Natural Products

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Amplification of the Cotton Effect of a Single Chromophore through Liposomal Ordering—Stereochemical Assignment of Plakinic Acids I and J**

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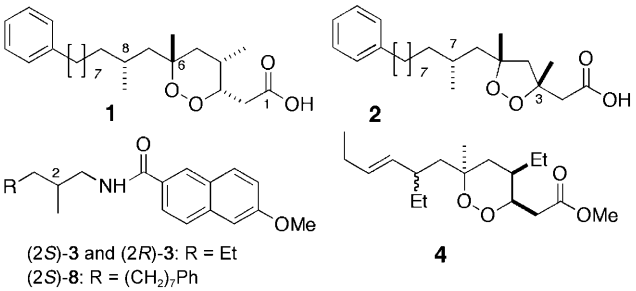
Circular dichroism (CD) is a powerful tool for assigning the configuration in natural products,[1] however, its use for acyclic molecules is limited by motional averaging that may reduce or eliminate Cotton effects. Recently, we reported the application of liposomal exciton-coupled CD (L-ECCD) for the determination of both the relative and absolute configuration of acyclic 1,*n*-diols ($n > 5$)[2] which exploited two properties: dual chromophores with very large electronic-charge-transition dipole moments and ordering of the long-chain carbon backbones within uniform unilamellar liposomes. This report now describes a sensitive technique—liposomal circular dichroism (L-CD)—for assigning the configurations at remote methyl-branched stereocenters in long-chain natural products at submicromol levels by exploiting a single chromophore appended to the chain terminus. L-CD reveals a general principle: simple Cotton effects (CEs) arising from perturbation of single chromophores may be amplified by constraining molecules within lipid bilayers. L-CD was applied to an outstanding problem: the configurational assignment of the remote stereocenters in methyl-branched polyketide peroxides (e.g., **1** and **2**) from marine sponges of the genera *Plakortis* and *Plakinastrella*.

The "remote-stereocenter problem" is illustrated with the enantiomeric naphthamides (Nps) (*S*)- and (*R*)-**3**. 2-Naphthamides exhibit strong charge-transfer bands that have been exploited in CD studies of chiral aminoalcohols.[1b] Despite the presence of a chiragenic center at C-2, (−)-(*S*)-**3** and (+)-(*R*)-**3**[3] showed essentially flatline CD spectra in MeOH (curve c of Figure 2) as a result of conformational averaging.



In contrast, when the compounds were formulated in highly uniform unilamellar liposomes from 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC; pressure extrusion through a 100 nm pore nylon membrane, $c(\text{DSPC}) = 2\ \text{mg mL}^{-1}$, lipid:naphthamide molar ratio 20:1, mean diameter $\phi \approx 30$ nm),[2] strong CEs appeared for (+)- and (−)-**3** (e.g., (*S*)-**3**: $\lambda = 206$ nm, $\Delta\varepsilon = +12.6$). Most importantly, the two spectra were mirror images of each other (curves a and b) and the effect was reproducible.

The antipodal CD curves suggested that the remote methyl branch induces asymmetric perturbation of the Np chromophore as a consequence of liposomal ordering of the chains, not as a result of diastereomeric interactions with the chiral polar head groups of DSPC. Consequently, L-CD appeared to be attractive for the interrogation of remote stereocenters in acyclic natural products.

With a method for CD amplification in hand, we turned our attention to plakinic acids I (**1**) and J (**2**), two ω-phenyl polyketide peroxides isolated from *Plakortis halichondroides* collected in the Bahamas. Compounds **1** and **2** are related to plakortin (**4**),[4a] also from *P. halichondriodes*, with submicromolar activity against the malaria parasite *Plasmodium falciparum*,[4b] and the cytotoxic plakinic and epiplakinic acids.[4c] Peroxides **1** and **2** showed differential inhibition of paired haplodeficient *lag1*Δ/*LAG1* strains of *S. cerevisae*,[5] suggesting interdiction of the yeast phosphoinositide pathway.

The absolute configurations of stereocenters around the 1,2-dioxane ring of **1** and the 1,2-dioxolane ring of **2** were determined conventionally by integrated ^{1}H NMR analysis including NOESY spectra and, for **1**, the Mosher ester[6] of a secondary alcohol obtained by hydrogenolysis (Pd/C, H_2) of **1** (for full characterization, see the Supporting Information).

