and H-8. The ^{1}H−^{1}H coupling constant (2.6 Hz) between H-11 and H-12 suggested a dihedral angle close to 53° with consideration of the NOESY correlation.[22] The absence of a NOESY correlation between H-12 and H-1 suggested a *trans* orientation. These relative configurations and the absolute configuration of **1** were established on the basis of a biomimetic synthesis of **1** from pseudopterolide (**7**). Pseudopterolide was treated with palmitic acid in the presence of a catalytic quantity of triethyl amine to afford a trace amount of lipidyl pseudopterane A (0.03% yield). Most of the product mixture was unreacted pseudopterolide. The production of **1** was confirmed by LC-MS analysis and comparison with an authentic sample of lipidyl pseudopterane A. The product and authentic standard of **1** had identical retention times and MS fragmentation patterns. The assignments of stereocenters depicted in structure **1** (1*R,* 7*R,* 8*R,* 11*S,* 12*R*) are therefore based on the absolute configuration of pseudopterolide.[1] Further confirmation of the absolute configuration at C-8 was achieved using CD data. Previous studies with the α,β-unsaturated γ-lactone moiety have shown that the sign of the Cotton effect due to the n−π* and π−π* transitions can be correlated directly with the absolute configuration of the stereogenic center at the γ-carbon.[23] The CD data for compound **1** (measured in chloroform at concentration 3.6×10^{-3} M: Δε (270 nm, n−π*) = +3.10, Δε (231 nm, π−π*) = +11.28) revealed that the configuration at C-8 was *R*.[21]

HRMS analysis (observed $[M + Na]^+ = 675.3854$, calculated $[M + Na]^+ = 675.3867$) of **2** indicated a molecular formula of $C_{39}H_{56}O_8$, which suggested a molecular weight of 26 mass units greater than **1** with an additional degree of unsaturation. The APCI-MS/MS fragmentation pattern of ions less than *m/z* 371 was exactly the same as that of **1**, while the peaks greater than *m/z* 371 in the MS/MS spectrum were all (26) greater than those of **1**. This suggested the presence of the same pseudopteranoid structure as in **1** but with a different lipid side chain. ^{1}H and ^{13}C NMR spectra of **2** (Table 2) showed the same chemical shifts of the pseudopterane core of **1**, which was confirmed by 2D NMR experiments. The ^{1}H NMR of **2** showed signals at δ 0.89 (t), 1.26 (m), 1.61 (m), 2.00 (4H, m), 2.38 (1H, m), 2.43 (1H, m), and 5.35 (2H, m), which, together with the mass spectrometry analysis, revealed the presence of a C-18 chain with one double bond. A base hydrolysis of **2** and subsequent GC-MS comparison identified the fatty acid as oleic acid. On the basis of the chemical shift of H-11 in **2** and comparison with **1**, the oleoyl group was assigned to C-11 in **2**.

The HRMS analysis (observed $[M + Na]^+ = 677.3978$, calculated $[M + Na]^+ = 677.4024$) of compound **3** (molecular formula $C_{39}H_{58}O_8$) revealed that the molecular weight of **3** was two mass units greater than **2** with one less degree of unsaturation, which suggested the hydrogenation of one double bond. The MS/MS pattern suggested the difference of two mass units was contributed by hydrogenation of the double bond in the fatty acid chain of **2**. Comparison of the ^{1}H and ^{13}C chemical shifts and 2D NMR data of **1** and **3** confirmed the structure (Table 2) of lipidyl pseudopterane C as the stearyl analogue of **1**. The diterpene moiety is common in lipidyl pseudopteranes A−C, and hence it was assumed that the absolute and relative configuration of **2** and **3** is the same as in **1**.

