JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Assignment of the Absolute Configuration of Phosphoeleganin via Synthesis of Model Compounds

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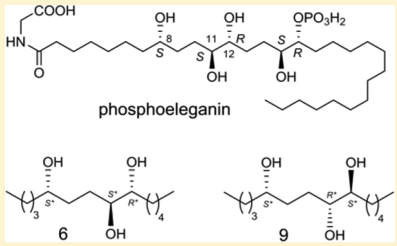
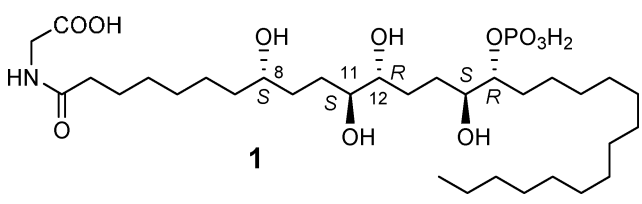
Supporting Information

ABSTRACT: The full absolute configuration assignment of phosphoeleganin (**1**), a recently discovered marine-derived phosphorylated polyketide with protein tyrosine phosphatase 1B inhibitory activity, was achieved. It was based on the synthesis of model diasteroisomeric compounds of the C-8−C-12 segment portion of phosphoeleganin, chiral derivatization methods, and application of the universal NMR database concept.



The problem of relative and absolute configuration elucidation of acyclic scaffolds is an important challenge in natural products chemistry. Often, integrated approaches are needed, which require rigorously acquired data obtained by a combination of different tools, including chemical manipulation of the original molecule and organic synthesis.[1,2] Databases of spectroscopic information can be a great resource for stereostructure determination, too. For example, Kishi and co-workers, on the basis of their extensive experience with polyketide chemistry and synthesis, developed an NMR database of several motifs common to these molecules. This data collection, known as the "universal NMR database" (UDB), allowed simplified protocols to be used for the relative and absolute configuration assignment of unknown polyketides without degradation and/or derivatization work.[3−6]

We have recently isolated phosphoeleganin (**1**), a new phosphorylated polyketide with protein tyrosine phosphatase 1B (PTP1B) inhibitory activity, from the Mediterranean ascidian *Sidnyum elegans*.[7] By a combination of spectroscopic analysis and microscale chemical degradation and/or derivatization, we established the planar structure of **1**, as well as its configuration except for the absolute configurations of the carbinol centers C-11 and C-12. This provided two alternative stereostructures for the natural metabolite, corresponding to the 8*S*, 11*S*, 12*R*, 15*S*, 16*R* or 8*S*, 11*R*, 12*S*, 15*S*, 16*R* configurations.



RESULTS AND DISCUSSION

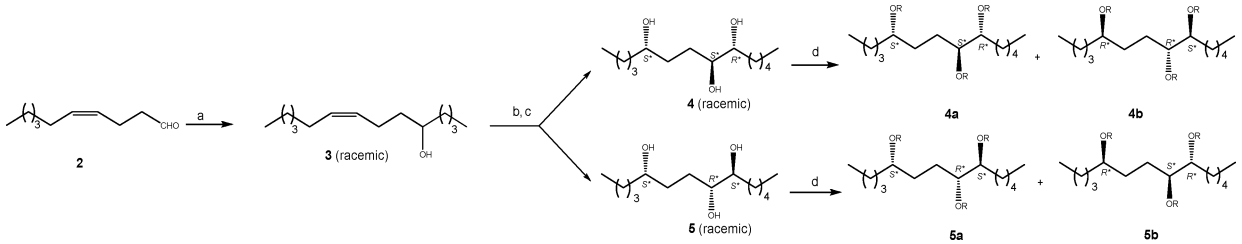
Here, we report the unequivocal 8*S*, 11*S*, 12*R*, 15*S*, 16*R* stereochemical assignment of phosphoeleganin, performed by a combination of organic synthesis and chiral derivatization methods directed to the application of the UDB concept. Indeed, the logic used in this approach is that, given a complex molecule composed of structural clusters of stereogenic centers connected by a number of methylene bridges, we can assume that (A) the structural properties of these clusters are inherent to the specific stereochemical arrangement of the substituents on the carbon backbone and (B) the structural properties of these clusters are independent from the rest of molecule, when they are sufficiently separated from each other.[6] In practice, the stereogenic subunits need only to be separated by two or more methylene groups so that they may be treated independently.

On this basis, we considered the C-8−C-12 stereocluster of phosphoeleganin, in which the *S* absolute configuration at C-8 and the *anti* relationship at C-11/C-12 had been previously assigned.[7] We decided to compare the ^{1}H and ^{13}C NMR chemical shifts of this stereocluster with those of synthetic compounds with known configuration. We did not find any suitable molecule for comparison in the Kishi's universal NMR database; therefore, we decided to prepare the 8,9-*anti* stereoisomers of tetradecane-5,8,9-triol to be used as model compounds. For this purpose, the *cis*-4-decenal (**2**) was converted to the (*Z*)-tetradec-8-en-5-ol (racemate) **3** by coupling with *n*-BuLi. Then, **3** was treated with N-methylmorpholine oxide (NMO) and 1% OsO_4 to give a mixture of the four 8,9-*anti* stereoisomers of tetradecane-5,8,9-triol. Separation by reversed-phase HPLC of the reaction

Received: May 5, 2017

DOI: 10.1021/acs.jnatprod.7b00397
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of the Four 8,9-*anti* Stereoisomers of 5,8,9-Tetradecanetriol[a,b]



