JOC Article

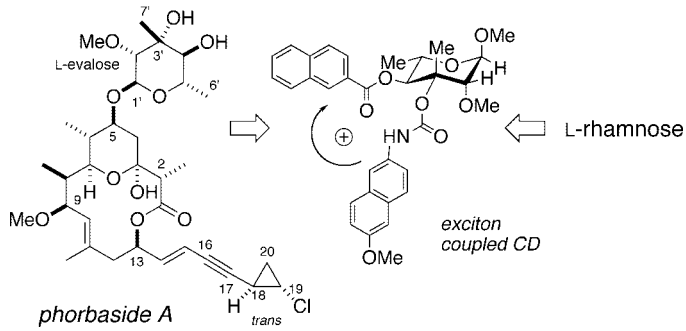
Phorbasides A−E, Cytotoxic Chlorocyclopropane Macrolide Glycosides from the Marine Sponge *Phorbas* sp. CD Determination of *C*-Methyl Sugar Configurations

John B. MacMillan,[‡,†] Guang Xiong-Zhou,[†] Colin K. Skepper,[§] and Tadeusz F. Molinski[§,‡,*]

Department of Chemistry and Biochemistry and Skaggs School of Pharmacy and Pharmaceutical Sciences, MC0358, 9500 Gilman Drive, La Jolla, California 92093-0358, and Department of Chemistry, University of California, Davis, One Shields Avenue, Davis, California 95616

tmolinski@ucsd.edu

Received November 8, 2007



Five new cytotoxic macrolide glycosides phorbasides A−E (**3**−**7**), each possessing a macrolide ring appended to a rare ene-yne-*trans*-2-chlorocyclopropane, were isolated from the same Western Australian sponge (*Phorbas* sp.) that provided phorboxazoles A and B. The structures of **3**–**7** were solved by analysis of spectroscopic data including NMR, MS, and CD. A synthesis of methyl 2-*O*-methyl-α-L-evalose from L-rhamnose was completed and used for configurational assignment of the sugar residue in **3**. Acid-catalyzed methanolysis of **3** followed by two-step derivatization of the liberated *O*-methyl glycoside gave a vicinal 4-*O*-naphthoyl/tertiary 3-*N*-(2-aminonaphthyl)carbamate derivative that exhibited exciton coupled CD identical with that of the derivative prepared from synthetic 1,2-*O*-dimethyl-α-L-evalose.

Introduction

In 1995 we reported the structures of phorboxazoles A (**1a**) and B (**1b**, Figure 1)[1] from the marine sponge *Phorbas* sp. collected off the Western Australian coastline. Recently, we disclosed the structures of two unrelated macrolides—phorbasides A (**3**) and B (**4**)—obtained from minor fractions of the sponge extract.[2] The phorbasides contain a common 14-membered macrolide ring, glycosylated at C5 by a 3-*C*-methyl sugar and terminated by a rare ene-yne 2-chlorocyclopropane. Glycosylated polyketides with similar macrolide rings have been found in cyanobacteria, (e.g., acutiphycin from *Oscillatoria acutissima*,[3] lyngbyaloside from *Lyngbya* sp.,[4] lyngbouilliside from *L. bouillani*[5]); however, the unique ene-yne 2-chlorocyclopropane group has been encountered only once before in callipeltosides A (**2**), B, and C from the New Caledonian sponge *Callipelta* sp.[6] We now convey a full account of structure

* To whom correspondence should be addressed. Phone: (858) 534-7115. Fax: (858) 822-0386.
‡ Present address: University of Texas, Southwestern Medical Center, Dept. of Biochemistry, 5323 Harry Hines Blvd., Dallas, TX 75390.
† University of California, Davis.
§ University of California, San Diego.

(1) (a) Searle, P. A.; Molinski, T. F. *J. Am. Chem. Soc.* **1995**, *117*, 8126. (b) Searle, P. A.; Molinski, T. F.; Brzezinski, L. J.; Leahy, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 9422. (c) Molinski, T. F. *Tetrahedron Lett.* **1996**, *37*, 7879.

(2) (a) Skepper, C. K.; MacMillan, J. B.; Zhou, G.-X.; Masuno, M. N.; Molinski, T. F. *J. Am. Chem. Soc.* **2007**, *129* (14), 4150–4151. (b) MacMillan, J. B. Ph.D. Thesis, University of California, Davis, 2004.

(3) Barchi, J. J.; Moore, R. E.; Patterson, G. M. L. *J. Am. Chem. Soc.* **1984**, *106*, 8193–8197.

(4) Luesch, H.; Yoshida, W. Y.; Harrigan, G. G.; Doom, J. P.; Moore, R. E.; Paul, V. J. *J. Nat. Prod* **2002**, *65*, 1945–1948.

(5) Tan, L. T.; Márquez, B. L.; Gerwick, W. H. *J. Nat. Prod.* **2002**, *65*, 925–928.

(6) (a) Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C.; Roussakis, C. *J. Am. Chem. Soc.* **1996**, *118*, 11085–11088. (b) Zampella, A.; D'Auria, M. V.; Minale, L.; Debitus, C. *Tetrahedron* **1997**, *53*, 3243–3248.

10.1021/jo702307t CCC: $40.75
Published on Web 04/16/2008

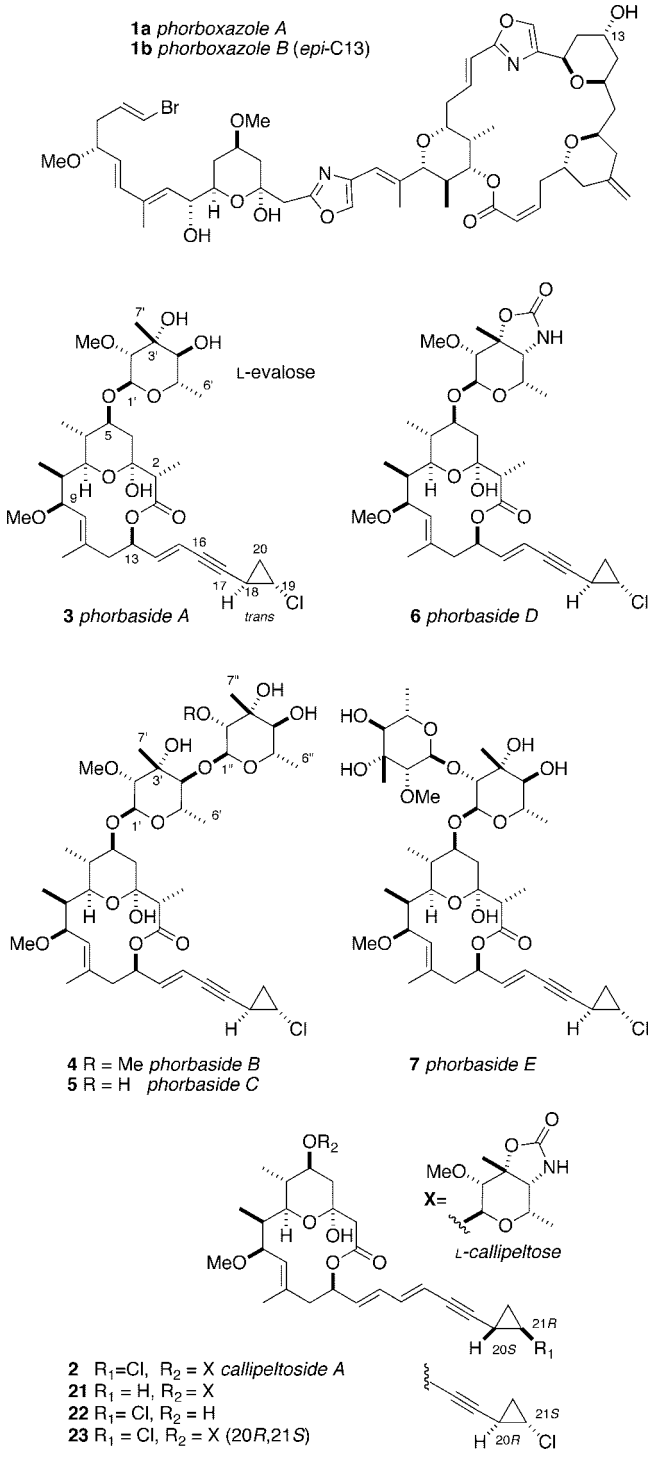


FIGURE 1. Structures of phorboxazoles A and B (**1a** and **b**),[1] phorbasides A−E (**3**−**7**), callipeltoside A (**2**),[6a] and derivatives.[19]

elucidation of phorbasides A and B (**3** and **4**, Figure 1), and three previously unreported minor metabolites, phorbasides C−E (**5**–**7**).