Table 2. NMR Data for Lipidyl Pseudopteranes B and C (**2**, **3**) in $CDCl_3$

	2		**3**	
position	^{13}C (mult.)[a]	^{1}H mult (*J* Hz)	^{13}C (mult.)[a]	^{1}H mult (*J* Hz)
1	43.1 (CH)	3.33 dd (13.4, 3.1)	43.1 (CH)	3.33 dd (12.7, 3.5)
2	31.9 (CH_2)	3.61 dd (15.7, 13.1)	31.9 (CH_2)	3.62 dd (15.7, 13.1)
		2.89 dd (15.7, 3.7)		2.88 dd (15.7, 3.5)
3	160.2 (C)		160.2 (C)	
4	116.3 (C)		116.3 (C)	
5	110.2 (CH)	6.38 s	110.2 (CH)	6.38 s
6	150.4 (C)		150.4 (C)	
7	48.3 (CH)	3.82 nr[d]	48.3 (CH)	3.82 nr
8	79.9 (CH)	5.41 nr	79.9 (CH)	5.41 nr
9	147.9 (CH)	6.89 br s	148.0 (CH)	6.89 br s
10	131.7 (C)		132.4 (C)	
11	75.2 (CH)	5.40 nr	75.2 (CH)	5.40 nr
12	75.2 (CH)	3.16 br d (7.9)	75.2 (CH)	3.14 dd (10.5, 2.3)
		2.43 nr		2.43 nr
13	144.2 (C)		144.2 (C)	
14	116.9 (CH_2)	5.30 s	116.9 (CH_2)	5.31 s
		5.17 s		5.16 s
15	24.0 (CH_3)	2.07 s	24.0 (CH_3)	2.07 s
16	163.8 (C)		163.8 (C)	
17	141.0 (C)		141.0 (C)	
18	115.4 (CH_2)	5.06 s	115.4 (CH_2)	5.06 s
		4.83 s		4.83 s
19	21.6 (CH_3)	1.96 s	21.6 (CH_3)	1.96 s
20	170.4 (C)		170.4 (C)	
21	51.5 (CH_3)	3.82 s	51.6 (CH_3)	3.82 s
FA 1′[b]	173.8(C)		173.8 (C)	
2′	33.7 (CH_2)	2.43 m	33.8 (CH_2)	2.43 m
		2.38 m		2.39 m
3′	24.6 (CH_2)	1.61 m	24.6 (CH_2)	1.62 m
8′	27.2 (CH_2)	2.00 m		
9′	129.9 (CH)	5.35 nr		
10′	129.8 (CH)	5.35 nr		
11′	27.2 (CH_2)	2.00 m		
16′[c]	31.9 (CH_2)	1.26 m	31.9 (CH_2)	1.26 m
17′[c]	22.7 (CH_2)	1.26 m	22.7 (CH_2)	1.26 m
18′	14.1 (CH_3)	0.89 t (6.8)	14.1 (CH_3)	0.89 t (6.9)

[a] Multiplicities were obtained from DEPTQ experiment. [b] Signals for the fatty acid chain other than mentioned in Table 2: For compound **2** ^{1}H NMR δ 1.26 (H-4′−H-7′, H-12′−H-15′); ^{13}C NMR δ 29.1−29.8 (C-4′−C-7′, C-12′−C-15′); for compound **3** ^{1}H NMR δ 1.26 (H-4′−H-15′); ^{13}C NMR δ 29.1−29.7 (C-4′−C-15′). [c] Signals were assigned on the basis of the HMBC spectrum. [d] Not resolved.

The HRMS (observed $[M + Na]^+ = 633.3746$, calculated $[M + Na]^+ = 633.3762$) of lipidyl pseudopterane D (**4**; molecular formula $C_{37}H_{54}O_7$) suggested one oxygen atom less than **1**. The comparison of the ^{1}H and ^{13}C NMR spectra of **1** and **4** (Table 3) indicated the hydroxyl group at C-12 in lipidyl pseudopterane A (**1**) was absent in **4** (Figure 1). The ^{13}C chemical shift assigned to C-12 at δ 39.0 (CH_2) with HMQC cross-peaks with protons at δ 2.24 (nr) and 1.2 (d, $J = 15.5$ Hz) supported the structure. COSY and HMBC correlations confirmed the overall structure of lipidyl pseudopterane D as **4**. The relative configuration of **4** was established on the basis of comparison with **1**−**3**; however the relative configuration of C-11 could not be unambiguously established.