[a]Reagents and conditions: (a) *n*-BuLi, anhydrous THF, −78 °C (10 min), rt (8 h); (b) NMO, 1% OsO_4, acetone/H_2O, 9:1, rt overnight; (c) HPLC (Luna C18 10 μm, MeOH/H_2O, 7:3); (d) EDC, DMAP, (*R*)-MPA, CH_2Cl_2, rt overnight. [b]R = (*R*)-MPA.

mixture afforded two diastereoisomeric fractions (**4** and **5**) as racemates. The chemical characterization of compounds **4** and **5** was performed through spectroscopic analyses (Experimental Section). Both **4** and **5** were separately derivatized with three equivalents of (*R*)-methoxyphenylacetic acid (*R*-MPA) to afford, in each case, a diastereoisomeric mixture of tris-(*R*)-MPA esters, **4a**/**4b** and **5a**/**5b**, respectively (Scheme 1). The four (*R*)-MPA derivatives were obtained as individual compounds by HPLC separation on a silica gel column, and their ^{1}H NMR spectra were registered. According to the approach proposed by Riguera's group for the assignment of the relative configuration of acyclic *sec*/*sec*-1,*n*-diols by double derivatization,[8,9] we calculated the chemical shift differences ($\Delta\delta$) in the pairs **4a**/**4b** and **5a**/**5b**. It is to be noted that in our case it was not necessary to prepare the two (*R* and *S*) tris-chiral derivatizing agent esters since the (*S*)-MPA derivatives of **4a** and **5a** would be enantiomeric to the (*R*)-MPA derivatives of **4b** and **5b**, respectively, and, consequently, their NMR chemical shifts would be identical.[10] Calculation of the $\Delta\delta$ values in the **4a**/**4b** pair (δH-4a − δH-4b or δH-4b − δH-4a) led to negative (or positive) values for the C-1−C-5 segment and positive (or negative) values for the C-8−C-14 segment (Figure 1a); in contrast, all positive (or negative) $\Delta\delta$ (δH-5a − δH-5b or δH-5b − δH-5a) values were obtained for the C-5−C-8 segment in the **5a**/**5b** couple (Figure 1b). Finally, comparison of the sign distributions in both pairs with the trends evidenced in every isomer of acyclic *sec*/*sec*-1,4-diols[9] suggested a 5,8-*anti* relative configuration in the **4a**/**4b** couple and a 5,8-*syn* relative configuration in the **5a**/**5b** pair (Figure 1).

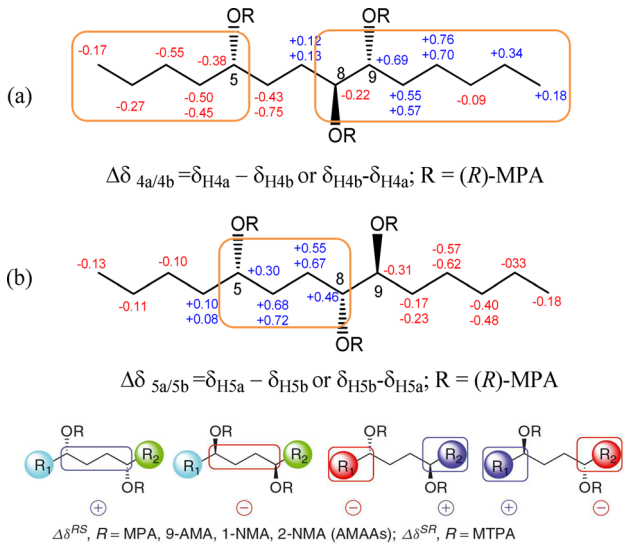


Figure 1. (a) $\Delta\delta$ sign distribution patterns for the MPA esters of the pairs **4a**/**4b** and (b) **5a**/**5b**.

Actually, as depicted in Figure 1, the patterns in the $\Delta\delta$ values' sign distribution were not perfectly homogeneous, especially in the pair **4a**/**4b**, where anomalies were detected for the protons whose signals are diagnostic. Combined shielding/deshielding effects of the MPA units close enough to each other possibly produce these anomalies and increase the degree of uncertainty in the stereochemical assignment.[11] Therefore, we decided to substantiate the (5*S**,8*S**,9*R**) and the (5*S**,8*R**,9*S**) relative configuration assignments in compounds **4a**/**4b** and **5a**/**5b**, respectively, by chemical means.

The enantiomerically pure tetradecane-5,8,9-triol **6** was obtained from the base-catalyzed methanolysis of the tris-(*R*)-MPA ester **4a**. Then, we performed the stereoselective synthesis of a tetrahydrofuran ring through a one-pot method involving the formation of cyclic ortho esters generated in situ via trimethyl orthoacetate[12] (Scheme 2). The ionization of the intermediate ortho ester **7** with the Lewis acid BF_3 led to an acetoxonium species, which, upon nucleophilic intramolecular displacement with the hydroxy group at C-5, afforded the cyclized ether **8** in excellent yield (Scheme 2). Analogously, compound **9**, derived from the base-catalyzed methanolysis of **5a**, was converted into the ether **11** (Scheme 2).