Each of the five compounds differs only in the structure of the 3-*C*-methyl sugars attached at C5. To define the configuration of the glycone residues, we developed a method for assignment of *C*-methyl sugars by circular dichroism (CD) that exploits exciton coupling between vicinal tertiary carbamate and 2-naphthoate ester groups. The latter was used to confirm the L-evalose configuration in phorbaside A (**1b**). Phorbaside C (**5**) appears to be the most potent member of this family of cytotoxic compounds ($IC_{50} = 2\ \mu M$, against HCT-116 cells) reported to date, which suggests that the cytotoxicity of glycosidic ene-yne chlorocyclopropane macrolides is largely modulated by the structures of the sugars.

Results

Examination of minor fractions obtained from the same specimen of *Phorbas* sp. that yielded **1a** (0.04% dry weight, w/w) and **1b** (0.017% w/w) showed minor components that were further purified by reversed phase C_{18} HPLC to provide phorbaside A (**3**, 1.29 mg, 5.5×10^{-3} % w/w), phorbaside B (**4**, 1.40 mg, 6.0×10^{-3} % w/w), phorbaside C (**5**, 3.0 mg, 1.3×10^{-2} % w/w), phorbaside D (**6**, 0.78 mg, 3.4×10^{-4} % w/w), and phorbaside E (**7**, 0.84 mg, 3×10^{-4} % w/w). Structure elucidation of these very small quantities of compounds was greatly assisted by 2D NMR experiments carried out at 600 MHz on an NMR spectrometer equipped with a cryoprobe that provides a 4–5-fold greater ^1H signal-to-noise than conventional probes.

The molecular formula of phorbaside A (**3**), $C_{33}H_{49}ClO_{10}$, was derived from HRFABMS (*m*/*z* $[M + Na]^+$, 663.2885) while that of phorbaside B (**4**) was $C_{41}H_{63}ClO_{14}$ (*m*/*z* $[M + Na]^+$ 837.4049). The UV spectra of both compounds exhibited chromophores (λ_{max} 245 nm, ϵ 12000) that were assigned to a conjugated 1-chloro-2-(*E*-alk-3′-en-1′-ynyl)cyclopropane group attached to a 14-membered-ring macrolide as described in our preliminary communication.[2] Extensive analysis of 2D NMR spectra of **3** (COSY, ROESY, HSQC, HMBC, and HSQC-TOCSY) established the constitution and relative configuration of the macrolide ring and attachment of the sugar group at C5. Quantitative CD analysis of the hyperconjugated ene-yne chromophore secured the absolute configuration of both the macrolide ring and the *trans*-2-chlorocyclopropyl ring in **3** and **4**, as depicted.[2] Analysis of UV, IR, and NMR spectroscopic data showed that each of the phorbasides A−E (**3**−**7**) had an identical macrolide ring (aglycone), but differed only in the nature of the sugar group.

The sugar unit in **3** was identified as 2-*O*-methylevalose (6-deoxy-3-*C*-2-*O*-dimethyl-*manno*-pyranoside) by analysis of vicinal coupling constants, ROESY correlations, and comparison of ^1H and ^{13}C NMR chemical shifts with the evalose residue in the glycoside callipeltoside C, but this did not reveal the absolute configuration. A number of interesting features were seen for the C5-glycone including a *C*-methyl group at C3′ and an *O*-methoxyl at C2′. The constitution was determined on the basis of COSY correlations from the anomeric proton H1′ (δ 4.92, s) to H2′ (δ 3.11, s) and from H4′ (δ 3.35, d, $J = 9.6$ Hz) to H5′ (δ 3.64, dq, $J = 9.6, 6.6$ Hz). The ^1H NMR coupled spin system of the pyranose residue was discontinuous at C3′ due to the presence of the quaternary center; however, HMBC correlations were observed from H2′, H3′, and H4′ to C3′-Me (C7′, δ 17.8, q) and from H2′ to C3′ (δ 72.3, s, see the Supporting Information in ref 2).

Phorbaside B (4). The molecular formula for phorbaside B (**4**), $C_{41}H_{63}ClO_{14}$, differed from that of **3** by addition of the elements of $C_8H_{14}O_4$ suggesting the presence of an additional *O*-methylevalose residue.[2] Differences in the ^1H NMR spectrum of **3** and **4** (ref 2,) were found only in the sugar portion of the spectra (Table S2 in the Supporting Information in ref 2); the ^1H NMR spectrum of **4** contained two anomeric protons (δ 4.83, s, H1′; δ 5.71, s, H1″) and an additional three methyl signals

(δ 3.48, s; 1.29, d, J = 6.6 Hz; 1.35, s). gCOSY and HMBC cross peaks located the second 2-*O*-methylevalose residue at C4′ of the first evalose residue [HMBC; δ 3.37, d, J = 9.7 Hz, H4′ to δ 92.4, C1″]. Therefore, phorbaside B had a 1″ → 4′ disaccharide linkage.

Phorbaside C (5). The molecular formula for phorbaside C (**5**) was $C_{40}H_{61}ClO_{14}$ (HRFABMS m/z $[M + Na]^+$ 823.3615), or one CH_2 group less than the formula of phorbaside B (**4**). ^{1}H NMR spectrum of **5** (Supporting Information, Figure S1) showed only two OMe singlets (δ 3.23 and 3.38 ppm) compared to the three found in **4**. The remainder of the ^{1}H NMR chemical shifts for the macrolide ring of **5** matched those of **4**, including the shifts for H9 (δ 3.75, dd, J = 9.0, 1.8 Hz), implying the MeO group at C9 was intact. The ^{1}H NMR chemical signals of the C5-*O*-evalose residue of **5** showed similarity with those of **4**, but the second sugar residue showed major changes. The signal for the anomeric proton H1″ of **5** (δ 5.42, s) was shifted 0.28 ppm upfield from that of **4** (δ 5.71, s), while H2″ (δ 3.58, s) shifted 0.48 ppm downfield with respect to that of **4** (δ 3.10, s). The absence of an HMBC cross peak from the singlet H2″, together with the differences of the ^{1}H NMR spectrum in the vicinity of C2″ strongly suggested the *O*-2″-desmethyl structure **5**. The remaining HMBC correlations and ^{1}H NMR shifts were consistent with a 1″ → 4′ disaccharide linkage, as in **4**.

Phorbaside D (6). The molecular formula for phorbaside D (**6**) is $C_{34}H_{48}ClNO_{14}$. Since the ^{1}H signals for the macrolide rings in **5** and **6** were similar (Supporting Information, Figure S2), the N atom was located within the sugar group, which strongly suggested the presence of one residue of callipeltose (see Figure 1, 3-*O*-4-*N*-carbamoyl-[4-amino-4,6-dideoxy-2-*O*,-3*C*-dimethyl-α-*talo*pyranose).[6] The IR spectrum of **6** revealed a second carbonyl stretch at ν 1710 cm^{-1}, while the ^{13}C NMR spectrum exhibited an extra sp^2 carbon (δ 158.0, s)—both associated with a cyclic carbamate between C3′ and C4′. This was verified by an HMBC cross peak between signals of H4′ (δ 3.35, s) and the C8′ C═O carbon (δ 158.0, s). The ^{1}H NMR signal for H4′ was now a singlet, as a consequence of flattening of the pyranose ring by the imidazolone ring, inversion of configuration at C4′, and a *syn* relationship between H4′ and H5′ (δ 3.91, d, J = 6.0 Hz). This is consistent with a boat configuration of the pyranose ring as assigned for callipeltoside A by Zampella and co-workers.[6] The latter relative configuration was verified by the appearance of a ROESY cross peak between H4′ and H5′ signals. Formally, callipeltose derives from intramolecular S_N2 displacement of the C4′-OH group by the pendant carbamoyl NH_2 group with inversion at C4′. A putative precursor for the oxazolidinone ring of callipeltose, 2-*O*-methyl-3-*O*-carbamoylevalose, has been reported in the structures of glycosylated macrolides auriside B[7] and callipeltoside B.[6]

