Bioactivity Profiling Using HPLC/Microtiter-Plate Analysis: Application to a New Zealand Marine Alga-Derived Fungus, *Gliocladium* sp.

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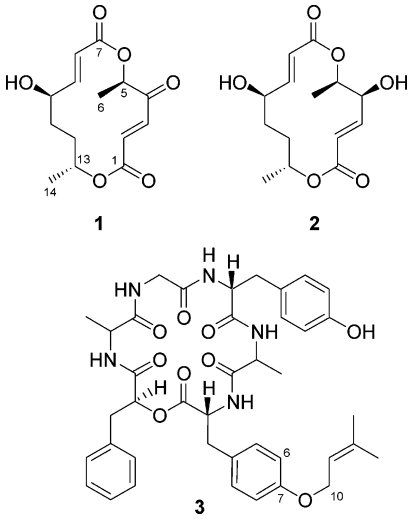
Using HPLC/microtiter-plate-based generation of activity profiles the extract of a marine alga-derived fungus, identified as *Gliocladium* sp., was shown to contain the known strongly cytotoxic metabolite 4-keto-clonostachydiol (**1**) and also clonostachydiol (**2**) as well as gliotide (**3**), a new cyclodepsipeptide containing several D-amino acids. The absolute configuration of **1** was elucidated by reduction to **2**, and two further oxidized derivatives of clonostachydiol (**5**, **6**) were prepared and evaluated for biological activity.

Fungi from the marine environment are well-known producers of novel and pharmacologically active secondary metabolites.[1] Chemically productive strains have been isolated from a wide variety of marine substrates, e.g., sponges,[2] algae,[3] driftwood,[4] and sediment.[5] During the investigation of fungi derived from marine algae and driftwood collected around the South Island of New Zealand, the highly cytotoxic and antibacterial extract of a *Gliocladium* sp., cultured from a thallus sample of the macroalga *Durvillaea antarctica*, attracted our attention. Fungi of this genus have been described before as producers of structurally diverse metabolites, e.g., glycosylated polyketides,[6] cytotoxic diketopiperazines,[7] and cyclopeptides.[8]

Results and Discussion

To identify which of the components (HPLC analysis) of the *Gliocladium* extract was responsible for the biological activities observed, a "bioactivity chromatogram" was generated. To achieve this, the eluent of an analytical HPLC separation of the extract (100 μg) was collected over 22 min into a 96-well microtiter plate (88 × 0.25 mL fractions), and a daughter plate was then prepared (5 μL transferred from each well of the master plate; equivalent to a total of 2 μg of extract). This daughter microtiter plate, after drying, was inoculated with P388 cells and incubated for 3 days. The distribution of the bioactivity across the plate was revealed using the yellow MTT dye, which is reduced by living cells to the purple MTT formazan.[9] Correlating the plate-reader output against well position (each well is equivalent to a 15 s fragment of the HPLC) allows generation of a HPLC-bioactivity profile. Typically, under the HPLC conditions used, peak widths are around 30−60 s, which corresponds to two to four consecutive wells on the microtiter plate, allowing ready identification of the bioactive peak(s) in an HPLC chromatogram (Figure 1). In our experience it has been useful to compare the bioactivity profile to both the ELSD data and the UV-DAD output, as the ELSD output reflects the actual mass of compound in a peak and is not biased toward compounds with strong UV chromophores.

In the same way a microtiter plate using 500 μg of extract was prepared and tested against *Bacillus subtilis*. For an appropriate response from this organism it is necessary to use a considerably higher concentration of sample per well. Both the resulting "cytotoxicity chromatogram" and the "anti-*Bacillus* chromatogram" (Figure 1) showed the bioactivity to be located around a retention time of 12.5 min, correlating to one of the major HPLC peaks.