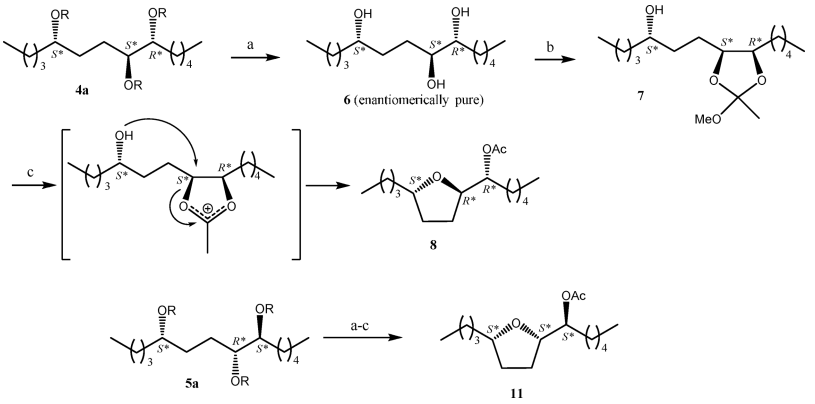
A *trans* and *cis* relationship across the tetrahydrofuran (THF) rings in compounds **8** and **11**, respectively, was then established by ROESY experiments. Particularly, strong dipolar couplings were observed between H-5 and H-8 in compound **11**, whereas we did not detect any ROE correlation between these two protons for compound **8** (Figure 2).

These results, combined with the stereoselective outcome of the cyclization reaction, where the configuration at C-8 is inverted, were in agreement with an *anti*−*anti* relative configuration in compound **7** and a *syn*−*anti* relative configuration in compound **10**. Thus, the (5*S**,8*S**,9*R**) and the (5*S**,8*R**,9*S**) relative configurations were definitely assigned to the tetradecane-5,8,9-triols **6** and **9**, respectively. Finally, the NMR data of the tetradecane-5,8,9-triols **6** and **9** were compared to those of the C-8−C-12 portion of the natural phosphoeleganin (**1**). This evaluation provided evidence that proton and carbon NMR resonances of this portion match with those of (5*S**,8*S**,9*R**)-tetradecane-5,8,9-triol (**6**) [mean average error (MAE): ^{13}C = 0.16; ^{1}H = 0.02 for compound **6** vs ^{13}C = 0.64 and ^{1}H = 0.07 for compound **9**, Figure 3]. This suggested an 8,11-*anti* relative configuration in **1**.

The absolute configuration at C-8 in phosphoeleganin has been previously established as *S*. Thus, the assignment of the relative configuration of the C-8−C-11 segment as *anti* required an absolute configuration at C-11 of *S*. Analogously, the C-11−C-12 *anti* configuration, previously established, too, required an

DOI: 10.1021/acs.jnatprod.7b00397
J. Nat. Prod. XXXX, XXX, XXX−XXX

Scheme 2. Preparation and Stereoselective Cyclization of Tetradecane-5,8,9-triols 6 and 9[a,b]



[a]Reagents and conditions: (a) NaOH, MeOH, rt, overnight; (b) $MeC(OMe)_3$ (1.2 equiv), PPTS (0.1 equiv), CH_2Cl_2, 15 min, rt; (c) $BF_3{\cdot}Et_2O$ (0.1 equiv), 0 °C, H_2O/acetone, 9:1. [b]R = (*R*)-MPA.

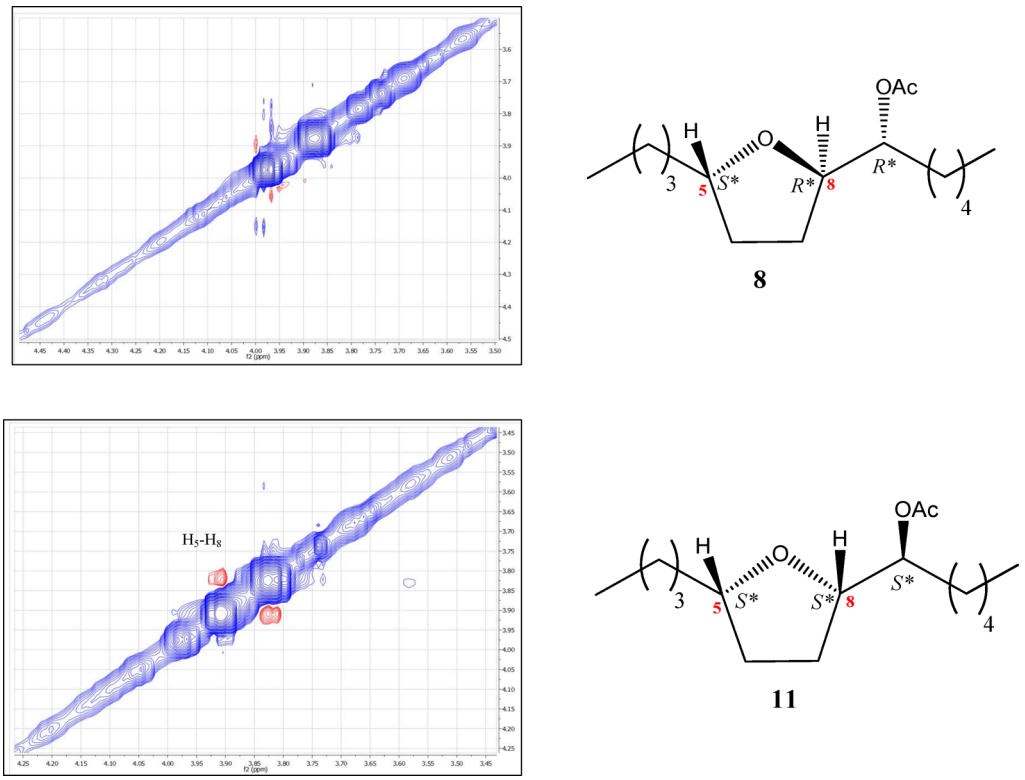


Figure 2. Key ROE correlations for **8** and **11**.

absolute configuration at C-12 of *R*. Therefore, the absolute configuration of phosphoeleganin was completely determined as 8*S*, 11*S*, 12*R*, 15*S*, 16*R*.