Lipidyl pseudopterane E (**5**) was isolated as a colorless oil and found to have a molecular formula of $C_{37}H_{52}O_7$, indicating 12 degrees of unsaturation (observed $[M + Na]^+ = 631.3586$, calculated $[M + Na]^+ = 631.3605$). The MS/MS fragmentation of **5** showed *m/z* 353.13 as a daughter ion (base peak) with the associated neutral loss of 256, corresponding to palmitic acid. Full MS (APCI mode) with source fragmentation comparison of **5** and **4** suggested that both possess the same fatty acid chain with one extra double bond in the pseudopterane skeleton of **5**. HMBC correlations between C-1′ at δ 173.4 (C) and H-15 at δ 4.74 (2H, s) confirmed the position of the palmityl group at C-15 (Figure 1). The location of the double bond in the pseudopterane ring was assigned on the basis of ^{1}H and ^{13}C chemical shifts (Table 3) as well as COSY correlations. On the basis of NMR data, **5** can be

Table 3. NMR Data for Lipidyl Pseudopteranes D and E (**4**, **5**) in $CDCl_3$

	4		**5**	
position	^{13}C (mult.)[a]	^{1}H mult (*J* Hz)	^{13}C (mult.)[a]	^{1}H mult (*J* Hz)
1	39.0 (CH)	3.23 m	39.7 (CH)	4.12 m
2	32.4 (CH_2)	3.33 dd (14.4, 12.8) 2.83 dd (14.5, 3.3)	29.7 (CH_2)	3.57 dd (15.4, 12.8) 2.90 (15.3, 3.7)
3	161.3 (C)		161.2 (C)	
4	115.7 (C)		115.3 (C)	
5	110.1 (CH)	6.37 s	111.5 (CH)	6.41 s
6	150.3 (C)		150.3 (C)	
7	48.5 (CH)	3.81 nr[d]	49.7 (CH)	3.83 d (4.8)
8	79.7 (CH)	5.38 dd (4.1, 1.3)	79.8 (CH)	5.39 d (4.7)
9	146.6 (CH)	6.78 br s	145.5 (CH)	6.58 dd (1.4, 1.3)
10	134.8 (C)		131.8 (C)	
11	69.9 (CH)	5.49 br s	119.5 (CH)	5.96 dd (11.6, 0.9)
12	39.0 (CH_2)	2.24 nr 1.20 d (15.5)	137.1 (CH)	5.47 (11.3, 10.4)
13	148.5 (C)		145.9 (C)	
14	111.6 (CH)	5.04 s 4.84 s	111.3 (CH_2)	5.20 s 5.19 s
15	19.9 (CH_3)	1.84 s	65.5 (CH_2)	4.74 s
16	164.0 (C)		163.9 (C)	
17	141.1 (C)		140.4 (C)	
18	115.3 (CH)	5.06 s 4.87 s	115.2 (CH_2)	5.05 s 4.86 s
19	21.6 (CH_3)	1.97 s	21.6 (CH_3)	1.99 s
20	171.0 (C)		171.5 (C)	
21	51.4 (CH_3)	3.81 s	51.4 (CH_3)	3.80 s
FA 1′[b]	173.8 (C)		173.4 (C)	
2′	33.9 (CH_2)	2.37 m 2.32 m	34.3 (CH_2)	2.41 m
3′	24.5 (CH_2)	1.62 m	25.0 (CH_2)	1.66 m
14′[c]	31.9	1.26 m	31.9 (CH_2)	1.26 m
15′[c]	22.7	1.26 m	22.7 (CH_2)	1.26 m
16′	14.1	0.89 t (6.4)	14.1 (CH_3)	0.89 t (6.7)

[a] Multiplicities were obtained from DEPTQ and HMQC experiments. [b] Signals for the fatty acid chain other than mentioned in Table 2: for compounds **4** and **5** ^{1}H NMR δ 1.26 (H-4′−H-13′); ^{13}C NMR δ 29.1−29.8 (C-4′−C-13′). [c] Signals were assigned on the basis of the HMBC spectrum. [d] Not resolved.