The methyl-branched center C-8 of **1** is effectively insulated from the rest of the molecule by the quaternary center C-6. Force-field calculations of the staggered conformations around C-6–C-7 show they are equally populated.[7]

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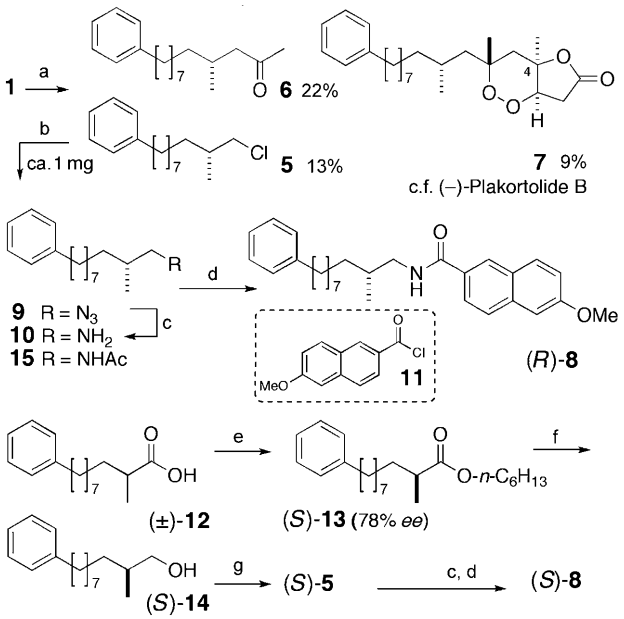
Lack of conformational constraints between C-6 and C-8 compromises the assignment of the C-8 configuration based on $^{2}J_{CH}$,$^{3}J_{CH}$ and NOE effects, but L-CD analysis bypassed this limitation as follows.

In order to segregate the C-8 stereocenter, we first cleaved the C-6–C-7 bond by using a ligand-directed Fe^{II}-promoted fragmentation of **1** to give three products (Scheme 1): **5** (13%



Scheme 1. Degradation of **1** and synthesis of authentic standards. Reagents and conditions: a) $FeCl_2$, CH_3CN/H_2O (degassed), RT, 45 min; b) NaN_3, DMF, 100 °C; c) H_2, Pd/C (hexane/EtOH); d) **11**, Et_3N, CH_2Cl_2; e) *Candida rugosa* lipase, 1-hexanol, cyclohexane, 50 h; f) $LiAlH_4$, Et_2O, RT; g) PPh_3, CCl_4.

yield), **6** (22%), and (−)-**7** (9%).[8] The formation of **5** is rationalized in Figure 1. The carboxylato–Fe^{II} species **I** promotes homolytic reduction of the O–O bond by single-electron transfer and the incipient *tert*-alkoxy radical **II** collapses by β scission along two paths, a and b. Compound **5** is formed by a "chloro-Fenton" reaction,[9] in which cleavage of the C–C bond along path a is followed by rebound and abstraction of Cl at the Fe center. Ketone **6** arises from the alternative β fragmentation path b, while (−)-**7** is formed from a different radical reaction.[10] The relative

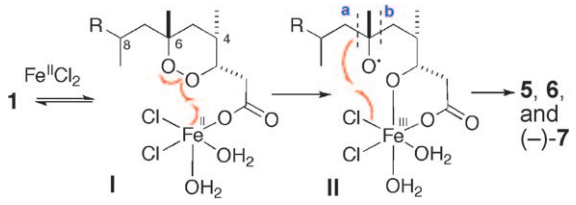


Figure 1. Proposed mechanism of the intramolecular "chloro-Fenton" reaction[9] of peroxide **1** with $FeCl_2$ in CH_3CN/water to give **5**–**7**. For clarity, an axial H_2O ligand has been removed from Fe in **I**.

configuration of (−)-**7** was secured from NOESY experiments.

Alkyl chloride **5** (ca. 1 mg) was transformed by a three-step sequence (Scheme 1): S_N2 displacement of the chloride by N_3^- to give **9**, which was hydrogenolyzed to primary amine **10** that was N-acylated with 6-methoxy-2-naphthoyl chloride (**11**) to give **8** (purified by HPLC, ca. 140 μg). Standard (*S*)-**8** was prepared as follows (Scheme 1): kinetic resolution of racemic 2-methyl-10-phenyldecanoic acid ((±)-**12**)[11] by esterification with 1-hexanol in the presence of *Candida rugosa* lipase[12] gave the (*S*)-*n*-hexyl ester **13** (78% *ee*), which was reduced to the corresponding alcohol (*S*)-**14** and sequentially transformed into (*S*)-**5** and, finally, (*S*)-**8** as described above. Optically pure naphthamides (*S*)- and (*R*)-**8** (>99% *ee*) were also prepared from (±)-**11** via enantiopure amines (*S*)- and (*R*)-**10** by using a modification of a method described earlier.[3]