In summary, the design and synthesis of model diastereoisomeric compounds of the C-8−C-12 segment portion of phosphoeleganin enabled the full absolute configuration assignment of the natural metabolite. Moreover, the whole of our studies have great significance for those who are working in stereochemical assignments of complex flexible acyclic scaffolds, such as polyketides. First, in this context, the usefulness of NMR databases clearly appeared. Particularly, in the logic of the UDB concept, the possibility that a small motif present in a larger natural product could be easily obtained by limited synthetic effort and used as a model compound represents an efficient and reliable simplified protocol in stereostructure determination. When this procedure is performed for several molecules within a pool of structurally analogous compounds, representing a class of molecules, it is conceivable to assemble a dedicated NMR database for this class, thus reducing the synthetic efforts. Finally, it is to be noted that, although Riguera's approach for the configuration assignments of acyclic 1,*n*-diols has been rigorously validated,[9,11] in our attempt to determine the absolute configuration of the synthetic tetradecane-5,8,9-triols by analysis of their poly-MPA derivatives, the obtained data appeared not completely reliable due to the combined anisotropy effects. In these cases, 1,2,*n*-triol stereocontrolled cyclization strategies, such as the practical cyclization to construct THF rings from 1,2,5-triols based on the Lewis acid-mediated cyclization of cyclic ortho-esters, was proved to be a valuable conclusive proof.

DOI: 10.1021/acs.jnatprod.7b00397
J. Nat. Prod. XXXX, XXX, XXX−XXX

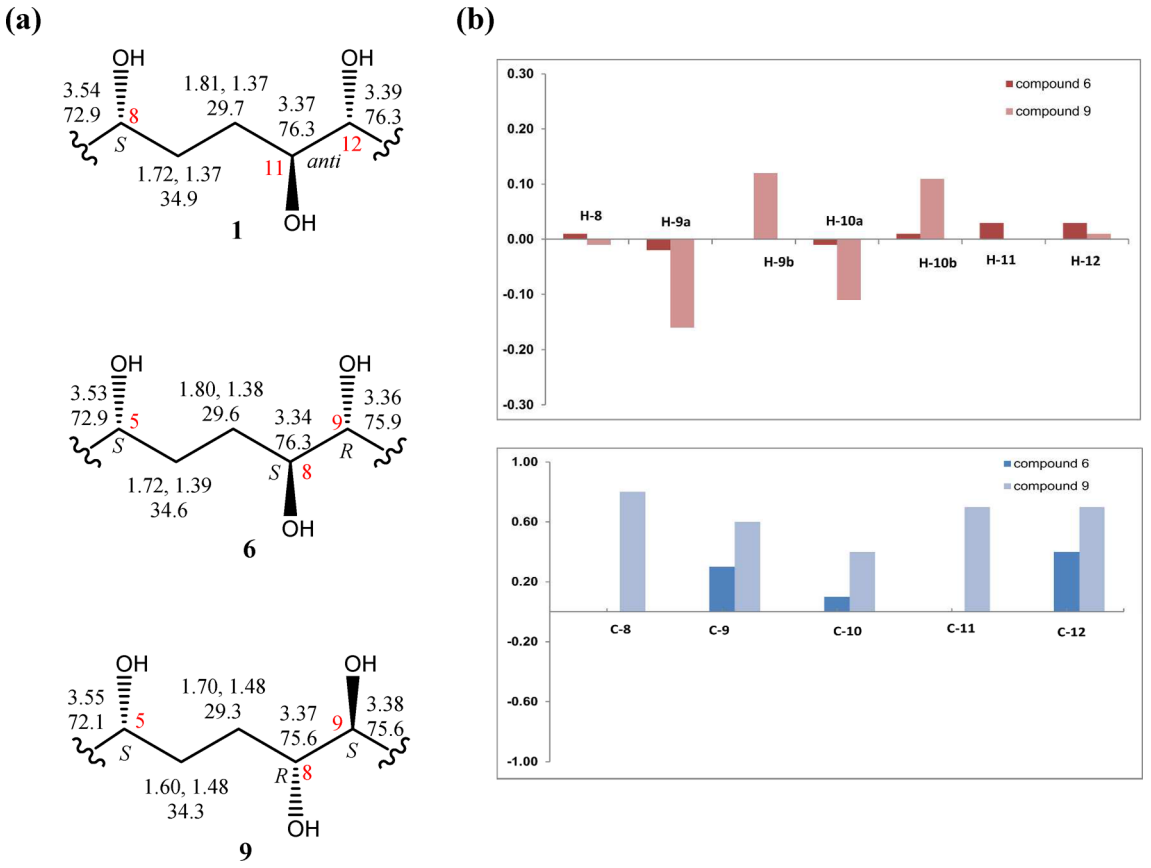


Figure 3. (a) ^{1}H and ^{13}C chemical shift values (CD_3OD) of the similar stereoclusters in phosphoeleganin (**1**), **6**, and **9**; (b) their calculated $\Delta\delta H$ ($\delta H_1 - \delta H_{6\text{ or }9}$) and $\Delta\delta C$ ($\delta C_1 - \delta C_{6\text{ or }9}$).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured at 589 nm with a Jasco P-2000 polarimeter using a 10 cm microcell. ECD spectra were recorded with a J-710 spectropolarimeter (Jasco) with J-710 for Windows software (Jasco). ^{1}H (700 MHz) and ^{13}C (175 MHz) NMR spectra were recorded on a Agilent INOVA spectrometer; chemical shifts were referenced to the residual solvent signal (CD_3OD: δ_H = 3.31, δ_C = 49.0; $CDCl_3$: δ_H = 7.26, δ_C = 77.0). Homonuclear ^{1}H connectivities were determined by COSY experiments. Two- and three-bond $^{1}H-^{13}C$ connectivities were determined by gradient 2D HMBC experiments optimized for a $^{2,3}J$ of 8 Hz. HRMS (ESI positive mode) was performed with a Thermo LTQ Orbitrap XL mass spectrometer. The spectra were recorded by infusion into the ESI source using MeOH as solvent. HPLC separation was achieved on a Knauer K-501 apparatus equipped with an Knauer K-2301 RI detector.