considered to be the palmitate ester of 15-hydroxypseudopteradiene.[24] The relative configuration in **5** was assigned on the basis of the comparison of ^{13}C chemical shifts of **5** and pseudopteradiene.[24]

The MS/MS fragmentation of $[M + H]^+$ ion of compound **6**, molecular formula $C_{37}H_{54}O_9$ (observed $[M + Na]^+ = 665.3651$, calculated $[M + Na]^+ = 665.3660$), gave a base peak at *m/z* 387.17, representing a loss of 256 mass units, corresponding to palmitic acid. The fragmentation pattern of **6** was substantially different from that of **1**, suggesting a structural difference in the pseudopterane ring. A comparison of the ^{13}C spectra of **4** and **6** revealed that most of the chemical shifts were very similar other than the signals at δ 161.3 (C), 115.7 (C), 110.1 (CH), and 150.3 (C) of **4** were shifted to 102.0 (C), 60.5 (C), 54.7 (CH), and 96.2 (C) in **6**. The molecular formula revealed 11 degrees of unsaturation with the addition of two oxygen atoms relative to **4**. ^{13}C and ^{1}H chemical shifts of **6** with HMBC (Figure 2) and COSY correlations (Table 4) suggested the structure shown in Figure 1.

The comparison of the assigned ^{1}H and ^{13}C chemical shifts of the diepoxyfuran ring of **6** with that of the previously reported diepoxygorgiacerone[19,25] confirmed the presence of the diepoxyfuran moiety in lipidyl pseudopterane F. The relative configuration of **6** was assigned on the basis of ^{1}H and ^{13}C NMR chemical shifts (Table 4) as well as 2D NOESY correlations. The two epoxide functionalities are shown as being on the α-face of **6**, which was suggested by $^{1}H-^{1}H$ NOESY cross-peaks between H-5 (4.23 ppm) and one of the C-18 exomethylene protons (5.34 ppm). The ^{13}C NMR signals ascribed to C-3, C-4, C-5, and C-6 in **6** were similar to those reported for diepoxygorgiacerone, whose epoxy groups were demonstrated to be α-oriented by X-ray crystallographic analysis.[25]

Several other less abundant lipidyl pseudopteranoids were observed in the relatively nonpolar fractions of the organic extract

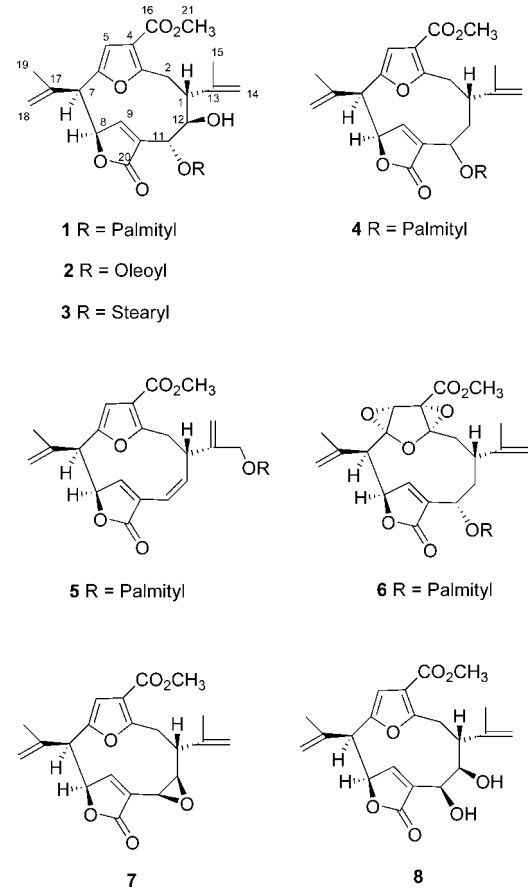

Figure 1. Structures of lipidyl pseudopteranes A−F.