Phorbaside E (7). Phorbaside E (**7**), $C_{40}H_{61}ClO_{14}$ (HR-FABMS m/z $[M + Na]^+$ 823.3750) is isomeric with phorbaside C (**5**). Analysis of the ^{1}H NMR, COSY, and HMBC spectral data (Supporting Information, Figure S3) revealed differences in the sugar moieties between **7** and **5** that suggested the glycoside linkage between the two evalose sugar residues was 1″→2′ rather than 1″→4′ found in phorbasides B and C. Phorbaside E (**7**) also differs in location of the MeO group (C2″ in **7**, C2′ in **5**).The key differences were the downfield shift of H2′ in **7** to δ 3.57 ppm from 3.15 ppm in **5**, and HMBC correlations revealed cross-peaks between H2′ (δ 3.57, s) and the C1″ anomeric carbon (δ 95.6 ppm). The sugar MeO group

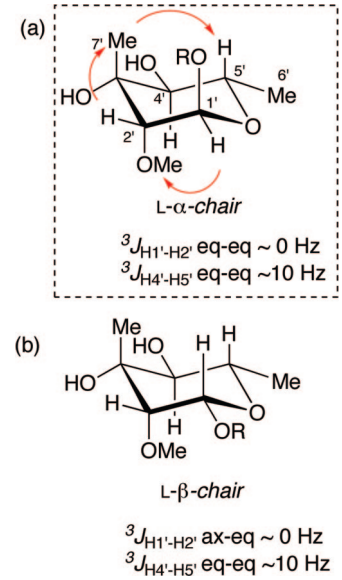


FIGURE 2. Conformations of α- and β-anomers of L-2-*O*-methylevalose glycosides (L-2-*O*-methyl-6-deoxy-3-*C*-methyl-*manno*-pyranoside) and predicted ^{1}H NMR $^3J_{HH}$ values. A arrows show ROESY correlations (600 MHz, t_m = 400 mS) observed for the corresponding glycone in **3**.

was positioned on C2″ based on HMBC correlations from the methyl singlet at δ 3.39 ppm to C2″ (δ 85.6).

Configuration of L-2-*O*-Methylevalose Residues. Evaluation of both vicinal homonuclear J couplings and ROESY correlations in the evalose ring system of **3** defined the sugar relative configurations in **3** and **4** (Figure 2). Vicinal J couplings for the sugar group were limited due to interruption of the vicinal proton pairs by the C3′ quaternary carbon and a J = 0 Hz coupling for H1′−H2′. Two pyranose conformations consistent with the latter data and a large *trans*-diaxial J coupling between H4′ and H5′ (J = 9.6 Hz) are the L-α-*chair* and the L-β-*chair* (Figure 2).

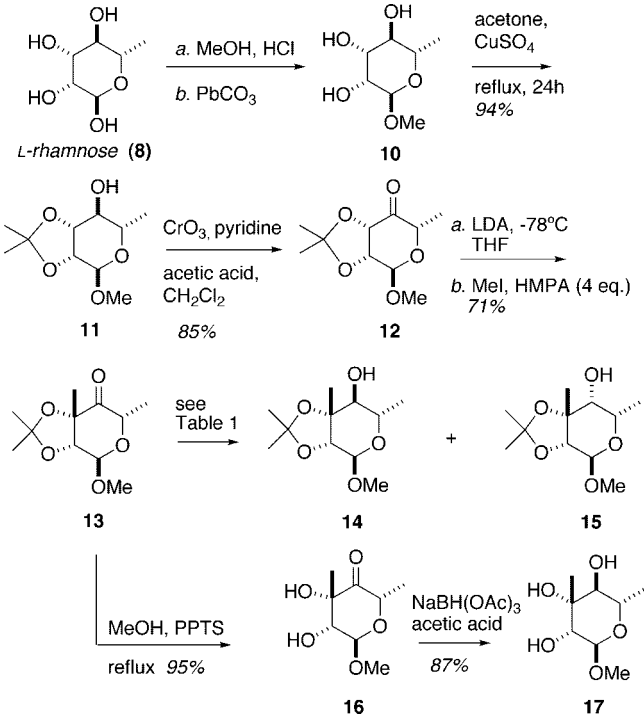
Configuration (b) was proposed for the β-2-*O*-methylevalose residue in callipeltoside C on the basis of interpretation of NOE's;[6b] however, our measurements of **3** support configuration (a) with an axial α-glycoside linkage (L-α-chair) at C5 as follows. ROESY (600 MHz, t_m = 400 mS) correlations were observed in **3** from the anomeric H1′ proton to H5 on the macrolide ring and from H1′ to the *syn*-facial C2′-OMe group (δ 3.45, s), but not from H1′ to H5′ or H1′ to H$_3$-7′. These results are consistent only with an axial L-α-glycoside linkage.[8] Similar ROESY correlations were seen in the 2-*O*-methylevalose residue at C5 in **4** (see the Supporting Inforamtion in ref 2a). Additional ROESY cross peaks were observed in **3** from C7′ to H2′ (δ 3.11, s)—confirming the *syn* relationship between the oxygenated substituents at C2′ and C3′—and from the C2′ MeO proton signal to the H23 methyl group (δ 0.89, d, J = 6.6 Hz). Since the absolute configuration of the macrolide ring in **3** had been assigned,[2a] the latter correlation was suggestive of an L-sugar, although the relatively weak magnitude of the cross peak did not lend itself to an unequivocal assignment.

To resolve this matter, we assigned the sugar configuration in **3** independently by synthesis of the methyl glycoside of α-L-2-*O*-methylevalose and a novel application of exciton coupled

(7) Sone, H.; Kigoshi, H.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 8956–8960.

(8) The α-configuration of the glycoside linkages in **3** and **4** contrasts with the β-configuration assigned to callipeltoside C (ref 6b; note, 2-*O*-methylevalose is depicted in the D-configuration in this paper).

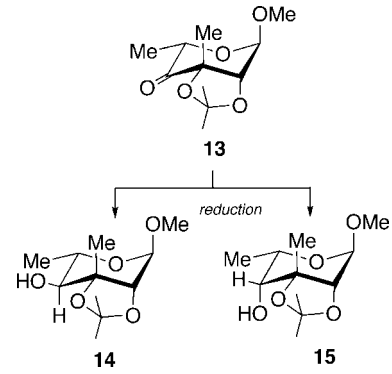
SCHEME 1. Synthesis of Methyl α-L-Evalopyranoside (17)



CD. Pair-wise exciton coupling CD (ECCD) of dibenzoate derivatives has been used to assign absolute configuration in pyranoses;[9] however, these methods are applicable only to secondary and primary OH groups. The evalose sugar residue poses two problems for analysis by ECCD; evalose is not commercially available and the C3 tertiary alcohol is resistant to benzoylation. These difficulties were overcome by a multistep synthesis of a bis-chromophoric derivative of L-evalose, **9**, from L-rhamnose (**8**). Compound **9** embodies an *N*-aryl carbamate derivative of the sterically hindered C3 tertiary alcohol and provides an opportunity to interrogate absolute configuration through exciton coupling with the adjacent 2-napthoate ester.