The cytotoxic compound (**1**) and the other two major compounds (**2** and **3**) were then isolated by semipreparative HPLC. Evaluation of NMR and MS data showed the active compound to be the macrodiolide 4-keto-clonostachydiol (**1**), a structure recently patented for its cytotoxic and antibacterial activities.[10] Compound **2** was, by analysis of the NMR and MS data, unequivocally identified as clonostachydiol,[11] a related fungal macrodiolide. Comparison of NMR data and the optical rotation of **2** with values published for the natural product and two diastereomers[12,13] confirmed the stereochemistry to be that of the previously described natural product. The relative and absolute configuration of **1** was established by reduction of the compound with $NaBH_4/CeCl_3$. This reaction resulted in two products, **4a** and **4b** (Scheme 1). Compound **4a** was, by HPLC retention time, ^{1}H NMR, and optical rotation, identical to clonostachydiol **2**, thus indicating for **1** a stereochemistry analogous to that of **2**, i.e., (5*R*,10*R*,13*R*). Compound **4b** was the previously undescribed 4-*epi*-clonostachydiol.

In comparison with 4-keto-clonostachydiol (**1**), which is strongly cytotoxic against P388 cells (IC_{50} 0.55 μM) and active against *Bacillus subtilis* and the fungi *Trichophyton mentagrophytes* and *Cladosporium resinae*, clonostachydiol (**2**) is significantly less cytotoxic and does not possess antimicrobial activity (Table 1). To explore the biological activity potential of related structures, **2** was oxidized with Dess−Martin periodinane (Scheme 2). Under these conditions the alcohol at C-10 was readily oxidized to the ketone, while oxidation at C-4 proceeded only slowly. This made it possible to isolate compound **5**, an isomer of 4-keto-clonostachydiol (**1**) with the oxidation states of C-4 and C-10 interchanged. Full oxidation

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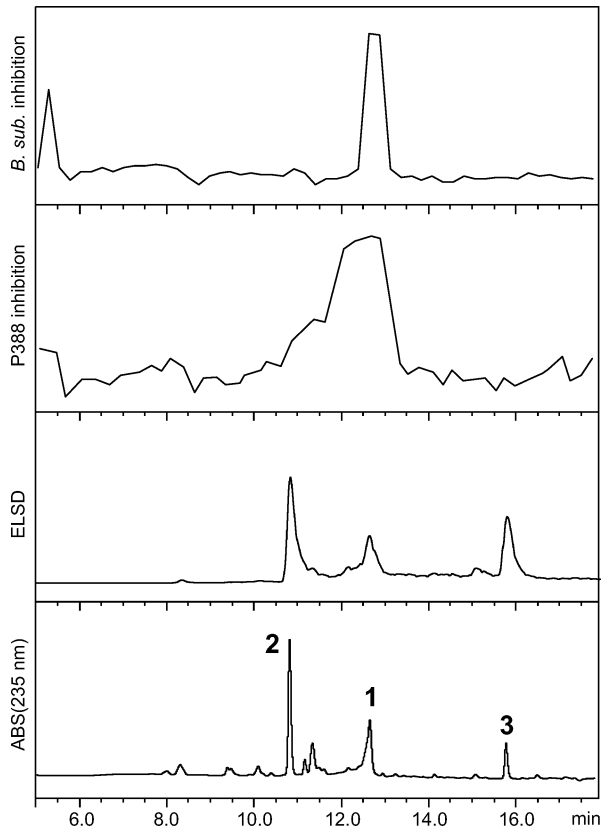


Figure 1. HPLC of the *Gliocladium* extract: UV absorption at 235 nm, ELSD signal, cytotoxicity against P388 cells, and anti-*B. subtilis* activity.

Scheme 1. Reduction of 4-Keto-clonostachydiol (**1**)

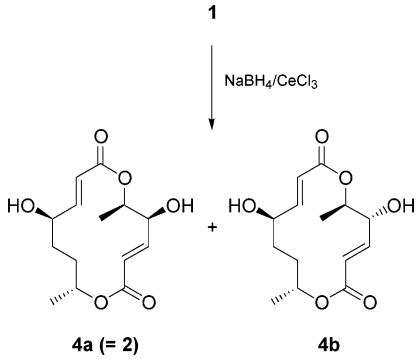


Table 1. Cytotoxic and Antimicrobial[a] Activities of Compounds **1**, **2**, **5**, and **6**