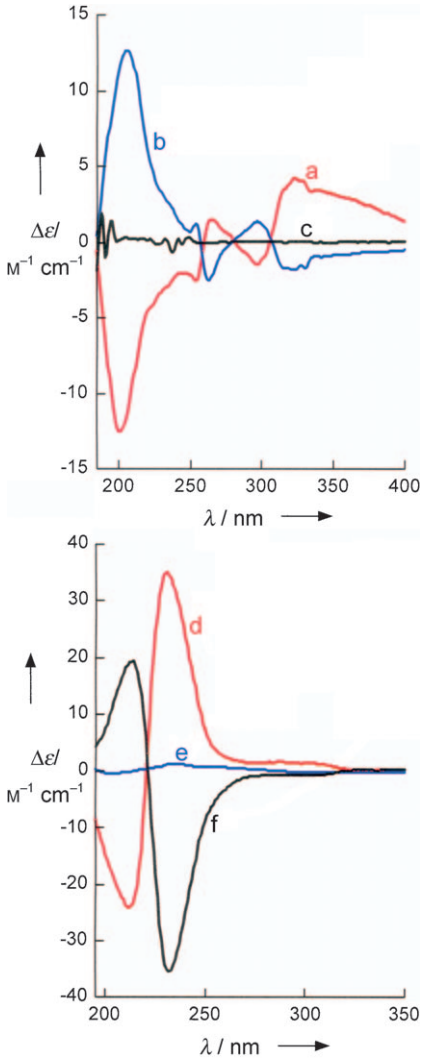


Figure 2. CD spectra of naphthamides (c = 0.23 mM, T = 23 °C). L-CD a) of (*R*)-**3**, b) of (*S*)-**3**; c(DSPC) = 2 mg mL^{-1}. c) CD of (*S*)-**3** in MeOH. L-CD d) of synthetic (*S*)-**8** (>99% *ee*), e) of (±)-**8**, f) of (*R*)-**8**, derived from **1**. See the Supporting Information for preparation of the liposomes.

The CD spectra of **8**, derived from either **1** or **2**, and standard (*S*)-**8** are shown in Figure 2. Whereas (*S*)-**8** and (±)-**8** measured in MeOH (see the Supporting Information), or (±)-**8** measured in DSPC liposomes gave only baseline CD spectra, the CD spectra of natural product derived **8** and synthetic (*S*)-**8** in DSPC liposomes showed strong bisignate CEs ($\lambda = 213$ nm, $\Delta\varepsilon = +20$; 232, −36, peak-to-trough, $A = 56$) of essentially equal magnitudes but opposite signs. Note, **10** and **5** have no significant dichroism in isotropic media and very weak rotations (e.g., synthetic (*S*)-**5**: $[\alpha]_D = -1.3\ \mathrm{deg\,cm^3\,g^{-1}\,dm^{-1}}$ ($c = 0.102\ \mathrm{g\,cm^{-3}}$, hexane). Therefore, the complete configurations of **1** and **2** are 3*S*,4*S*,6*R*,8*R* and 3*R*,5*R*,7*R*, respectively.[13]

The liposomes used in these L-CD experiments were very stable at room temperature; the CE of freshly prepared DSPC liposomes of (*S*)-**8** was evident within 20 min of sample preparation and unchanged after 44 days at room temperature. In order to better understand the origin of the L-CD signals, their temperature dependence was examined by measuring the CD spectra of liposomal preparations of (*S*)-**8** at $T = 4$–90 °C (Figure 3 A, B), which spans the gel phase transition temperature of DSPC liposomes ($T_c = 54.5$ °C).[14] The L-CD spectrum was largely unchanged from 4 to 40 °C, but above 40 °C the CE significantly decreased. At 90 °C, the CE had diminished in magnitude ($\lambda = 213$ nm, $\Delta\varepsilon = +8.32$; $\lambda = 232$, $\Delta\varepsilon = -4.72$) to less than 10 % of its value at 23 °C. The L-CD spectrum of (*S*)-**8** was partly restored upon cooling the sample to room temperature (Figure 3 C). These results are consistent with a reversible transition from a gel phase to a liquid phase in the liposome bilayer and an attendant disruption of liposomal ordering of the embedded methyl-branched alkyl chain of (*S*)-**8**.