L-Rhamnose (**8**) was converted to the *O*-methyl glycoside (**10**, Scheme 1) that was further protected as the acetonide **11** (acetone, $CuSO_4$, reflux).[10] Oxidation of **11** (CrO_3, pyridine, CH_2Cl_2, CH_3CO_2H) gave the ketone **12** in 85% yield.[11] The C3-methyl branch was introduced according to Klemer by kinetic deprotonation of **12** (1 equiv, LDA, −78 °C) followed by the addition of MeI to give **13** (71% yield).[12] Reduction of the carbonyl group in **13** by hydride under thermodynamic conditions was expected to give the desired C4 equatorial alcohol **14**; however, literature precedence[12] suggested that $NaBH_4$ reduction proceeds under kinetic control by axial attack of hydride from the less hindered β-face to give the undesired equatorial alcohol **15**. Attempted reduction of ketone **13** under a variety of kinetic and thermodynamic conditions (Table 1) produced only **15** or poor conversion to **14** (entries 6 and 7, <8%). Successful conversion of **13** to the desired diastereomer **14** was achieved by using a variant of Lipták's procedure that allowed hydride reduction to proceed under the direction of the C3 hydroxyl.[13] Removal of the acetonide in **13** (acetone, PPTS, reflux) gave keto-diol **16**, which was reduced ($NaBH(OAc)_3$, AcOH) to alcohol **17** exclusively.[13] Reprotection of **17** as a C2/C3 acetonide (acetone, $CuSO_4$, reflux) gave **14** (Scheme 2), which was treated with 2-naphthoyl chloride (pyridine, CH_2Cl_2)

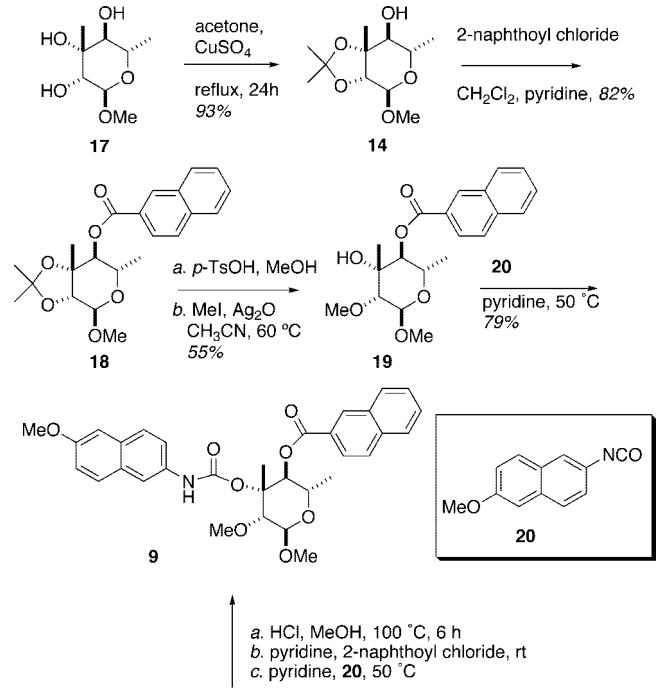
TABLE 1. Reduction of 13



entry no.	reagent	solvent	*T* (°C)	*t* (h)	% yield	**14:15**
1	$NaBH_4$	EtOH	0	0.16	97	0:100
2	L-selectride	THF	0	0.5	86	0:100
3	SmI_2	THF/ *i*-PrOH	rt → reflux	16	[b]	
4	Na°	EtOH	0	1	[b]	
5	Na°	*i*-PrOH	−10	2	[b]	
6	Li°	*i*-PrOH	−10	1.7	3[a]	100:0
7	Na°	*t*-BuOH/ NH_3(l)	−30	0.7	8[a]	100:0

[a] Based on NMR integration of the anomeric H1 signal. [b] Decomposition.

SCHEME 2. Synthesis of Dichromophoric Derivative 9 from Phorbaside A (3) and Methyl 2-*O*-methyl-α-L-evalose (17)



(9) Chang, M.; Meyers, H. V.; Nakanishi, K.; Ojika, M.; Park, J. H.; Park, M. H.; Takeda, R.; Vázquez, J. T.; Wiesler, W. T. *Pure Appl. Chem.* **1989**, *61*, 1193–1200.

(10) Jary, J.; Capek, K.; Kovar, J. *Collect. Czech. Chem. Commun.* **1963**, *28*, 2171–2176.

(11) Garegg, P. J.; Samuelsson, B. *Carbohydr. Res.* **1978**, *67*, 267–270.

(12) Klemer, A.; Stegt, H. *J. Carbohydr. Chem.* **1985**, *4*, 205–213.

(13) Gyergyói, K.; Tóth, A.; Bajza, I.; Lipták, A. *Synlett* **1998**, 127–128.

to provide the 4-*O*-naphthoate ester **18** in 82% yield. Removal of the acetonide (MeOH, *p*-TsOH, reflux) followed by *O*-methylation of the secondary OH group (Ag_2O, CH_3I, CH_3CN) provided 1,2-*O*-dimethyl-4-*O*-naphthoyl-L-evalose (**19**) in 55% yield.

To introduce a second chromophore into the sugar by acylation of the sterically hindered tertiary alcohol **19**, we chose to use an *N*-aryl isocyanate as a more reactive electrophile for conversion of the tertiary OH group to a carbamate. 6-Methoxynaphthylisocyanate (**20**),[14] prepared from commercially available 6-methoxy-2-naphthoic acid by Curtius rearrangement of the corresponding acyl azide (diphosphoryl azide, reflux, benzene), added smoothly to **19** (pyridine, 60 °C) to give carbamate **9**.

Phorbaside A (**3**) was degraded to **9** in three steps (Scheme 2): microscale methanolysis of **3** (50 μg, MeOH, HCl, 80 °C) followed by removal of the volatiles, treatment of the crude product with 2-naphthoyl chloride (pyridine, CH_2Cl_2), and heating the residue with **20** (pyridine, 60 °C). Purification of the crude product by normal phase HPLC (4% *i*-PrOH:*n*-hexane) gave **9** with a retention time identical with the synthetic standard prepared from L-rhamnose. The collected HPLC fraction containing pure **9** was used directly for CD measurements, as follows (Figure 3).

The CD spectrum of **9** (Figure 3a), derived from L-rhamnose (4% *i*-PrOH:*n*-hexane), gave a weak positive bisignate Cotton effect [λ_{max} 241 nm ($\Delta\epsilon$ + 2.6), 226, (−1.5); A = 4.1]. This is consistent with a conformation of **9** (Figure 3b) that subtends a positive helicity between the C−O bonds at C3 and C4. The CD spectrum of the *O*-methyl glycoside **9** prepared from phorbaside A was identical in sign and magnitude with that of authentic **9** prepared from L-rhamnose.

Thus, the C3′-methyl sugar in **3** was verified as L-2-*O*-methylevalose. By association, the evalose units in the co-occurring natural products **4**, **5**, and **7** are also likely to have the L-configuration. Since the absolute configurations at C13, C18, and C19 in **3** and **4** had been assigned earlier from analysis of the CD spectrum of the native ene-yne chromophore,[2] and the relative configuration of the macrolide ring established from ROESY correlations, the complete configuration of **3**–**4** can be stated as 2*S*,3*S*,5*S*,6*R*,7*R*,8*R*,9*R*,13*R*,18*R*,19*S*,1′*R*,2′*R*,3′*R*,4′*S*,5′*S*.[15]

Discussion

Determination of configuration in *C*-methyl sugars adds a level of difficulty to carbohydrate analysis for a number of reasons. *C*-Branched sugars exhibit more conformational heterogeneity due to lower barriers of inversion between chair conformers or the presence of other conformers (e.g., twist-chair and boat). Earlier, we had assigned the absolute configuration of the aglycon in **3** and **4** by analysis of macrolide dipolar couplings from ROESY spectra and independent CD analysis of the ene-yne chlorocyclcopropane group, which relayed the configurations of C18 to C13.[2] Although the configuration of callipeltose in **6** was most *likely* to be the same as that in callipeltoside A, the sugar configuration of evalose residues in **3**, **4**, **5**, and **7** was not so obvious for two reasons.