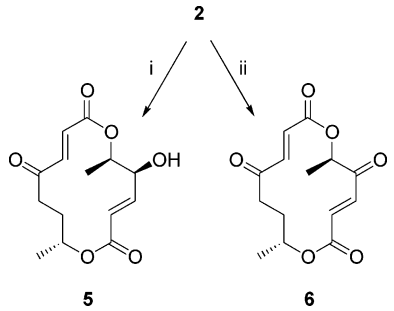
	IC_{50}(P388) [μM]	*B. subtilis*	*T. ment.*	*C. resinae*
1	0.55	6	2	1
2	25	not active	not active	not active
5	1.9	3	3	5
6	3.9	1	1	not active

[a] Widths of inhibition zones (in mm) around disks loaded with 40 μg of compound.

of **2** to the diketone **6** was achieved with MnO_2. Both oxidation products of clonostachydiol are antimicrobial and markedly cytotoxic (Table 1), but less so than the natural product **1**. This suggests that the presence of at least one α,β-unsaturated ketone moiety is essential for the biological activity. The reduced activity of **6**, in comparison to **1** and **5**, may be due to the nonpolar nature of this compound, which is barely soluble in water.

HRESIMS indicated a *m*/*z* of 742.3424, corresponding to a molecular formula of $C_{40}H_{48}N_5O_9$, for the $[M + H]^+$ ion of compound **3**, which was named gliotide after the producing

Scheme 2. Oxidation of Clonostachydiol (**2**): (i) Dess−Martin, (ii) MnO_2



organism. The ^{1}H NMR spectrum was characteristic of a peptide, with six partially overlapping signals for the α-protons of the α-amino acid and the α-hydroxy acid residues between 3.8 and 5.2 ppm. A TOCSY experiment revealed the presence of five amino acid units, namely, glycine, two alanine, and two tyrosine residues and an α-hydroxy acid, 3-phenyllactic acid. Long-range H,C-correlations of the α-protons to the carbonyl carbons of the adjacent amino acid provided the information necessary for assembling the sequence of the amino acids and the 3-phenyllactic acid (Figure 2). The carbonyl resonances were narrowly spaced in the range of 171 to 175 ppm, so the IMPRESS technique[14] was applied, as this approach provides better resolution in the F1 dimension than a conventional HMBC experiment. This established that the peptide had the sequence phenyllactoyl-Ala-Gly-Tyr-Ala-Tyr with the ring-closing ester bond between the hydroxyl group of the phenyllactic acid and the second tyrosine residue. The ^{1}H NMR resonances of two singlet methyl groups, one double-bond proton, and an oxymethylene group were also identified and assigned to an *O*-prenyl residue. This *O*-prenyl group was attached to the *C*-terminal tyrosine unit, as the methylene protons (H-10) showed an HMBC correlation to C-7 as well as an ROE interaction with one of the aromatic proton signals (H-6/H-8) of this amino acid (Figure 2). A further IMPRESS experiment was required to establish that these aromatic protons did not belong to one of the two other aromatic groups.

Acid hydrolysis of **3** and Marfey's analysis[15] of the amino acid mixture showed that both tyrosine units were of D-configuration, while one of the alanines was D- and the other L-configured. The 3-phenyllactic acid was found to have an *S*-configuration by reacting the acid hydrolyzate of **3** with (*S*)-MTPA-Cl and HPLC comparison of the resulting (*R*)-MTPA-phenyllactic acid ester with standards. The only stereochemical detail remaining unclear was which alanine was of L- and which was of D-configuration. The conformational flexibility of the molecule limits the value of the observed ROE correlations when applied to potential molecular modeling studies of the two stereochemical options, while attempts to crystallize the compound have been as yet unsuccessful. Gliotide (**3**) was inactive in the cytotoxicity and antimicrobial assays.

The value of the microtiter-plate-based system lies in the simple generation of bioactivity profiles of the HPLC chromatogram of

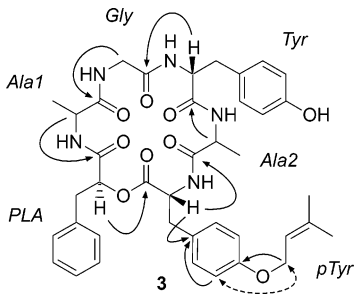


Figure 2. Long-range H,C-couplings (arrows) and one ROE interaction (dashed arrow) define the amino acid sequence of **3** and the position of the prenyl group.