Synthesis of (*Z*)-Tetradec-8-en-5-ol (3). (*Z*)-Tetradec-8-en-5-ol was prepared from a solution of *cis*-4-decenal (424 mg, 2,75 mmol, 1.0 equiv) in anhydrous THF (20 mL) to which was added *n*-BuLi dropwise (2.06 mL, 1.6 M in hexane, 3.30 mmol, 1.2 equiv). The reaction mixture was stirred at −78 °C for 10 min and then for an additional 8 h at room temperature (rt). The reaction was quenched with saturated NH_4Cl and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4, and solvent removed with rotary evaporation to yield the desired alcohol in 90% yield (524 mg). ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.84 (6H, m, H-1 and H-14), 1.20−1.51 (14H, overlapped, H-2/H4, H-9, H-11/H-13), 1.96 (2H, m, H-5), 5.32 (2H, m, H-6 and H-7), 2.10, (1H, m, H-8a), 2.06 (1H, m, H-8b), 3.54 (1H, m, H-10); ^{13}C NMR (175 MHz, $CDCl_3$) δ 14.1 (CH_3, C-1 and C14), 22.5 (CH_2), 22.7 (CH_2), 22.4 (CH_2), 27.1 (CH_2), 27.7 (CH_2), 29.3 (CH_2), 31.4 (CH_2), 37.0 (CH_2), 37.1 (CH_2), 71.5 (CH, C-5), 129.1 (CH), 130.5 (CH); ESIMS *m/z* 213.22 $[M + H]^+$.

Synthesis of 4 and 5. (*Z*)-Tetradec-8-en-5-ol (495 mg, 2.3 mmol, 1 equiv) was dissolved with acetone/H_2O (9:1, 10 mL), and NMO (410 mg, 3.5 mmol, 1.5 equiv) and OsO_4 (1 mol %, 6 mg) were added. The reaction mixture was stirred overnight at rt. The reaction was quenched with saturated Na_2SO_3 solution and extracted with EtOAc (3×). The organic layer was dried over anydrous Na_2SO_4, filtered, and concentrated in vacuo. Part of the organic layer was purified by reversed-phase HPLC (Luna C18 10 μ MeOH/H_2O, 7:3), providing two diastereoisomeric fractions, **4** (55.1 mg, t_R 12.6 min) and **5** (61.4 mg, t_R 14.4 min), as enantiomeric mixtures. **4**: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.92 (3H, t, *J* = 7.6 Hz, H-1), 1.43 (1H, overlapped, H-2a), 1.32 (1H, overlapped, H-2b), 1.32 (2H, overlapped, H-3), 1.46, (1H, m, H-4a), 1.42 (1H, overlapped, H-4b), 3.53 (1H, m, H-5), 1.72 (1H, m, H-6a), 1.39 (1H, overlapped, H-6b), 1.80 (1H, m, H-7a), 1.39 (1H, overlapped, H-7b), 3.34 (1H, m, H-8), 3.36 (1H, m, H-9), 1.60 (1H, overlapped, H-10a), 1.36 (1H, overlapped, H-10b), 1.54 (1H, m, H-11a), 1.33 (1H, overlapped, H-11b), 1.32 (2H, overlapped, H-12), 1.34 (2H, overlapped, H-13), 0.92 (3H, t, *J* = 7.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 14.1 (CH_3, C-1), 28.7 (CH_2, C-2), 32.8 (CH_2, C-3), 37.9 (CH_2, C-4), 72.9 (CH, C-5), 34.6 (CH_2, C-6), 29.6 (CH_2, C-7), 76.3 (CH, C-8), 75.9 (CH, C-9), 33.4 (CH_2, C-10), 26.4 (CH_2, C-11), 32.8 (CH_2, C-12), 23.7 (CH_2, C-13), 14.1 (CH_3, C-14); ESIMS *m/z* 247.22 $[M + H]^+$.

5: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.92 (H-1, t, *J* = 7.6 Hz 7.6, 3H,), 1.44 (1H, overlapped, H-2a), 1.33 (1H, overlapped, H-2b), 1.32 (2H, overlapped, H-3), 1.46 (1H, m, H-4a), 1.42 (1H, overlapped, H-4b), 3.55 (1H, m, H-5), 1.60 (1H, overlapped, H-6a), 1.53 (1H, overlapped, H-6b), 1.70 (1H, overlapped, H-7a), 1.48 (1H, overlapped, H-7b), 3.37 (1H, m, H-8), 3.38 (1H, m, H-9), 1.60 (1H, overlapped, H-10a), 1.36 (1H, overlapped, H-10b), 1.54 (1H, overlapped, H-11a), 1.33 (1H, overlapped, H-11b), 1.32 (2H, overlapped, H-12), 1.34 (2H, overlapped, H-13), 0.92 (3H, t, *J* = 7.6 Hz 7.6, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC

DOI: 10.1021/acs.jnatprod.7b00397
J. Nat. Prod. XXXX, XXX, XXX−XXX

data) δ 14.1 (CH_3, C-1), 28.7 (CH_2, C-2), 32.8 (CH_2, C-3), 37.9 (CH_2, C-4), 72.1 (CH, C-5), 34.3 (CH_2, C-6), 29.3 (CH_2, C-7), 75.6 (CH, C-8), 75.6 (CH_2, C-9), 33.4 (CH_2, C-10), 26.4 (CH_2, C-11), 32.8 (CH_2, C-12), 23.7 (CH_2, C-13), 14.1 (CH_3, C-14); ESIMS m/z 247.25 $[M + H]^+$.