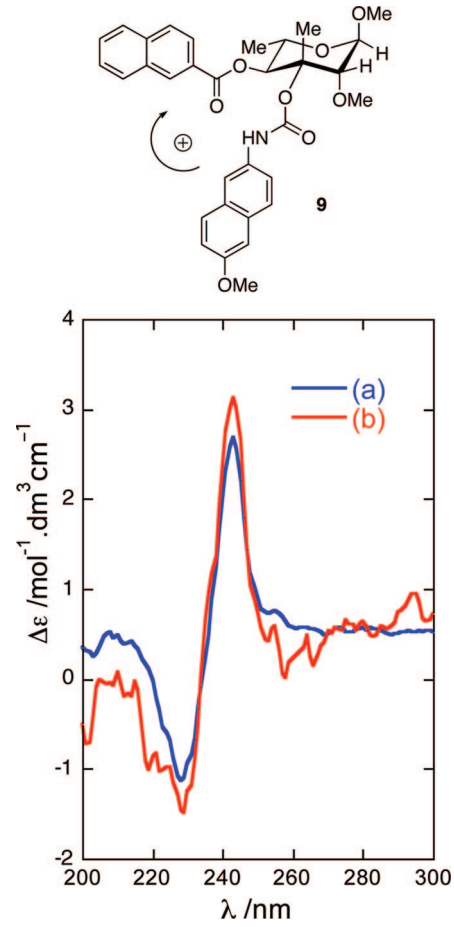


FIGURE 3. CD spectra (4% *i*-PrOH:*n*-hexane): (a) synthetic **9** (blue, solid line, see Schemes 1 and 2) and (b) **9** derived from phorbaside A (red line). The electronic transition dipole moments of the two chromophores in **9** constitute a positive helicity.

Evalose and callipeltose have *manno*-pyranoside and *talo*-pyranoside relative configurations, respectively; however, evalose occurs in nature in both L- and D-configurations.[16] We chose to evaluate the sugar configuration of **3** using an independent approach. The method described here is a variant on the well-established sugar dibenzoate method developed by Nakanishi and co-workers.[9] Use of the dibenzoate method is precluded for assignment of *C*-methyl sugars due to the lack of reactivity at tertiary OH groups, but the present variation circumvents this limitation by exploiting sequential *O*-naphthoylation of a secondary OH group followed by smooth reaction of the tertiary OH group with an aryl isocyanate.

The ^{1}H and ^{13}C NMR values of the α-L-2-*O*-methylevalose residue in **3** and **4** were entirely consistent and similar to those of methyl α-L-evalopyranoside (cf. Figure 2a, R = Me), the anomeric configuration of which was established beyond doubt by Rheingold and co-workers by use of X-ray crystallography.[17] Coincidently, methanolysis of L-evalose glycosides is known to give the methyl α-L-evalose, not methyl β-L-evalose. This is also readily apparent from the present work where microscale methanolysis of **3** gave methyl α-L-2-*O*-methylevalose identical

(14) Asis, S. E.; Bruno, A. M.; Martinez, A. R.; Sevilla, M. V.; Gaozza, C. H.; Romano, A. M.; Coussio, J. D.; Ciccia, G. *Farmaco* **1999**, *54* (8), 517–523.

(15) The sugar units in phorbasides C-E **5**–**7** have analogous configurations, the only difference being 4′*R* for the callipeltose unit of **6**.

(16) For example, in the orthosomycin antibiotics, everninomicin: (a) Ganguly, A. K.; Saksena, A. K. *J. Chem. Soc., Chem. Commun.* **1973**, *15*, 531–532. (b) Ollis, W. D.; Smith, C.; Wright, D. E. *J. Chem. Soc., Chem. Commun.* **1974**, 881–882.

(17) Giuliano, R. M.; Kasperowicz, S.; Boyko, W. J.; Rheingold, A. L. *Carbohydr. Res.* **1989**, *185*, 61–67.

TABLE 2. Cytotoxicity of Phorbasides (3–7), Callipeltoside A (2), and Analogues

compd	cell line	IC_{50} (μM)	ref
3	HCT-116	30.0	*a*
4	HCT-116		*a*
5	HCT-116	2.0	*a*
6	HCT-116	61.9	*a*
7	HCT-116	10.2	*a*
2	NSCLCN6	16.6[d]	*b*
2	P388	22.4[d]	*b*
2	A2780	20.0	*c*
21	A2780	17.0	*c*
22	A2780	>100	*c*
23	A2780	7.0	*c*

[a] This work. Inhibition of cell growth and IC_{50}'s determined from dose responses of MTS end points. [b] Zampella et al. (ref 6a), [c] Trost et al. (ref 19). [d] Values converted from literature values of IC_{50} in μg/mL.

with that synthesized from methyl α-L-rhamnoside (**10**). The favored axial α-anomeric configuration is also seen in each glyoside linkage of **3**–**7**.

The L-absolute configuration of the 2-*O*-methylevalose residue in **3** was unambiguously defined by CD correlation with our synthetic standard. The split-Cotton effect of the vicinal carbamate−naphthoate pair in **9** is relatively weak ($A \approx 4.5$) compared to typical values for vicinal dibenzoyl pyranoses ($A \approx 62$)[8] for a number of reasons. Greater rotational freedom of the O−(CO)−N bond allows reorientation through several conformers that are separated by relatively low energy barriers, with consequent averaging of the effective electronic dipole moment vector. A weaker transition dipole moment of the *N*-arylamine group in **9** compared to *O*-benzoate esters may further reduce the *A* value. The latter limitation may be overcome by the use of *N*-acylisocyanates to prepare tertiary *N*-aryl carbamates of tertiary alcohols with larger electronic transition dipole moments, but the limitation of conformational averaging remains. Nevertheless, vicinal *N*-arylcarbamate−*O*-naphthoate pairs give CD spectra with sufficient signal at submicromole amounts and an unequivocal sign for the split Cotton effect allows unambiguous configurational assignment of *C*-3-methyl sugars. The method should be applicable to other bioactive glycosidic macrolides containing *C*-methyl pyranosides with tertiary OH groups and possibly extendable to *C*-methyl furanosides (e.g., trachycladine).[18]

Biological Activity. Phorbasides exhibited moderate to potent cytotoxicity against cultured HCT 116 cells (human colon cancer, Table 2). Phorbaside C (**5**) appears to be the most potent member among the family of phorbasides and callipeltosides with an IC_{50} of 2.0 μM, while phorbaside E (**7**) was somewhat less active (IC_{50} = 10.2 μM). Both phorbasides A (**3**) and D (**5**) were substantially less cytotoxic (IC_{50} = 30 and 61.9 μM, respectively). On the basis of these results it appears that a free hydroxyl at C2′ or C2″ is required for enhanced cytotoxic activity. Trost and co-workers had earlier shown that the sugar residue of callipeltoside A (**2**) was essential for activity;[19] the removal of the chlorine atom from the cyclopropane ring did not affect activity, but the configuration of the *trans*-2-chlorocyclopropane did. Incubation of **2** with the A2780 human ovarian carcinoma cell line showed modest inhibitory activity (IC_{50} = 20.0 μM), similar to the des-chloro derivative **21** (IC_{50} = 17.4 μM); however, the algycon **22** was essentially inactive (IC_{50} > 100 μM).[19] Interestingly, a synthetic diastereomer **23** of callipeltoside A with *opposite* configurations at the two chlorocyclopropane stereocenters C20, C21 (i.e., the *same* as the C18,C19 stereocenters of phorbasides A−E) was about twice as potent (IC_{50} 7.0 μM) under the same conditions.[19]

Callipeltoside A showed modest activity against NSCLCN6 human bronchopulmonary nonsmall cell lung carcinoma cells (IC_{50} = 11.26 μg/mL) and the murine cell line P388 (15.16 μg/mL), but appeared to induce a blockade of cell proliferation at the G1 phase in the cell cycle.[6a] These results and the higher potency of the disaccharide phorbaside C (**5**) suggest the latter may be an interesting synthetic target for the preparation of additional quantities for advanced biological evaluation.

None of the phorbasides showed antifungal activity in a standard paper disk assay at 10 μg per disk against *Candida albicans* ATCC 14503, *C. glabrata*, *C. krusei*, and *C. albicans* UCD-FR1.