Table 2. NMR Data of Gliotide (**3**; 500 MHz, MeOH-d_4)

position	δ_C		δ_H	position	δ_C		δ_H
Ala1				pTyr[a]			
1	174.7			1	173.8		
2	50.3		4.39 dd 7.0, 7.0	2	57.6		4.48 dd 9.1, 5.3
3	18.2		1.09 d 7.2	3	37.3	a	3.05 m
Gly						b	2.92 dd 13.5, 9.2
1	172.5			4	129.4		
2	43.9	a	3.88 d 16.2	5/9	131.8		7.00 d
		b	3.47 d 16.2	6/8	116.3		6.58 d 8.6
Tyr				7	159.9		
1	173.6			10	66.0		4.43 d 6.5
2	58.2		4.31 m	11	121.5		5.34 bt 6.1
3	37.2	a	3.01 m	12	138.9		
		b	2.82 dd 14.1, 9.7	13	18.4		1.64 s
4	129.1			14	26.1		1.67 s
5/9	131.5		7.00 d m	PLA[b]			
6/8	116.6		6.68 d 8.6	1	171.2		
7	157.7			2	76.1		5.19 dd 5.7, 4.2
Ala2				3	38.8	a	3.03 m
1	174.5					b	2.74 dd 14.3, 3.9
2	50.8		4.31 m	4	136.9		
3	18.9		1.35 d 7.2	5/9	131.5		6.88 bd 7.7
				6/8	129.5		7.19 m
				7	128.4		7.18 m

[a] *O*-Prenyltyrosine. [b] 3-Phenyllactic acid.

crude extracts. This then allows strategic decisions to be made for the subsequent purification steps. The bioactivity profile can also be used to assist in the dereplication of extracts containing known bioactive compounds.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter and UV spectra on a GBC UV/vis 920 spectrometer. NMR spectra were recorded on a Varian (UNITY INOVA) AS-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons (δ_H 3.30 and 2.60 for MeOH-d_4 and DMSO-d_6, respectively) and the solvent carbons (δ_C 49.3 and 39.6 for MeOH-d_4 and DMSO-d_6, respectively) as internal references. HRESIMS were acquired using a Micromass LCT TOF mass spectrometer, which was also used for LC-MS. HPLC was performed on a Dionex system equipped with an ISCO Foxy Jr. sample collector using a reversed-phase analytical column (Phenomenex Prodigy C18, 4.6 × 250 mm, 5 μm) with photodiode array (DAD) and evaporative light scattering detection (ELSD; Alltech). Solvents used for extraction and isolation were distilled prior to use.

Biological Assays. For the P388 cytotoxicity assays the following medium was used: MEM, fetal calf serum (10%), penicillin (266 u/mL), streptomycin (132 μg/mL), L-glutamine (2 mM), $NaHCO_3$ (2.2 g/L), HEPES (7.4 mM). For the *Bacillus subtilis* assay a RPMI-1640 medium was used, supplemented with L-glutamine (2 mM) and MOPS (34.53 g/L) and adjusted to pH 7 with NaOH (1 M). Cytotoxicity against P388 cells and antimicrobial activities were measured using standard protocols.[16]

Fungus. A small cube from the interior of the alga *Durvillaea antarctica*, collected at the coast of Tauranga Bay, New Zealand, was placed on filtered seawater agar (fresh filtered seawater, agar 15 g/L, chloramphenicol 100 μg/L, ampicillin 100 μg/L, and streptomycin sulfate 50 μg/L). After cultivation at 15 °C for 7 days, hyphae growing on the substrate were transferred onto seawater potato dextrose agar (fresh filtered seawater, Gibco PDA 39 g/L, chloramphenicol 100 μg/L, ampicillin 100 μg/L, and streptomycin sulfate 50 μg/L). One of the isolates was identified as a species of *Gliocladium* due to the densely penicillate conidiophores bearing one-celled, ellipsoidal, smooth-walled conidia in slimy heads.[17]

Extraction. For chemical investigation the *Gliocladium* sp. was grown at 26 °C in half-strength potato dextrose broth (PDB; 3 × 500 mL). The cultures were agitated on a shaker (first 14 days of growth) and then left growing under static conditions (16 more days). The cultures were filtered, and broth and cells were extracted exhaustively with ethyl acetate. The combined organic phases were concentrated, partitioned against water, and dried to give a crude extract, which was subsequently partitioned between petroleum ether and methanol/water (9:1). The resulting methanol/water phase was concentrated to dryness (184 mg).