Compounds 4a,b and 5a,b. The esterification reaction was carried out on both fractions **4** (5.1 mg, 0.048 mmol) and **5** (18.1 mg, 0.074 mmol) each dissolved in dry CH_2Cl_2 (3 mL). To a solution of **4** was added ethylene dichloride (EDC) (37.4 mg, 0.195 mmol), 4-dimethylaminopyridine (DMAP) (11.8 mg, 0.096 mmol), and (*R*)-methoxyphenylacetic acid (6.32 mg, 0.195 mmol). Instead, to a solution of **5** was added EDC (63.3 mg, 0.334 mmol), DMAP (18.1 mg, 0.148 mmol), and (*R*)-methoxyphenylacetic acid (54.8 mg, 0.334 mmol). Each mixture was stirred overnight under a N_2 atmosphere at rt. After solvent evaporation, HPLC purification of the two mixtures on a SiO_2 column (Luna SiO_2 3 μm), eluting with hexane/EtOAc, 85:15 (v/v), gave two diastereoisomeric triesters, **4a** (2.1 mg, t_R 3.74 min), **4b** (2.3, t_R 4.64 min), **5a** (7.8 mg, t_R 3.12 min), and **5b** (8.0 mg, t_R 4.32 min).

Compound 4a: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.67 (3H, t, *J* = 7.6 Hz, H-1), 0.98 (2H, overlapped, H-2), 0.61 (2H, m, H-3), 0.98 (1H, overlapped, H-4a), 0.94 (1H, overlapped, H-4b), 4.42 (1H, m, H-5), 0.88 (1H, overlapped, H-6a), 0.56 (1H, m, H-6b), 1.14 (1H, m, H-7a), 0.93 (1H, overlapped, H-7b), 4.66 (1H, m, H-8), 5.09 (1H, m, H-9), 1.39 (1H, overlapped, H-10a), 1.36 (1H, overlapped, H-10b), 1.22 (2H, overlapped, H-11), 1.22 (2H, overlapped, H-12), 1.25 (2H, overlapped, H-13), 0.86 (3H, t, *J* = 7.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 13.5 (CH_3, C-1), 22.1 (CH_2, C-2), 26.2 (CH_2, C-3), 33.4 (CH_2, C-4), 74.9 (CH, C-5), 29.1 (CH_2, C-6), 24.0 (CH_2, C-7), 74.7 (CH, C-8), 74.1 (CH, C-9), 29.9 (CH_2, C-10), 24.9 (CH_2, C-11), 31.1 (CH_2, C-12), 22.3 (CH_2, C-13), 13.7 (CH_3, C-14); ESIMS m/z 713.36 $[M + Na]^+$.

Compound 4b: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.84 (3H, t, *J* = 7.6 Hz, H-1), 1.25 (2H, overlapped, H-2), 1.16 (2H, overlapped, H-3), 1.48 (1H, m, H-4a), 1.39 (1H, m, H-4b), 4.80 (1H, m, H-5), 1.31 (2H, overlapped, H-6), 1.02 (1H, m, H-7a), 0.80 (1H, overlapped, H-7b), 4.88 (1H, m, H-8), 4.40 (1H, m, H-9), 0.84 (1H, overlapped, H-10a), 0.79 (1H, overlapped, H-10b), 0.52 (1H, m, H-11a), 0.46 (1H, m, H-11b), 1.31 (2H, overlapped, H-12), 0.91 (2H, m, H-13), 0.68 (3H, t, *J* = 7.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 13.8 (CH_3, C-1), 22.3 (CH_2, C-2), 27.1 (CH_2, C-3), 33.9 (CH_2, C-4), 74.7 (CH, C-5), 30.4 (CH_2, C-6), 25.5 (CH_2, C-7), 74.4 (CH, C-8), 74.7 (CH, C-9), 26.9 (CH_2, C-10), 23.9 (CH_2, C-11), 30.3 (CH_2, C-12), 22.1 (CH_2, C-13), 13.5 (CH_3, C-14); ESIMS m/z 713.36 $[M + Na]^+$.

Compound 5a: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.68 (3H, t, *J* = 7.6 Hz, H-1), 1.05 (2H, m, H-2), 0.83 (2H, overlapped, H-3), 1.31 (1H, overlapped, H-4a), 1.29 (1H, overlapped, H-4b), 4.83 (1H, m, H-5), 1.46 (1H, overlapped, H-6a), 1.42 (1H, overlapped, H-6b), 1.44 (1H, overlapped, H-7a), 1.37 (1H, overlapped, H-7b), 5.07 (1H, m, H-8), 4.69 (1H, m, H-9), 0.99 (2H, overlapped, H-10), 0.59 (1H, overlapped, H-11a), 0.54 (1H, overlapped, H-11b), 0.82 (1H, overlapped, H-12a), 0.74 (1H, m, H-12b), 0.91 (2H, m, H-13), 0.67 (3H, t, *J* = 7.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 13.7 (CH_3, C-1), 22.1 (CH_2, C-2), 26.7 (CH_2, C-3), 33.4 (CH_2, C-4), 74.2 (CH, C-5), 29.9 (CH_2, C-6), 25.9 (CH_2, C-7), 74.5 (CH, C-8), 74.8 (CH, C-9), 27.6 (CH_2, C-10), 23.9 (CH_2, C-11), 30.9 (CH_2, C-12), 22.1 (CH_2, C-13), 13.7 (CH_3, C-14); ESIMS m/z 713.37 $[M + Na]^+$.