Conclusion

The complete stereostructures of phorbasides A−E (**1**–**5**) are defined. The structures of phorbasides contain the same macrolide ring but differ in the sugar units. A novel application of exciton coupling CD, which employs pairwise interactions between the vicinal tertiary *N*-carbamoyl-2-aminonaphthyl group and a secondary *O*-naphthoate ester, was used to define the L-configuration of evalose sugar units in phorbasides A and B. The compounds exhibit variable cytotoxicity that appears to depend upon the presence of a free 2′- or 2″-hydroxyl group.

Experimental Section

General Procedures. 1D and 2D NMR spectra for phorbasides A−E were measured on an NMR spectometer equipped with a 14 T cryomagnet and a {^{13}C}−^{1}H/^{15}N cold probe with a preamplifier cooled to $T = \sim$20 K. FTIR spectra were acquired on thin films dispersed on NaCl or ZnSe plates. Circular dichroism spectra were measured on a grating spectropolarimeter equipped with a photoelastic modulator. NMR spectra of synthetic compounds were acquired on an NMR spectrometer equipped with a 9.3 T cryomagnet and an inverse detect {^{13}C}^{1}H cryoprobe or 7T with a ^{1}H,^{13}C dual-tuned probe. High-resolution mass spectra were measured by R. New (University of California, Riverside, Mass Spectrometry Facility). All solvents were HPLC grade or distilled from glass.

Isolation of Phorbasides A−E. The sponge *Phorbas* sp. was collected in Muiron Island, Western Australia in 1993. The sample was immediately frozen and stored at −20 °C until extraction (~2 months). Two batches of the $CHCl_3$-soluble fraction (93-054-C3 and 93-054-C2) of the MeOH extract of *Phorbas* sp. (93–054)[1] were separated under HPLC conditions A (C_{18}, 5 μ, 100 Å, 78:22 MeOH/H_2O, 3 mL/min) as described previously.[2] The second-eluting peaks (94-054-C3-1-2 and 94-054-C2-2-2) were pooled and further fractionated under HPLC conditions B (C_{18}, 3 μ, 60:40 CH_3CN/H_2O, 1.0 mL/min, UV detect λ 254 nm) to give 93-054-C3-1-2a, phorbaside D (**6**, 0.78 mg). The third-eluting peaks and fourth-eluting peaks were similarly separated under conditions B to give phorbasides A (**3**,1.29 mg) and phorbaside B (**4**, 1.30 mg), as reported earlier.[2] Peaks (93-054-C3-3, 11 mg and 93-054-C2-4, 9.0 mg) were separated in parallel by HPLC under conditions A. The pooled third-eluting peaks (1.79 mg) were further purified under conditions B to give 93-054-C2-C4-2a, phorbaside E (**7**, 0.84 mg). The CCl_4-soluble fraction of the MeOH extract of the sponge[1] was purified under condition A to give five peaks. Peaks 2 and 3 were found to contain additional phorboxazoles A (**1a**, 1.44 mg) and B

(18) Searle, P. A.; Molinski, T. F *J. Org. Chem.* **1995**, *60* (13), 4296–4298.
(19) Trost, B. M.; Gunzner, J. L.; Dirat, O.; Rhee, Y. H. *J. Am. Chem. Soc.* **2002**, *124* (35), 10396–10415.

(**1b**, 1.00 mg), respectively,[1] and the fifth-eluting peak (93-054-B-2A-3-1-3, 3.52 mg) was resolved under conditions B to give 93-054-B-2A-3-7A, phorbaside C (**5**, 1.40 mg).

Phorbaside A (3). UV (MeOH) λ_{max} 236 nm (ϵ 12000); IR (ZnSe) ν 3455, 2923, 1740 cm^{-1}; for ^{1}H and ^{13}C NMR see the Supporting Information in ref 2. HRFABMS *m*/*z* [M + Na]$^+$ 663.2885 (calcd for $C_{33}H_{49}ClO_{10}Na$, 663.2912).

Phorbaside B (4). UV (MeOH) λ_{max} 235 nm (ϵ 12000); IR (ZnSe) ν 3457, 2920, 1741 cm^{-1}; for ^{1}H and ^{13}C NMR see the Supporting Information in ref 2. HRFABMS *m*/*z* [M + Na]$^+$ 837.4049 (calcd for $C_{41}H_{63}ClO_{14}Na$, 837.3804).

Phorbaside C (5). UV (MeOH) λ_{max} 235 nm (ϵ 12000); IR (ZnSe) ν 3450, 2925, 1740 cm^{-1}; for ^{1}H and ^{13}C NMR and HMBC, see Table S1, Supporting Information. HRFABMS *m*/*z* [M + Na]$^+$ 823.3615 (calcd for $C_{40}H_{61}ClO_{14}Na$, 823.3648).

Phorbaside D (6). UV (MeOH) λ_{max} 236 nm (ϵ 12300); IR (ZnSe) ν 3450, 2925, 1740, 1701 cm^{-1}; for ^{1}H,^{13}C NMR, and HMBC, see Table S2, Supporting Information. HRFABMS *m*/*z* [M + Na]$^+$ 688.2885 (calcd for $C_{34}H_{48}ClNO_{10}Na$, 688.2864).

Phorbaside E (7). UV (MeOH) λ_{max} 235 nm (ϵ 12000); IR (ZnSe) ν 3448, 2922, 1742 cm^{-1}; for ^{1}H, ^{13}C NMR and HMBC, see Table S3, Supporting Information. HRFABMS *m*/*z* [M + Na]$^+$ 823.3750 (calcd for $C_{40}H_{61}ClO_{14}Na$, 823.3648).

***O*-Methyl α-L-Rhamnose (10).** Trimethysilyl chloride (15 mL) was added to anhydrous MeOH (100 mL) followed by L-rhamnose (25 g, 0.15 mol). The mixture was heated at reflux for 19 h with stirring, cooled, and neutralized by addition of $PbCO_3$. Lead salts were removed by filtration and the filtrate was concentrated under reduced pressure to give a syrupy residue. The residual water was removed by azeotropic distillation under reduced pressure with toluene (2 × 200 mL) to give the crude methyl glycoside as a white foam. The product was taken up in 5% MeOH:CH_2Cl_2 and eluted through a plug of silica gel with the same solvent to give **10** as a 30:1 mixture of α-L:β-L anomers (20 g, 78% yield). $[\alpha]_D$ −61.9 (*c* 2.0, H_2O) {lit.[9] $[\alpha]_D$ −62.4 (*c* 9.87, H_2O)}; ^{1}H NMR (CD_3OD) δ 4.55 (s, 1H), 3.78 (d, 1H, J = 2.6 Hz), 3.58 (dd, 1H, J = 7.2, 2.6 Hz), 3.55 (m, 1H), 3.38 (m, 1H), 3.37 (s, 3H), 1.3 (d, 3H, J = 6.4 Hz).

Methyl 6-Deoxy-2,3-isopropylidene-α-L-*talo*-pyranoside (11). Anhydrous $CuSO_4$ (1.2 g) was added to a solution of **10** (600 mg, 3.4 mmol) in acetone (100 mL) and the mixture was heated to reflux for 24 h. $CuSO_4$ was removed via filtration and the filtrate concentrated under reduced pressure to give **11** as a clear oil (660 mg, 94% yield). $[\alpha]_D$ −10.2 (*c* 2.0, H_2O) {lit.[9] $[\alpha]_D$ −10.8 (*c* 1.65, H_2O)}; ^{1}H NMR ($CDCl_3$) δ 4.82 (s, 1H), 4.1 (d, 1H, J = 6.0 Hz), 4.04 (dd, 1H, J = 6.4, 6.0 Hz), 3.6 (m, 1H), 3.37 (m, 1H), 3.35 (s, 3H), 1.51 (s, 3H), 1.31 (s, 3H), 1.27 (d, 3H, J = 6.4 Hz); ^{13}C ($CDCl_3$) 109.5, 98.1, 78.3, 75.7, 74.4, 65.7 54.9, 27.9, 26.1, 17.5.