Generation of Bioactivity Profiles. An aliquot of the extract (100 μg) was analyzed by HPLC (solvents: (A) H_2O + 0.05% TFA, (B) MeCN; gradient: 0 min 10% B, 2 min 10% B, 14 min 75% B, 24 min 75% B, 26 min 100% B; flow: 1 mL/min; 40 °C). The eluent from the DAD was split in a 1:10 ratio between the ELSD and the fraction collector configured to collect into a 96-well microtiter plate (15 s/well). A total of 88 wells were collected (2.5−24.5 min). A daughter plate was prepared by transferring an aliquot (5 μL) from each well of the master plate. After complete evaporation of the solvent the wells in the plate were analyzed for activity against P388 murine leukemia cells as follows: aliquots of P388 cell suspension (150 μL; 1.26 × 10^4 cells) were added to each well except wells H1 and H2, which as a positive control were loaded with medium (150 μL) only. After incubation (36 °C, 3 days) an MTT solution was added to each well (20 μL, 3.8 mg/mL in PBS) and incubated (4 h at 36 °C). To dissolve the formazan product, HCl in isopropanol (170 μL, 0.08 M) was added to each well.[9] Cell viability was assessed by measuring the absorption of each well at 540 nm, subtracting the absorption at 690 nm and using the negative control (wells H1 and H2) and the analyte free cell control (wells H3−H5) as 0% and 100% growth reference, respectively.

For the antibacterial profile a second microtiter plate was prepared using identical conditions, but injecting 500 μg of extract. After evaporation of the solvent this plate was tested directly for activity against *B. subtilis*: 200 μL of a *B. subtilis* suspension (OD(610 nm) = 0.04) was added to each well except the negative control wells H1 and H2, which were loaded with 200 μL of medium each. After incubation (24 h; 30 °C) a resazurin solution (0.2 mg/mL PBS; 30 μL) was added to all wells except H4 and the plate further incubated (15 min; 30 °C). Cell viability was assessed the same way as described above for the cytotoxicity profile, but measuring the absorption at 600 nm and subtracting the absorption at 690 nm.

Isolation. The extract was subjected to semipreparative HPLC (Phenomenex Luna C18, 10 × 250 mm, 5 μm; H_2O + 0.05% TFA (A), MeCN (B); 0 min 20% B, 13 min 75% B; 5 mL/min; 40 °C). Clonostachydiol (**2**; 13.2 mg), 4-keto-clonostachydiol (**1**; 8.2 mg), and gliotide (**3**; 7.1 mg) were eluted after 5.9, 8.4, and 12.5 min, respectively.

4-Keto-clonostachydiol (1): colorless oil; $[\alpha]^{20}_D$ +49 (*c* 0.33, MeOH); ^{1}H and ^{13}C NMR data were consistent with those reported in the literature;[10] for full 1D and 2D NMR data, see Supporting Information; ESIMS *m/z* 283.1 $[M + H]^+$.

Clonostachydiol (2): white solid; $[\alpha]^{20}_D$ +90 (*c* 1.0, MeOH) (lit.[10] +103, *c* 1.0 MeOH); ^{1}H and ^{13}C NMR data and results from CIGAR

and COSY were identical with, or consistent with, reported data;[11] ESIMS *m/z* 285.2 $[M + H]^+$.