Compound 5b: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.81 (3H, t, *J* = 7.6 Hz, H-1), 1.16 (2H, overlapped, H-2), 0.93 (2H, overlapped, H-3), 1.21 (1H, overlapped, H-4a), 0.99 (1H, overlapped, H-4b), 4.53 (1H, m, H-5), 0.74 (2H, overlapped, H-6), 0.89 (1H, overlapped, H-7a), 0.77 (1H, overlapped, H-7b), 4.61 (1H, m, H-8), 5.00 (1H, m, H-9), 1.22 (1H, overlapped, H-10a), 1.16 (1H, overlapped, H-10b), 1.16 (2H, overlapped, H-11), 1.22 (2H, overlapped, H-12), 1.24 (2H, overlapped, H-13), 0.85 (3H, t, *J* = 7.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 13.8 (CH_3, C-1), 22.4 (CH_2, C-2), 27.1 (CH_2, C-3), 33.4 (CH_2, C-4), 73.9 (CH, C-5), 28.8 (CH_2, C-6), 22.6 (CH_2, C-7), 74.2 (CH, C-8), 74.4 (CH, C-9), 29.6 (CH_2, C-10), 29.4 (CH_2, C-11), 31.4 (CH_2, C-12), 22.4 (CH_2, C-13), 13.9 (CH_3, C-14); ESIMS m/z 713.36 $[M + Na]^+$.

Compounds 6. Pure triol enantiomer **6** was obtained from **4a** (2 mg, 0.003 mmol) by a hydrolysis reaction with NaOH (400 mg, 10 mmol) in MeOH (3 mL) and a few drops of H_2O. The mixture was stirred at rt overnight, then diluted with HCl (1% solution) and washed with brine, and the triol was extracted with 20 mL of BuOH (3×). The organic extract was dried over anhydrous Na_2SO_4 and then concentrated in vacuo. Reversed-phase HPLC (Luna 5 μm C18), eluting with MeOH/H_2O, 7:3, allowed triol purification. (2 mg, t_R 12.6 min). $[\alpha]^{25}_D$ +3.7 (*c* 0.009, MeOH); ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.92 (3H, t, *J* = 7.6 Hz, H-1), 1.43 (1H, overlapped, H-2a), 1.32 (1H, overlapped, H-2b), 1.32 (2H, overlapped, H-3), 1.46 (1H, m, H-4a), 1.42, (1H, overlapped, H-4b), 3.53 (1H, m, H-5), 1.72 (1H, m, H-6a), 1.39 (1H, overlapped, H-6b), 1.80 (1H, m, H-7a), 1.39 (1H, overlapped, H-7b), 3.34 (1H, m, H-8), 3.36 (1H, m, H-9), 1.60 (1H, overlapped, H-10a), 1.36 (1H, overlapped, H-10b), 1.54 (1H, m, H-11a), 1.33 (1H, overlapped, H-11b), 1.32 (2H, overlapped, H-12), 1.34 (2H, overlapped, H-13), 0.92 (3H, t, *J* = 7.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 14.1 (CH_3, C-1), 28.7 (CH_2, C-2), 32.8 (CH_2, C-3), 37.9 (CH_2, C-4), 72.9 (CH, C-5), 34.6 (CH_2, C-6), 29.6 (CH_2, C-7), 76.3 (CH, C-8), 75.9 (CH, C-9), 33.4 (CH_2, C-10), 26.4 (CH_2, C-11), 32.8 (CH_2, C-12), 23.7 (CH_2, C-13), 14.1 (CH_3, C-14); ESIMS m/z 247.22 $[M + H]^+$ HREIMS m/z 247.2266 (calcd for $C_{14}H_{31}O_3$, 247.2268).

Compound 9. Pure triol enantiomer **9** (3.6 mg, t_R 14.4 min) was obtained by the same reaction and purification process (reversed-phase HPLC MeOH/H_2O, 7:3) as for **6** reported above, using 4.8 mg (0.007 mmol) of **5a**. $[\alpha]^{25}_D$ +1.5 (*c* 0.015, MeOH); ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.92 (H-1, t, *J* = 7.6 Hz 7.6, 3H), 1.44 (1H, overlapped, H-2a), 1.33 (1H, overlapped, H-2b), 1.32 (2H, overlapped, H-3), 1.46 (1H, m, H-4a), 1.42, (1H, overlapped, H-4b), 3.55 (1H, m, H-5), 1.60 (1H, overlapped, H-6a), 1.53 (1H, overlapped, H-6b), 1.70 (1H, overlapped, H-7a), 1.48 (1H, overlapped, H-7b), 3.37 (1H, m, H-8), 3.38 (1H, m, H-9), 1.60 (1H, overlapped, H-10a), 1.36 (1H, overlapped, H-10b), 1.54 (1H, overlapped, H-11a), 1.33 (1H, overlapped, H-11b), 1.32 (2H, overlapped, H-12), 1.34 (2H, overlapped, H-13), 0.92 (3H, t, *J* = 7.6 Hz 7.6, H-14); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 14.1 (CH3, C-1), 28.7 (CH_2, C-2), 32.8 (CH_2, C-3), 37.9 (CH_2, C-4), 72.1 (CH, C-5), 34.3 (CH_2, C-6), 29.3 (CH_2, C-7), 75.6 (CH, C-8), 75.6 (CH_2, C-9), 33.4 (CH_2, C-10), 26.4 (CH_2, C-11), 32.8 (CH_2, C-12), 23.7 (CH_2, C-13), 14.1 (CH_3, C-14); ESIMS m/z 247.25 $[M + H]^+$; HREIMS m/z 247.2267 (calcd for $C_{14}H_{31}O_3$, 247.2268).