Methyl 6-Deoxy-2,3-isopropylidene-α-L-*lyxo*-pyranosid-4-ulose(12). Chromium(VI) oxide (1.4 g, 14 mmol) was added to a mixture of CH_2Cl_2 (80 mL) and pyridine (8 mL). After 10 min, when the mixture had turned maroon, alcohol **11** (4 g, 18 mmol) was added to form a thick slurry. Acetic anhydride (8 mL) was added and the mixture was vigorously stirred for 30 min. The crude mixture was filtered through a plug of silica gel in ethyl acetate and the filtrate concentrated under reduced pressure to give a pale yellow oil. Separation of the crude product by flash chromatography (silica, 10:90 ethyl acetate:*n*-hexane) gave ketone **12**[12] as a clear oil (3.6 g, 85% yield). ^{1}H NMR ($CDCl_3$) δ 4.80 (s,1H), 4.42 (s,1H), 4.22 (q, 1H, J = 6.4 Hz), 3.46 (s, 3H), 3.38 (s, 1H), 1.48 (s, 3H), 1.40 (d, 3H, J = 6.4 Hz), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 204.2, 111., 98.2, 78.7, 75.9, 69.8, 55.8, 26.7, 25.5, 16.0. The product was used immediately in the next step.

Methyl 6-Deoxy-2,3-isopropylidene-3-*C*-methyl-α-L-*lyxo*-pyranosid-4-ulose (13).[12] Freshly distilled dry diisopropylamine (0.46 mL, 3.3 mmol) was added to a 3-necked flask containing THF (40 mL). *n*-Butyllithium (2.4 M in hexanes, 1.4 mL, 3.3 mmol) was added dropwise at 0 °C and allowed to stir for 30 min. The temperature was lowered to −78 °C and ketone **12** (650 mg, 3.09 mmol) was added dropwise via cannula to give a deep yellow solution. After 30 min, dry HMPA (4.7 mL, 26 mmol) and methyl iodide (1.6 mL, 26 mmol) were added by syringe and the mixture was stirred for 3 h at −78 °C. The mixture was quenched by addition of saturated NH_4Cl (aq, 30 mL) and extracted with Et_2O (2 × 40 mL). The combined ether layers were washed with brine, dried over $MgSO_4$, and concentrated to give a yellow oil that was purified by flash chromatography (silica, 10:90 ethyl acetate:*n*-hexane) to give **13** as a clear oil (0.7 g, 71% yield). $[\alpha]_D$ −108.2 (*c* 3, $CHCl_3$) {lit.[12] $[\alpha]_D$ −114.6 (*c* 1.5, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$) δ 4.88 (s, 1H), 4.30 (q, 1H, J = 6.4 Hz), 4.04 (s, 1H), 1.45 (s, 3H), 1.38 (s, 3H), 1.37 (s, 3H), 1.35 (d, 3H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) 204.9, 110.7, 98.3, 83.2, 68.9, 56.2, 27.6, 27.4, 21.7, 15.4.

Methyl 6-Deoxy-2,3-*O*-isopropylidene-α-L-*talo*-pyranoside (15). A stirred solution of ketone **13** (200 mg, 0.87 mmol) in EtOH (10 mL) was treated portionwise with $NaBH_4$ (60 mg,1.6 mmol) over 5 min After an additional 15 min, the mixture was quenched by the addition of solid NH_4Cl (60 mg), and the mixture was extracted with Et_2O (2 × 15 mL). The combined ether layers were dried over $MgSO_4$ and concentrated to give **15** as a clear oil (194 mg, 96% yield). $[\alpha]_D$ −54 (*c* 2, $CHCl_3$) {lit.[13] $[\alpha]_D$ −55.0 (*c* 1, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$) 4.91 (s, 1H), 3.88 (q, 1H, J = 6.4 Hz), 3.39 (s, 3H), 3.15 (s, 1H), 2.42 (br s, 1H), 1.55 (s, 3H), 1.40 (s, 3H). 1.36 (s, 3H), 1.34 (d, 3H, J = 6.4 Hz); ^{13}C ($CDCl_3$) δ 109.4, 98.5, 79.7, 75.2, 67.6, 55.2, 27.8, 26.3, 21.9, 15.4.

4,5-Dihydroxy-6-methoxy-2,4-dimethyldihydropyran-3-one (16). A solution of ketone **13** (700 mg, 3.24 mmol) in methanol (25 mL) containing *p*-toluenesulfonic acid hydrate (60 mg) was heated at reflux for 24 h. The volatiles were removed under reduced pressure to give a yellow oil that was taken up in 1:1 EtOAc/*n*-hexane and eluted through a plug of silica gel with the same solvent to give **16** as a clear foam (612 mg, 97% yield). $[\alpha]_D$ −102.2 (*c* 2.5, $CHCl_3$) {lit.[20] $[\alpha]_D$ −105 (*c* 2.0, $CHCl_3$)}; ^{1}H NMR ($CDCl_3$) δ 4.87 (s, 1H), 4.51 (d, 1H, J = 6.4 Hz), 3.52 (s, 3H), 3.43 (s, 1H), 1.54 (s, 3H), 1.34 (d, 3H, J = 6.4 Hz); ^{13}C NMR ($CDCl_3$) δ 203.9, 100.3, 86.6, 78.3, 66.8, 56.0, 23.9, 13.8.

Methyl 6-Deoxy-3-*C*-methyl-α-L-*manno*-pyranoside (17). Ketone **16** (550 mg, 2.86 mmol) was dissolved in glacial acetic acid (15 mL) and the solution was treated with $NaBH(OAc)_3$ (1.8 g, 8.6 mmol) in three portions. After 5 h, H_2O (15 mL) was added, followed by 6 M (NaOH) until the mixture was neutral. The mixture was extracted with Et_2O (2 × 40 mL) and the combined organic phases dried over Na_2SO_4 and concentrated under reduced pressure to give a white foam. Purification of the residue by flash chromatography afforded **17** as a white solid (493 mg, 87%). $[\alpha]_D$ −81.5 (*c* 1.2, MeOH) {lit.[13] $[\alpha]_D$ −83.3 (*c* 0.85, MeOH)}; ^{1}H NMR (CD_3OD) δ 4.56 (s, 1H), 3.58 (dd, 1H, J = 9.8, 6.4 Hz), 3.47 (s,1H), 3.39 (d, 1H, J = 9.8 Hz), 3.34 (s, 3H), 1.26 (d, 3H, J = 6.4 Hz), 1.24 (s, 3H); ^{13}C δ 103.2, 76.4, 76.1, 73.6, 68.6, 55.3, 19.2, 18.5.

Methyl 6-Deoxy-2,3-*O*-isopropylidene-3-*C*-methyl-α-L-*manno*-pyranoside (14). Alcohol **17** (450 mg, 2.34 mmol) was dissolved in acetone (15 mL) and $CuSO_4$ (300 mg) and the mixture was heated at reflux for 16 h. $CuSO_4$ was removed by filtration and the volatiles were removed under reduced pressure to give **14** as a clear oil (490 mg, 93% yield). ^{1}H NMR and ^{13}C NMR data were consistent with literature values.[13]

Methyl 6-Deoxy-2,3-*O*-isopropylidene-3-*C*-methyl-4-*O*-naphthoyl-α-L-*manno*-pyranoside (18). To a solution of **14** (490 mg, 2.11 mmol) in CH_2Cl_2 (25 mL) was added 2-naphthoyl chloride (521 mg, 2.7 mmol) in CH_2Cl_2 over 5 min. Pyridine (250 μL) was added and the reaction mixture was stirred for 8 h at room temperature then quenched with NH_4Cl (aq). The mixture was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic

(20) Chatterjee, D.; Bozic, C.; Aspinalli, G. O.; Brennan, P. J. *J. Biol. Chem.* **1988**, *263*, 4092–4094.

layers were dried over $MgSO_4$ and concentrated to give a yellow oil that was purified by chromatography (silica, 10:90 EtOAc:*n*-hexane) to afford **18** as a yellow oil (682 mg, 82%). $[\alpha]_D$ −94.3 (*c* 1.4, $CHCl_3$); IR ν 2820, 1738, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.29–7.46 (m, 7H), 5.1 (d, $J = 9.4$ Hz), 4.88 (s, 1H), 3.81 (s, 1H), 3.64 (dd, 1H, $J = 9.4$, 6.4 Hz), 3.34 (s, 3H), 1.54 (s, 3H), 1.46 (d, 3H, $J = 6.4$ Hz); ^{13}C NMR ($CDCl_3$) δ 178.2, 135.6, 133.2, 132.5, 131.4, 131.1, 130.6, 129.7, 128.2, 126.5, 124.4, 109.3, 98.2, 85.9, 83.1, 67.3, 61.2, 55.2, 27.09, 26.9, 18.1, 17.2; HR FABMS *m/z* $[M + H]^+$ 387.1801 (calcd for $C_{22}H_{27}O_6$, 387.1808).