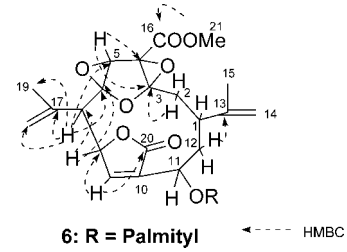

Figure 2. $^{1}H-^{13}C$ HMBC correlations of **6**.

of *P. acerosa*. The MS analysis, GC-MS and LC-MS, along with ^{1}H NMR of some of these minor compounds indicated the presence of pseudopteranoids with the same diterpene moiety as **1** but with different fatty acid side chains including C16:2, C17:0, C18:2, and C18:3. Unfortunately, there was insufficient sample to allow full characterization of these compounds.

Lipidyl pseudopteranes A−F (**1**−**6**) as well as pseudopterolide (**7**) and gorgiceradiol (**8**) were subjected to a primary screen (at a concentration of 100 μg/mL) for inhibition of a panel of protein tyrosine phosphatases. Compounds **1** and **4** were the most active, and interestingly, these diterpenes were active against PTP1B and inactive against other members of the PTP family (including LAR, SHP-1, and MKPX). The most active compound was lipidyl pseudopterane A, with an IC_{50} of 71 μg/mL. It is important to note that genetic as well as pharmacological studies demonstrated that inhibiting PTP1B at various levels has led to a corresponding beneficial effect in animal models of diabetes, obesity, and cancer. Therefore, the therapeutic window of inhibition for PTP1B is relatively large. The intrinsic qualities of selectivity and diffusibility of the lipidyl pseudopteranes even within relatively modest inhibitory activity may still be of great value for therapeutic intervention.

Table 4. NMR Data for Lipidyl Pseudopterane F (**6**) in $CDCl_3$

position	^{13}C (mult.)[a]	^1H mult (*J* Hz)	$^1H-^1H$ COSY	$^1H-^{13}C$ HMBC
1	38.0 (CH)	2.50 nr[d]	2a, 2b, 12a	
2	36.7 (CH_2)	2.33 nr	2b, 1	
		1.75 nr	2a, 1	3
3	102.0 (C)			
4	60.5 (C)			
5	54.7 (CH)	4.23 s		4, 3
6	96.2 (C)			
7	48.2 (CH)	3.28 br s	8(w)	19, 5, 6, 18, 17
8	80.2 (CH)	5.32 dd (<1)	9, 7(w)	6, 7
9	150.3 (CH)	7.20 dd (1.53, 1.09)	8	8, 20
10	132.8 (C)			
11	69.2 (CH)	5.64 d (2.6)	12a, 12b	
12	41.4 (CH_2)	2.50 nr	12b, 11, 1	
		1.70 nr	12a, 11	13
13	148.7 (C)			
14	111.0 (CH_2)	4.79 s	14b, 15	15, 1
		4.70 s	14a, 15	15, 1
15	18.9 (CH_3)	1.67 s	14a, 14b	1, 14, 13
16	164.5 (C)			
17	141.0 (C)			
18	115.7 (CH_2)	5.34 s	18b, 19	19, 7
		5.04 s	18a, 19	19, 7
19	24.5(CH_3)	1.85 s	18a, 18b	7, 17, 18
20	170.4(C)			
21	53.2 (CH_3)	3.87 s		16
FA 1′[b]	173.8 (C)			
2′	33.8 (CH_2)	2.33 nr	3′	1′
		2.33 nr	3′	1′
3′	24.9 (CH_2)	1.60 nr	2'a, 2'b, FAC	
14′[c]	31.9 (CH_2)	1.26 br m		
15′[c]	22.9 (CH_2)	1.26 br m		
16′	14.1 (CH_3)	0.89 t (6.7)	FAC	14′, 15′

[a] Multiplicities were obtained from DEPTQ experiment. [b] Signals for the fatty acid chain other than mentioned in Table 3: ^1H NMR δ 1.26 (H-4′−H-13′); ^{13}C NMR δ 29.1−29.7 (C-4′−C-13′). [c] Signals were assigned on the basis of the HMBC spectrum. Fatty acid chain represented as FAC. [d] Not resolved.