Cyclization of Enantiomerically Pure 6. Compound **6** (10.4 mg, 0.0422 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (5 mL), and trimethyl orthoacetate (7 μL, 1.2 equiv) and pyridinium *p*-toluenesulfonate (PPTS) (0.100 mg, 0.01 equiv) were added at rt. After 15 min, $BF_3{\cdot}Et_2O$ (0.53 μL, 0.1 equiv) was added at 0 °C, and when TLC showed the absence of the orthoester, the mixture was quenched with H_2O/acetone (9:1) and the solvent was removed in vacuo. Cyclized product **8** was obtained quantitatively. NMR data: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.87 (3H, t, *J* = 7.6 Hz, H-1), 1.30 (2H, overlapped, H-2), 1.31 (1H, overlapped, H-3a), 1.25 (1H, m, H-3b), 1.56 (1H, m, H-4a), 1.38, (1H, overlapped, H-4b), 3.88 (1H, m, H-5), 1.98 (1H, m, H-6a), 1.44 (1H, overlapped, H-6b), 1.96 (1H, m, H-7a), 1.58 (1H, overlapped, H-7b), 3.98 (1H, m, H-8), 4.86 (1H, m, H-9), 1.53 (2H, overlapped, H-10), 1.27 (2H, overlapped, H-11), 1.28 (2H, overlapped, H-12), 1.30 (2H, overlapped, H-13), 0.92 (3H, t, *J* = 7.6 Hz, H-14), 2.08 (3H, s, $COOCH_3$); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 14.1 (CH_3, C-1), 22.8 (CH_2, C-2), 28.4 (CH_2, C-3), 35.5 (CH_2, C-4), 79.6 (CH, C-5), 32.4 (CH_2, C-6), 28.4 (CH_2, C-7), 79.3 (CH, C-8), 75.7 (CH, C-9), 31.1 (CH_2, C-10), 31.9 (CH_2, C-11), 25.6 (CH_2, C-12), 22.8 (CH_2, C-13), 14.1 (CH_3, C-14), 21.3 (CH_3, $COOCH_3$), 171.4 (C, $COOCH_3$); ESIMS m/z 271.22 $[M + H]^+$; HREIMS m/z 271.2270 (calcd for $C_{16}H_{31}O_3$, 271.2273).

Cyclization of Enantiomerically Pure 9. Using 10.5 mg of **7** (0.0422 mmol, 1.0 equiv), compound **11** was obtained under the same conditions as for **8** reported above. NMR data: ^{1}H NMR (700 MHz, $CDCl_3$, *J* in Hz) δ 0.87 (3H, t, *J* = 7.6 Hz, H-1), 1.30 (2H, overlapped,

DOI: 10.1021/acs.jnatprod.7b00397
J. Nat. Prod. XXXX, XXX, XXX−XXX

H-2), 1.31 (1H, overlapped, H-3a), 1.25 (1H, m, H-3b), 1.56 (1H, overlapped, H-4a), 1.40, (1H, overlapped, H-4b), 3.81 (1H, m, H-5), 1.92 (1H, overlapped, H-6a), 1.44 (1H, overlapped, H-6b), 1.89 (1H, overlapped, H-7a), 1.62 (1H, overlapped, H-7b), 3.91 (1H, m, H-8), 4.87 (1H, m, H-9), 1.56 (2H, overlapped, H-10), 1.27 (2H, overlapped, H-11), 1.28 (2H, overlapped, H-12), 1.29 (2H, overlapped, H-13), 0.87 (3H, t, J = 7.6 Hz, H-14), 2.09 (3H, s, COOCH_3); ^{13}C NMR ($CDCl_3$, signals assigned from HSQC data) δ 14.1 (CH_3, C-1), 22.9 (CH_2, C-2), 28.5 (CH_2, C-3), 35.5 (CH_2, C-4), 80.1 (CH, C-5), 31.2 (CH_2, C-6), 27.8 (CH_2, C-7), 79.6 (CH, C-8), 75.9 (CH, C-9), 31.2 (CH_2, C-10), 31.9 (CH_2, C-11), 25.2 (CH_2, C-12), 22.6 (CH_2, C-13), 14.1 (CH_3, C-14), 21.3 (CH_3, COOCH_3), 171.4 (C, $COOCH_3$); ESIMS m/z 271.22 $[M + H]^+$; HREIMS m/z 271.2269 (calcd for $C_{16}H_{31}O_3$, 271.2273).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00397.

Characterization data and ^{1}H and ^{13}C NMR spectra for compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was funded by Regione Campania under POR Campania FESR2007-2013−O.O. 2.1 (FarmaBioNet), EU project Bluegenics (grant number 311848), and Università di Napoli Federico II under the STAR project (SeaLEADS).

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DOI: 10.1021/acs.jnatprod.7b00397
J. Nat. Prod. XXXX, XXX, XXX−XXX