Methyl 4-*O*-(2′-Naphthoyl)-α-L-evalose (19). A solution of methyl 2,3-*O*-isopropylidene-4-*O*-(2′-naphthoyl)-α-L-evalose (**18**) (600 mg, 1.55 mmol) and *p*-TsOH (40 mg) in methanol (15 mL) was heated at reflux for 14 h. The volatiles were removed under reduced pressure to give the corresponding diol as a white solid (502 mg). A portion of the crude diol (350 mg, 1.0 mmol) in CH_3CN (15 mL) was treated with freshly prepared Ag_2O (916 mg, 4.0 mmol), followed by slow addition of iodomethane (187 mg, 1.3 mmol), and the mixture was then heated at reflux for 5 h. Ag_2O was removed by filtration to give a yellow oil that was purified by SiO_2 chromatography (2:8 EtOAc:*n*-hexane) to provide **19** as a white solid (200 mg, 55% yield). $[\alpha]_D$ −86.4 (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.28–7.46 (m, 7H), 5.14 (d, 1H, $J = 9.2$ Hz), 3.59 (dd, 1H, $J = 9.2$, 6.4 Hz), 3.41 (s, 3H), 3.32 (s, 3H), 3.14 (s, 1H), 1.51 (s, 3H), 1.42 (d, 3H, $J = 6.4$ Hz); ^{13}C NMR ($CDCl_3$) 178.4, 135.4, 133.5, 132.1, 131.3, 131.1, 129.8, 129.2, 128.2, 126.5, 124.1, 97.9, 85.6, 82.0, 67.4, 61.3, 55.2, 53.1, 18.1, 16.9; HR FABMS *m/z* 361.1672 $[M + H]^+$ (calcd for $C_{20}H_{25}O_6$, 361.1651).

2-Isocyanato-6-methoxynaphthalene (20). A stirred solution of 6-methoxy-2-naphthoic acid (201 mg, 1.03 mmol) in benzene (10 mL) was treated dropwise with diphenylphosphoryl azide (343 mg, 1.25 mmol). The mixture was stirred at 50 °C overnight, then heated to reflux (16 h total) to complete the reaction, before cooling and concentration. Filtration of the mixture through a plug of silica (1:1 EtOAc:hexane) gave pure **20**. IR ν 2250 cm^{-1}.[14]

***N*-(6″-Methoxy-2″-naphthyl)-[1,2-*O*-dimethyl-α-L-3-*C*-methyl-4-*O*-(2′-naphthoyl)-6-deoxy-*manno*-3-*O*-pyranosyl] Carbamate (9).** Evalose derivative **19** (25 mg, 0.07 mmol) was dissolved in pyridine (5 mL). Freshly prepared **20** (19.9 mg, 1.4 mmol)[14] was added as a solution in pyridine (1 mL) and the mixture was heated to 50 °C for 5 h. The mixture was quenched by the addition of 1 mL of saturated aq $NaHCO_3$, extracted with CH_2Cl_2, dried over $MgSO_4$, and concentrated to give a yellow solid that was purified by SiO_2 chromatography (1:9 EtOAc:*n*-hexane) to afford **9** as a yellow solid (32 mg, 79% yield). UV (MeOH) λ_{max} 238 nm (ϵ 52000), 270 (13000); $[\alpha]_D$ −67.2 (*c* 4, $CHCl_3$); ^1H NMR ($CDCl_3$) 7.2–7.45 (m, 13H), 5.32 (d, 1H, $J = 9.2$ Hz), 4.84 (s, 1H), 3.71 (dd, 1H, $J = 9.2$, 6.4 Hz), 3.31 (s, 3H), 3.24 (s, 3H), 3.12 (s, 1H), 1.52 (s, 3H), 1.45 (d, 3H, $J = 6.4$ Hz); ^{13}C δ 173.1, 157.6, 139.2, 137.6, 136.2, 136.1, 135.5, 132.7, 132.4, 131.6, 131.5, 130.9, 130.6, 130.2, 129.5, 128.2, 128.1, 126.3, 125.4, 124.2, 123.2, 97.2, 85.2, 84.9, 68.2, 59.2, 56.2, 53.1, 19.1, 18.4; HR FABMS *m/z* $[M + H]^+$ 560.2282 (calcd for $C_{32}H_{34}NO_8$, 560.2284).

Methanolysis of Phorbaside A and Derivatization. Phorbaside A (**3**, 50 μg) was dissolved in dry MeOH (1 mL) that had been purged with HCl (g) and heated to 100 °C for 6 h. The sample was concentrated to a yellow film, then dissolved in CH_2Cl_2 (200 μL) before addition of pyridine (20 μL) and 2-naphthoyl chloride (100 μg). The mixture was allowed to stir for 8 h and monitored by TLC for the formation of 4-*O*-naphthoate ester. The solvent was removed under a stream of N_2 and the residue dried under high vacuum. The sample was redissolved in pyridine (300 μL) and treated with 2-isocyanato-6-methoxynaphthalene (**20**, 100 μg) before being heated to 50 °C for 12 h. After removal of pyridine under reduced pressure, the sample was resuspended in EtOAc (100 μL) and the mixture centrifuged to remove solid material. The supernatant was concentrated and redissolved in EtOAc to give "sample A" (20 μL).

HPLC and CD Analysis of L-Evalose Derivative 9 Obtained from L-Rhamnose and Phorbaside A. HPLC analysis of synthetic **9** (silica, ISCO 5 μ, 4.6 × 100 mm, 4% *i*-PrOH:*n*-hexane, UV detection: λ =254 nm) gave a single peak t_R = 6.7 min. Analytical HPLC of sample "A" (above) gave an identical peak with the same retention time. The remainder of sample "A" (see above) was purified by semipreparative HPLC under the same conditions. The fractions eluting at t_R = 6.7 min from repetitive injections of the remainder of sample "A" were collected and measured directly by CD in the solvent used for elution (4% *i*-PrOH/*n*-hexane). The CD spectrum of synthetic **9** (ϵ 52000, $c = 7.9 \times 10^{-6}$ M) was measured under the same conditions and the concentration of sample "A" was normalized from the UV extinction coefficient (see Figure 3).

Cytotoxicity Measurements: HCT116. Compounds were assayed with compounds in DMSO (final concentration, 1% v/v) and run against etoposide as positive control. Human colon tumor cells (HCT-116) were incubated in 96-well plates for 72 h before addition of MTS ((3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt, Promega CellTiter 96 Aqueous cell proliferation assay, Technical Bulletin No. 169). Dose responses were obtained from well absorbances (λ 490 nm), after correction for background, and expressed as a percentage of the negative control (DMSO, only).

Acknowledgement. We thank S. Lievens (UC Davis) for cytotoxicity measurements of **3–7**, Sara Kelly and W. Fenical (Scripps Institution of Oceanography) for preliminary cytotoxicity measurements, J. DeRopp (UC Davis) for assistance with measurements of 600 MHz NMR spectra, and R. New (UC Riverside) for MS data. This research was supported by funding from NIH (AI39987 and CA122256).

Note Added in Proof. An independent confirmation of the L-2-*O*-methylevalose in callipeltoside C has been revealed by total synthesis. Carpenter, J.; Northrup, A. B.; Chung, deM.; Wiener, J. J. M.; Kim, S-G.; MacMillan, D. W. C. *Angew. Chem. Int. Ed.* **2008,** *early view*, DOI: 10.1002/anie.200800086

Supporting Information Available: ^1H NMR, HSQC, HMBC, and HSQC TOCSY of **3**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO702307T