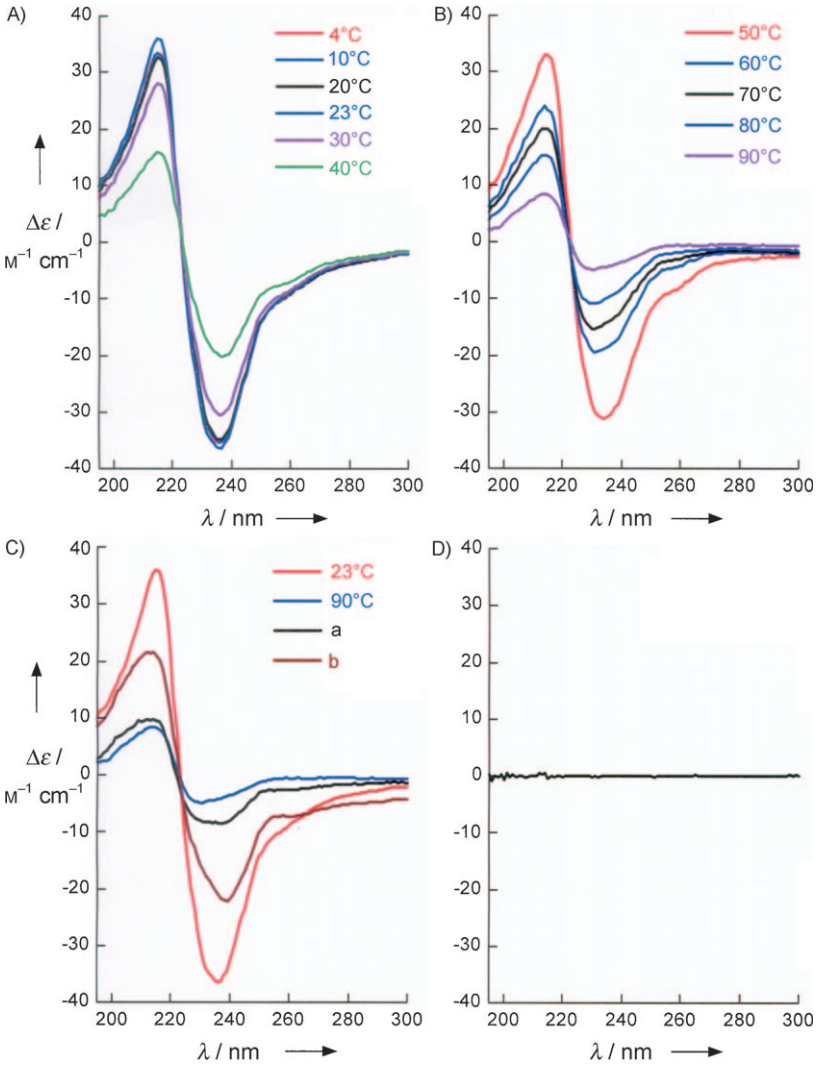


Figure 3. A), B) L-CD spectrum of (*S*)-**8** at different temperatures. C) Restoring of the L-CD spectrum of (*S*)-**8** upon cooling: a) 90 °C sample, cooled to 23 °C over 30 min; b) 90 °C sample, cooled to 23 °C, after 14 h. D) CD spectrum of (*S*)-**15** (78 % *ee*) with annealing.

Neither the chiral head groups of DSPC nor the terminal phenyl groups of **3** and **8** appear to be strongly involved in the observed L-CD CEs, however the presence of the naphthamide unit was critically important. For example, the L-CD spectrum of (*S*)-*N*-(2-methyl-10-phenyldecyl)acetamide (**15**), prepared by acetylation of (*S*)-**10** (Ac_2O, pyridine; see the Supporting Information) was essentially a baseline, even after repeated sonication and annealing at 60 °C (Figure 3 D). Similarly, the L-CD spectrum of the 6-methoxy-2-naphthamide of an achiral long-chain C_{14} amine (6-methoxy-*N*-myristyl-2-naphthamide, see **S11** in the Supporting Information) showed only a baseline under the same conditions.

The origin of amplified CEs in the L-CD spectra of **3** and **8** is more complex than simple intramolecular perturbation of the chromophore. Although it is clear that the L-CD CE originates in asymmetric perturbation of the naphthamide π-π* transitions by the remote stereogenic center bearing a β-methyl group, long-range intramolecular interactions are also operative.