Further studies in cells and animals will thus be required for clarifying their true potential as PTP1B inhibitors.

Experimental Section

Collection and Extraction Procedure. The coral specimens were collected from Sweetings Cay, Bahamas, in May 2005, and a voucher (#090607-02-009) is maintained at UPEI. Sun-dried coral (367 g) was blended with ethyl acetate and dichloromethane, and blending continued with fresh solvents until the color of the solvent became faint yellow (8 × 1 L) and then it was filtered. Evaporation of solvent in vacuo resulted in 27.6 g of crude extract. Separation was carried out by silica (170 g) flash column chromatography using a stepwise gradient (hexane to ethyl acetate). Lipidyl pseudopteranoids **1**−**3** were isolated from the 50% hexane extract, while **4**−**6** were isolated from the 60% hexane extract. These fractions were then subjected to silica column chromatography using an isocratic solvent system optimized by thin-layer chromatography (TLC) experiments. The aliquots were collected and combined on the basis of the TLC of each aliquot, which resulted in seven different fractions. Compounds **1**−**3** were isolated by semi-preparative HPLC (CH_3CN-H_2O gradient with a phenyl hexyl column) from fraction number 2 of the 50% hexane extract, and **4**−**6** were obtained from fraction numbers 3 and 4 of the 60% hexane extract.

Lipidyl pseudopterane A (1): colorless oil (8.48 mg), $[\alpha]_D$ +150 (*c* 0.27, $CHCl_3$); UV (acetonitrile) λ_{max} (ε), 218 nm (13 743), 254 nm (4949); IR (neat) 3529, 3087, 2929, 2858, 1765, 1737 and 1720; ^1H NMR and ^{13}C NMR ($CDCl_3$) refer to Table 1; Mass (LRMS) obsd [M + H]$^+$ 627.17 *m/z*, MS2 fragments (CID = 35) 609.3, 595.31 (BP), 371.1, 353.15, 321.16, 247.12; HRESIMS calcd [M + Na]$^+$ 649.3716, obsd 649.3715.

Lipidyl pseudopterane B (2): colorless oil (2.98 mg), $[\alpha]_D$ +120 (*c* 0.19, $CHCl_3$); UV (acetonitrile) λ_{max} (ε), 214 nm (18 325); ^1H NMR and ^{13}C NMR ($CDCl_3$) refer to Table 2; Mass (LRMS) obsd [M + H]$^+$ 652.94 *m/z*, MS2 fragments (CID = 35) 635.28, 621.41 (BP), 603.41, 442.35, 353.22, 321.19, 247.14; HRESIMS calcd [M + Na]$^+$ 675.3867, obsd 675.3854.

Lipidyl pseudopterane C (3): colorless oil (2.45 mg), $[\alpha]_D$ +130 (*c* 0.19, $CHCl_3$); UV (acetonitrile) λ_{max} (ε), 214 nm (7714); ^1H NMR and ^{13}C NMR ($CDCl_3$) refer to Table 2; Mass (LRMS) obsd [M + H]$^+$ 655.18 *m/z*, MS2 fragments (CID = 35) 637.38, 623.25 (BP), 567.33, 371.14, 353.15, 321.15, 247.15; HRESIMS calcd [M + Na]$^+$ 677.4024, obsd 677.3978.

Lipidyl pseudopterane D (4): colorless oil (4.34 mg), $[\alpha]_D$ +120 (*c* 0.45, $CHCl_3$); UV (acetonitrile) λ_{max} (ε), 216 nm (10 616), 248 nm (4623); IR (neat), 3510, 3082, 2930, 2859, 1765, 1721, 1649, 1615, 1579, 1439; ^1H NMR and ^{13}C NMR ($CDCl_3$) refer to Table 3; Mass (LRMS) obsd [M + H]$^+$ 610.87 and its fragment 355.01 *m/z*, MS2 fragments of 355.01 (CID = 35) 340.98, 323.09 (BP), 295.12, 246.00, 191.05; HRESIMS calcd [M + Na]$^+$ 633.3762, obsd 633.3746.