Gliotide (3): white solid; $[\alpha]^{20}_D$ +6.6 (*c* 0.33, MeOH); for ^1H and ^{13}C NMR data (in MeOH-d_4), see Table 2; for additional NMR data (HMBC, COSY, ROESY data in MeOH-d_4; ^1H, ^{13}C, and ROESY in DMSO-d_6), see Supporting Information; HRESIMS *m/z* 742.3424 $[M + H]^+$ (calcd for $C_{40}H_{48}N_5O_9$, 742.3452).

Preparation and Analysis of Marfey Derivatives. Compound **3** (0.8 mg) was hydrolyzed by heating (110 °C for 24 h) in HCl (6 M; 1 mL). After cooling, the solution was evaporated to dryness and redissolved in H_2O (100 μL). A 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent, N^{α}-(2,4-dinitro-5-fluorophenyl)-L-alaninamide)[15] in acetone was added to an aliquot (50 μL) of the acid hydrolysate solution (or to 50 μL of a 50 mM solution of the respective amino acid). After addition of $NaHCO_3$ solution (1 M; 20 μL) the mixture was incubated (1 h at 40 °C). The reaction was stopped by addition of HCl (2 M; 10 μL), the solvents were evaporated to dryness, and the residue was redissolved in MeOH−H_2O (1:1; 1 mL). An aliquot of this solution (10 μL) was analyzed by HPLC (Phenomenex Luna C18, 250 × 4.6, 5 μm; solvents: A H_2O + 0.05% TFA, B MeCN; linear gradient: 0 min 35% B, 30 min 45% B; 25 °C; 1 mL min^{-1}). Retention times (min) of the amino acid derivatives were as follows: L-Ala (6.4), D-Ala (7.6), L-Tyr (25.1), and D-Tyr (29.5).

Preparation and Analysis of (*R*)-MTPA Esters of 3-Phenyllactic Acid. To (*R*)- or (*S*)-3-phenyllactic acid (0.1 mg), synthesized from D- or L-phenylalanine, respectively,[18] or to dried acid hydrolysate of **3** (from 0.4 mg peptide) were added a solution of (*S*)-MTPA chloride (0.5 mg) in CH_2Cl_2 (0.5 mL), Et_3N (3 μL), and a small crystal of DMAP. After 3 h the solvent was evaporated, the residue dissolved in MeOH (0.5 mL), and the solution analyzed by HPLC (Phenomenex Luna C18, 250 × 4.6, 5 μm; solvents: A H_2O + 0.05% TFA, B MeCN; linear gradient: 0 min 10% B, 2 min 10% B, 14 min 75% B, 24 min 75% B; 40 °C; 1 mL min^{-1}). Retention times of the (*R*)- and (*S*)-3-phenyllactic acid esters were 17.86 and 18.00 min, respectively.

Reduction of 1. To a stirred solution of **1** (1.3 mg, 4.6 μmol) and $CeCl_3 \cdot 7H_2O$ (3 mg, 8.0 μmol) in MeOH (2.5 mL) was added $NaBH_4$ (2 mg, 53 μmol). After 5 min the solvent was evaporated in vacuo, dissolved in EtOAc (3 mL), and washed with HCl (0.5 M; 3 mL). Semipreparative HPLC on a C_{18} column yielded the two reduced products (0.3 mg of each). **4a**: $[\alpha]^{20}_D$ +100 (*c* 0.03, MeOH); ^1H NMR data and HPLC retention time identical to those of clonostachydiol (**2**). **4b** (4-*epi*-clonostachydiol): $[\alpha]^{20}_D$ +60 (*c* 0.03, MeOH); ^1H NMR (MeOH-d_4; 500 MHz) δ 6.84 (1H, dd, J = 15.8, 4.3 Hz, H-9), 6.77 (1H, dd, J = 15.7, 3.0 Hz, H-3), 6.14 (1H, dd, J = 15.7, 2.0 Hz, H-2), 5.85 (1H, dd, J = 15.8, 1.9 Hz, H-8), 5.25 (1H, qd, J = 6.5, 2.1 Hz, H-5), 5.12 (1H, m, H-13), 4.57 (1H, m, H-10), 4.40 (1H, m, H-4), 1.97 (1H, m, H_a-11), 1.70 (1H, m, H_b-11), 1.66 (1H, m, H_a-12), 1.50 (1H, m, H_b-12), 1.38 (3H, d, J = 6.5 Hz, H-6), 1.21 (3H, J = 6.5 Hz, H-14).

