Stereochemistry

Stereochemical Assignment in Acyclic Lipids Across Long Distance by Circular Dichroism: Absolute Stereochemistry of the Aglycone of Caminoside A**

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In memory of D. John Faulkner

The glycolipid caminoside A (**1**, Figure 1), isolated from the marine sponge *Caminus sphareoconia* from Dominica, was shown to be an inhibitor of a type III bacterial secretory system.[1] The components of type III secretory pathways mediate insertion of virulence factors into host cells by pathogenic bacteria including *Bordetella pertussis*, *Chlamydia trachomatis*, *Salmonella typhi*, and enterohemorrhagic *Escherichia coli* (EHEC); the later being responsible for "hamburger" disease. Caminoside A is an unusual marine glycolipid[2] with a non-glycerol aglycone (the C_{19} hydroxyketone **2**) that is glycosylated at the C10 hydroxy group by a tetrasaccharide consisting of D-deoxytalose, two D-glucose units, and L-quinovose that are linked through 1,2′ and 1,6′-*O*-glycosidic bonds. The secondary C10 OH group of **2** is separated from the chain termini by at least seven CH_2 groups, consequently, the configuration could not be assigned by conventional NMR spectroscopic methods. This situation typifies a well-recognized problem in natural products structure elucidation—assignment of stereochemistry at remote stereocenters in acyclic molecules.[3]

The problem can be described in terms of the logical order of analysis employed by natural products chemists when addressing absolute configuration by NMR spectroscopy. Assignment of configuration at stereogenic centers along an acyclic carbon chain requires reliable chemical shift assignments to reduce the problem to one of defining the stereotopicity of groups flanking the left and right sides of each substituted carbon in the chain (Figure 2). For example, the configurations of many secondary alcohols are conveniently assigned from their derived Mosher[4] or mandelate esters[5]

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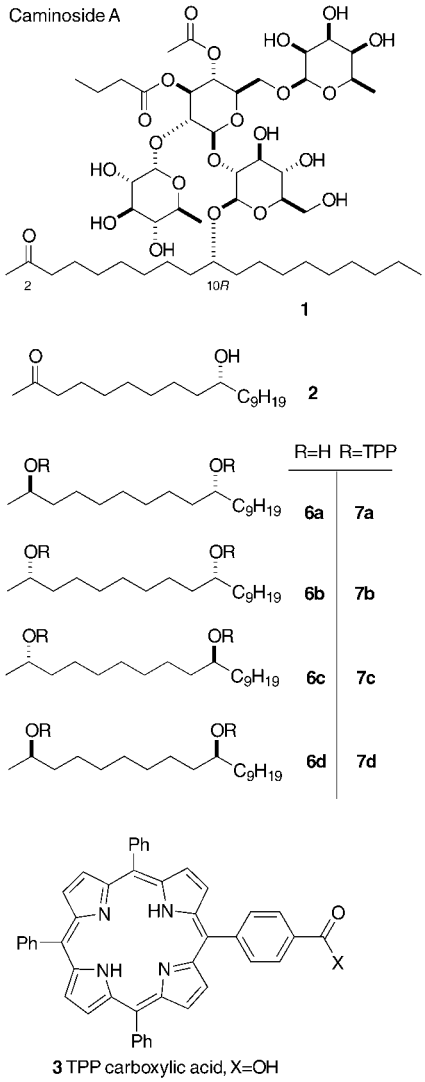


Figure 1. Caminoside A (**1**), the aglycone **2**, and the four possible stereoisomers of reduced aglycone (**6a–d**) and 5-(4′-carboxy)-5,10,15,20-tetraphenylporphyrin (TPP, X = OH, **3**).

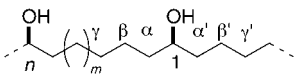


Figure 2. Acyclic 1,*n*-glycol. The configuration of C1 can deduced from NMR spectroscopic assignments of the stereotopicity of the two families of chemical shifts [α, β, γ...] and [α′, β′, γ′...]. To obtain the relative configuration of the 1,*n*-glycol, similar conditions must be satisfied for C*n*, or the C1 and C*n* configurations must be cross-correlated.

by interpretation of anisotropic shifts ($\Delta\delta$) of the families of chemical shifts [α, β, γ…] and [α′, β′, γ′…]. Data are fitted to an empirical model which orders the diastereotopic groups located to the left and right of the CH(OH) group by the sign of their $\Delta\delta$ values. The use of integrated ^{1}H-^{1}H and ^{1}H-^{13}C coupling constant analysis (Karplus-like analysis) provides assignments of stereochemistry from defined dihedral angle dependencies (*J*-based methods).[6] *J*-based analysis can relay stereochemical information along the chain (α to β to γ…) to reveal relative stereochemistry at contiguous carbon atoms (1,2-disubstituted) or even alternating substituted carbon atoms (1,3-disubstituted). The requirement (and limitation) of these methods is that stereotopicity of the signals of intervening CH_2 groups must be made by measurements of *J* coupling constants under favorable conditions of NMR spectroscopy signal dispersion and dihedral angles that conform to all-staggered molecular conformations. These methods, although usually successful for polyketides bearing contiguous 1,2 or 1,3-disubstituted stereoelements, become less reliable for molecules containing two or more stereocenters separated by three or more methylene bridges because the two diastereotopic chain segments subtended at each stereocenter cannot always be differentiated by NMR spectroscopy.[7]