Lipidyl pseudopterane E (5): colorless oil (4.00 mg), $[\alpha]_D$ +110 (*c* 0.39, $CHCl_3$); UV (acetonitrile) λ_{max} (ε), 244 nm (7196); IR (neat), 3453, 3093, 2929, 2858, 1765, 1720, 1655, 1619, 1579, 1439; ^1H NMR and ^{13}C NMR ($CDCl_3$) refer to Table 3; Mass (LRMS) obsd [M + H]$^+$ 609.18 *m/z*, MS2 fragments (CID = 35) 576.95, 353.13 (BP), 321.05, 275.13, 247.1, HRESIMS calcd [M + Na]$^+$ 631.3605, obsd 631.3586.

Lipidyl pseudopterane F (6): colorless oil (3.99 mg), $[\alpha]_D$ +60 (*c* 0.46, $CHCl_3$); UV (acetonitrile) λ_{max} (ε), 216 nm (11783); IR (neat), 3509, 3082, 2929, 2858, 1765, 1720, 1651, 1439; ^1H NMR and ^{13}C NMR ($CDCl_3$) refer to Table 4; Mass (LRMS) obsd [M + H]$^+$ 643.08 *m/z*, MS2 fragments (CID = 35) 625.26, 611.31, 405.06, 387.03(BP), 337.09, 259.15; HRESIMS calcd [M + Na]$^+$ 665.3660, obsd 665.3651.

Base Hydrolysis of Fraction Enriched with 1. Three milliliters of 0.5 M methanolic NaOH was added to the fraction enriched with **1** (100 μg), and the mixture was heated over a steam bath for 3 min. A solution of 5 mL of BF_3−methanol (14% solution) was then added to the mixture, which was boiled for another 3 min. The sample mixture was cooled and transferred into a separatory funnel containing 25 mL of hexane. A NaCl solution was added, and fatty acid methyl esters were extracted in hexane and analyzed by GC-MS. EIMS fragmentation patterns were compared with the NIST library database. The same protocol was used to identify the fatty acid components of **2**−**6**.

Synthesis of Lipidyl Pseudopterane A (1) from Pseudopterolide. A solution of palmitic acid (420 μg, 1.6 μmol) in dichloromethane (250 μL) with a catalytic amount of triethyl amine was added to pseudopterolide (590 μg, 1.6 μmol) in 250 μL of dichloromethane. The reaction mixture was stirred at ambient temperature, and after 26 h, methanol (50 μL) was added and the solvent evaporated under a stream of nitrogen. The product mixture was reconstituted with 0.5 mL of acetonitrile, and 20 μL was analyzed by LCMS. The production of **1** was confirmed by comparison of retention time, full MS, and MS^2 spectra of [M − 18]$^+$ (609 *m/z*) with an authentic standard. The percentage yield (0.03%) was calculated by plotting a calibration curve generated from an authentic standard (range = 0.1−10 ppm, R^2 = 0.9992).

PTP Assays. Assay buffer at pH 7.0 was prepared using HEPES (50 mM), DTT (3 mM final), and BSA (0.1 mg/mL). Assays were conducted at 25 °C in 96-well plates (Falcon) in a volume of 100 uL. Reaction rates were determined using a Varioskan plate reader (Thermo Electron). Using pNPP as a substrate, absorbance was monitored at 405 nm and measured every 30 s over 10 min, and the reaction rates were calculated by linear regression. Enzymes used were the catalytic domains of GST-tagged protein tyrosine phosphatases: GST-PTP1B, GST-LAR, GST-SHP-1, and GST-MKPX. PP1 was purchased at New England Biolabs. All inhibitor assays contained 1% DMSO (final). IC_{50} assays were conducted at substrate concentrations equal to the Km value for PTP1B. IC_{50} values were derived by fitting data to a sigmoidal dose−response (variable slope) curve (Prism software).

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Supporting Information Available: Copies of the HRMS, MS, ^1H NMR (500 and 300 MHz), ^{13}C NMR (125 and 75 MHz), DEPTQ,

COSY, NOESY, HMQC, and HMBC for compounds **1**−**6** are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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