Preparation of 5. A solution of clonostachydiol (**2**; 3.7 mg; 0.013 mmol) and Dess−Martin periodinane (20 mg; 0.047 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 3 h. The mixture was washed twice with saturated $NaHCO_3$ solution and dried over Na_2SO_4. Purification by chromatography on a RP18 cartridge (MeOH/H_2O) yielded compound **5** (1.9 mg; 0.007 mmol; 54%) as a white solid: $[\alpha]^{20}_D$ +35 (*c* 0.1, MeOH); ^1H NMR ($CDCl_3$; 500 MHz) δ 6.87 (1H, d, J = 16.0 Hz, H-9), 6.63 (1H, d, J = 15.7 Hz, H-8), 6.60 (1H, dd, J = 15.9, 8.4 Hz, H-3), 5.74 (1H, d, J = 15.8 Hz, H-2), 5.02 (1H, m, H-5), 5.02 (1H, m, H-13), 3.99 (1H, t, J = 8.8 Hz, H-4), 2.64 (1H, m, H_a-11), 2.55 (1H, m, H_b-11), 2.10 (1H, m, H_a-12), 1.97 (1H, m, H_b-12), 1.49 (3H, d, J = 6.2 Hz, H-6), 1.23 (3H, d, J = 6.2 Hz, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 201.0 (C, C-10), 167.5 (C, C-7), 166.2 (C, C-1), 147.2 (CH, C-3), 140.0 (CH, C-9), 132.6 (CH, C-8), 126.8 (CH, C-2), 77.7 (CH, C-4), 74.1 (CH, C-5), 72.6 (CH, C-13), 40.2 (CH_2, C-11), 33.4 (CH_2, C-12), 20.4 (CH_3, C-14), 18.3 (CH_3, C-6); HRESIMS *m/z* 283.1147 $[M + H]^+$ (calcd for $C_{14}H_{19}O_6$, 283.1182).

Preparation of 6. A solution of clonostachydiol (**2**; 2.8 mg; 0.010 mmol) in CH_2Cl_2 (3 mL) was stirred with freshly prepared MnO_2 (30 mg) at room temperature for 24 h. Filtering and drying the reaction mixture gave **6** (1.6 mg; 0.006 mmol; 60%) as a colorless oil: $[\alpha]^{20}_D$ +25 (*c* 0.1, MeOH); ^1H NMR ($CDCl_3$; 500 MHz) δ 7.06 (1H, d, J = 16.0 Hz, H-9), 6.97 (1H, d, J = 16.6 Hz, H-3), 6.75 (1H, d, J = 16.0 Hz, H-8), 6.45 (1H, d, J = 16.6 Hz, H-2), 5.32 (1H, q, J = 7.3 Hz, H-5), 5.10 (1H, m, H-13), 2.71 (1H, ddd, J = 13.5, 8.9, 3.3 Hz, H_a-11), 2.53 (1H, ddd, J = 13.5, 8.9, 3.3 Hz, H_b-11), 2.03 (2H, m, H-12), 1.57 (3H, d, J = 7.0 Hz, H-6), 1.30 (3H, d, J = 6.6 Hz, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 198.5 (C, C-10), 197.8 (C, C-4), 165.0 (C, C-7), 163.4 (C, C-1), 138.3 (CH, C-9), 135.0 (CH, C-3), 129.6 (CH, C-8), 129.6 (CH, C-2), 75.7 (CH, C-5), 71.6 (CH, C-13), 37.4 (CH_2, C-11), 30.4 (CH_2, C-12), 17.9 (CH_3, C-14), 15.3 (CH_3, C-6); HRMS (APCI, pos.) *m/z* 281.1028 $[M + H]^+$ (calcd for $C_{14}H_{17}O_6$, 281.1025).

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Supporting Information Available: ^1H and ^{13}C NMR spectra as well as tabulated NMR data of compounds **1** and **3** and HPLC of the 3-phenyllactic-acid (*R*)-MTPA esters are available free of charge via the Internet at http://pubs.acs.org.

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