The CEs arising from liposomal ordering of extended long chains appear also to be modulated by intermolecular π-π interactions of naphthamide chromophores in higher-order *J* aggregates within the bilayer. Evidence for delocalized (Frenkel) excitons[15] was most apparent in the L-CD spectra of (+)-**3** and (−)-**3** which revealed weaker, red-shifted transitions (e.g., $\lambda = 260$, 290, 320 nm; $\Delta\varepsilon < \pm 5$). The simplest interpretation of the L-CD would be that the major CE bands arise from 1,*n* pairwise exciton coupling of paired nearest-neighbor naphthamide groups ($n = 2$), held close by weak π-π interactions; however, quantitative analysis must await a more detailed photophysical description of L-CD.

In conclusion, the Cotton effects induced by liposomal circular dichroism of a single naphthamide chromophore—amplified by lipid ordering and second-order intermolecular

interactions—were used to assign the C-8 configuration of plakinic acids I (**1**) and J (**2**). The method is sensitive (the limit of detection for **8** is about 16 nmol) and suitable for "nanomol-scale" structure elucidation of natural products,[16] including other plakinic acids.[17]

The work presented herein demonstrates a specific case in application of L-CD—utilization of liposomes to amplify the CD spectrum of an acyclic chiral long-chain naphthamide for configurational assignment. L-CD should find general utility in the chiroptical analysis of acyclic methyl-branched long-chain polyketides where Cotton effects appear weak or even below the limits of detection.

Experimental Section

Experimental details, complete characterization of all synthetic products and general procedures can be found in the Supporting Information.

Plakinic acids I (**1**; 58 mg, 0.029% wet weight) and J (**2**; 47 mg, 0.023%) were isolated from the sponge *Plaktortis halichondroides*. **1**: colorless oil; $[\alpha]_D^{24} = -113$ deg cm^3 g^{-1} dm^{-1} ($c = 0.0437$ g cm^{-3}, $CHCl_3$), UV (MeOH): $\lambda_{max} = 260$ ($\varepsilon = 286$), 268 nm (200), FT-IR (ATR, neat): $\tilde{\nu} = 2921$, 2854, 1712, 1452, 1374, 1291, 1026, 738, 691 cm^{-1}; ^{1}H and ^{13}C NMR data: see Table S1 in the Supporting Information; HR-EI-MS: m/z: 404.2928 $[M]^+$, calcd 404.2921 for $C_{25}H_{40}O_4$. **2**: colorless oil; $[\alpha]_D^{24} = -43.4$ deg cm^3 g^{-1} dm^{-1} ($c = 0.0442$ g cm^{-3}, $CHCl_3$); UV (MeOH): $\lambda_{max} = 261$ ($\varepsilon = 183$), 261 nm (260); FT-IR (ATR, neat): $\tilde{\nu} = 2920$, 2850, 1715, 1452, 1371, 1305, 1218, 743, 697 cm^{-1}; ^{1}H and ^{13}C NMR: see Table S3 in the Supporting Information; HREIMS: m/z: 390.2773 $[M]^+$, calcd 390.2765 for $C_{24}H_{38}O_4$.

$FeCl_2$-promoted fragmentation of **1** and **2**: $FeCl_2 \cdot 4H_2O$ (AR grade, purified by washing with 6 M HCl) was prepared as a stock solution (1 M) in degassed, distilled H_2O. A solution of **1** (7.0 mg, 17.3 μmol) in CH_3CN/H_2O (8:2, 1.0 mL, de-aerated, N_2 purge, 40 min) was treated with this stock solution (74 μL, 51.9 μmol) and stirred under an atmosphere of N_2 for 30 min, before quenching with 4 drops of aqueous citric acid (1.0 M). The mixture was vortexed with hexane (4 volumes) for 1 min, and centrifuged to separate the organic layer. The aqueous layer was washed twice with hexane and the combined hexane layers were concentrated under reduced pressure. The residue was purified on a short pipet column (silica, 1:9, 2:8, and 3:7 EtOAc/hexanes) to give (*R*)-**5** as a colorless oil (0.89 mg, 13%), followed by **6** (1.5 mg, 22%) and (−)-**7** (0.59 mg, 9%). Treatment of **2** under the same conditions also gave (*R*)-**5** (19%).

Preparation of DSPC liposomes and L-CD measurements: Liposomal naphthamides were prepared using a modification of the previously described method.[2] Briefly, a solution of DSPC (2 mg mL^{-1} in $CHCl_3$) was added to a solution of the naphthamide in $CHCl_3$, contained in a 25 mL round bottom flask, and the solution was "shell-evaporated" under reduced pressure using a rotatory evaporator. To the dried residue was added HPLC-grade H_2O (2 mL) and the mixture was subjected to the following treatment: sonication for 2 min, heating (60 °C), and cooling (RT), repeated twice. Uniform liposomes were prepared from this mixture by repeated extrusion (25 times) through a 100 nm polycarbonate membrane secured between two 0.5 mL gas-tight syringes (Liposofast, Avestin, Toronto, Canada). CD measurements were carried out on the resulting clear preparations using the following parameters: $T = 23$ °C; sensitivity: 100 mdeg; scanning speed: 50 nm min^{-1}; wavelength from 180 to 400 nm; 15 accumulations. The CD spectra were subtracted from the blank spectra measured on DSPC liposomes prepared without added naphthamide. Sample concentrations were determined from absorbance at $\lambda = 238$ nm in MeOH. See the Supporting Information (Table S4) for tabulations of λ and $\Delta\varepsilon$ values for **3** and **8**.

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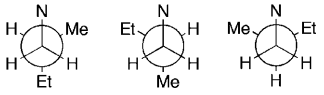
[7] Force field calculation of the relative energies of the three *gauche* conformers for a simple model of **1** (MMFF94, Spartan 04) show they differ by less than 0.3 kcal mol^{-1} ($E = 29.5$, 29.6, and 29.8 kcal mol^{-1}, respectively). Similarly, minimized C-1–C-2 *gauche* conformers of 6-methoxy-*N*-(2-methylbutyl)-2-naphthamide (see **3**) have similar energies: **A1**: 12.8; **A2**: 12.0; **A3**: 12.1 kcal mol^{-1}.



A1 **A2** **A3**

[8] Compound **7** is diastereomeric with plakortolide B, from *Plakinastrella onkodes*: P. A. Horton, R. E. Longley, M. Kelly-Borges, O. J. McConnell, L. M. Ballas, *J. Nat. Prod.* **1994**, *57*, 1374–1381.

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[10] Compound **2** also gave **5** under the same conditions. Evidence for ligand-directed free-radical cleavage is found in the treatment of the methyl ester of **2** with $FeCl_2$ which fails to give **5**. Formation of the bicyclic peroxy-γ-lactone (−)-**7** from **1** probably proceeds by a different radical pathway involving a complex of Fe^{II} ligated to two molecules of **1**. Fe^{II}-promoted scission of the O–O bond at one ligand **1** is followed by intramolecular H abstraction from C-4 by the alkoxy radical from the second ligand. Rebound of the C-centered radical to a carboxylato ligand gives (−)-**7**. Interestingly, the configuration at C-4 in (−)-**7** is inverted with respect to that in **1**. Since compound (−)-(**7**) is similar to plakortolides B[8] and G,[13] formation of (−)-

7 from **1** suggests a biomimetic transformation relevant to plakortolide biogenesis.

[11] All new compounds were fully characterized by HR-MS, FT-IR, ^{1}H and ^{13}C NMR (see the Supporting Information). Acid (±)-**12** was prepared by a malonic acid synthesis as follows: diethyl 2-methylmalonate was alkylated with (8-bromooct-1-ynyl)benzene (NaOEt) followed by hydrogenation (H_2, Pd/C), saponification (NaOH, H_2O/EtOH), and decarboxylation (100 °C, H_2SO_4(aq)); see the Supporting Information.

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[13] (+)-Plakortolide G, a peroxylactone similar to (−)-**7**, was assigned the configuration 2*S*,4*S*,6*R*,8*S*—opposite at C-8—by ab initio Hartree–Fock calculations of molar rotations: T. L. Perry, A. Dickerson, A. A. Khan, R. K. Kondru, D. N. Beratan, P. Wipf, M. Kelly, M. T. Hamann, *Tetrahedron* **2001**, *57*, 1483 – 1487. The absolute configuration of the 1,2-dioxolane ring in **2** was assigned by comparison of the $[\alpha]_D$ value of **2** with those of synthetic "plakinates": P. Dai, T. K. Trullinger, X. Liu, P. H. Dussault, *J. Org. Chem.* **2005**, *71*, 2283 – 2292.

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