We describe, herein, the configurational assignment of the aglycone of caminoside A (**2**) in which these limitations are overcome by exploiting long-range exciton-coupling circular dichroism (ECCD) of 1,*n*-glycol diesters in acyclic lipid chains that are formulated in highly uniform liposomes prepared from 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DPC). The success of the method (defined here as liposomal exciton-coupling circular dichroism, LECCD) relies on the structural order imposed on the long-chain lipid by the hydrophobic alignment of the 1,*n*-glycol diesters of *O*-tetraphenylporphyrincarboxylic acid (TPP, X = OH **3**) upon being constrained by the fatty acyl chains in small diameter, uniform DPC liposomes (average $\varnothing \approx 30$ nm, Figure 3). Partial ordering of the

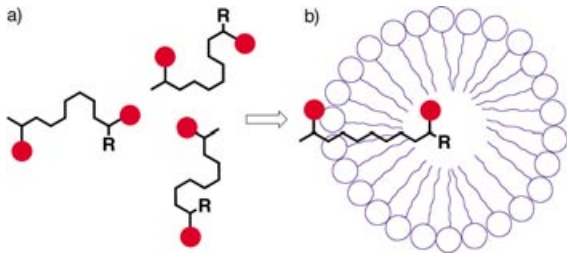


Figure 3. Imposing order upon random coiled, long-chain 1,*n*-diol TPP-carboxylate esters (a) by chain alignment within liposomes (b). Red circles TPP chromophores, blue DCP, R = alkyl

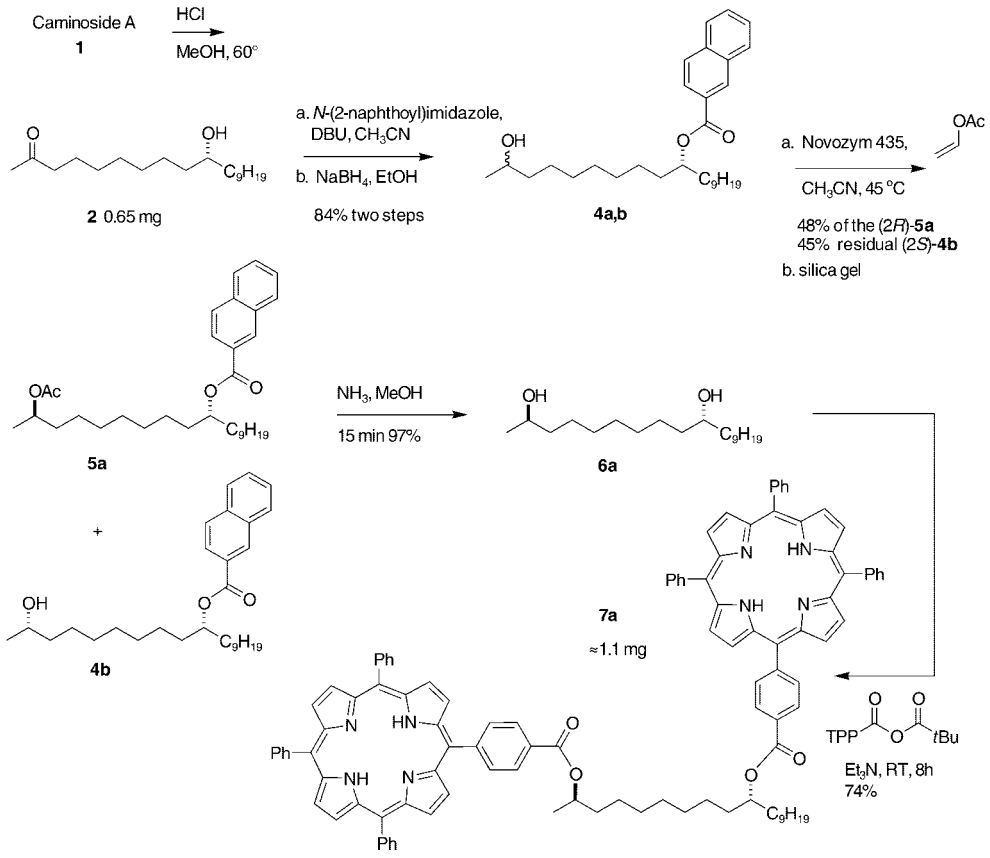
lipid chain limits conformational averaging and allows detection of ECCD that would not be observable in isotropic solution. The TPP chromophore was chosen from the many chromophores useful in ECCD[8] because of its exceptionally strong charge-transfer band and successful applications across long distances in conformationally rigid diols and amino-alcohols.[9] Since the TPP ester chromophore dipole moments are aligned predominantly along the C–O bond vectors of the ester groups at defined angular orientations, the sign of the resulting bisignate CD bands reveals the sign of the absolute helicity or "screw" angle subtended by the C–O bonds and the stereochemistry at the OH substituted carbon atoms.[10] Herein, we demonstrate an application to an otherwise intractable problem of natural product stereochemical assignment: the relative and absolute configurations of a natural product containing an OH group isolated within an acyclic

lipid chain. The LECCD achieves exceptional sensitivity (limit of detection ≈40 pmol) through two factors: the use of liposomes with uniform, small diameters ($< \lambda_{max}$) that reduce CD signal loss from non-Rayleigh scattering and the colossal charge-transfer dipole moment of the TPP chromophore ($\lambda =$ 418 nm, ε 350000[9b]) which allows propagation of relative stereochemical information in substituted acyclic lipids over distances of at least 10 Å.

We recently showed that the stereochemistry of *meso* and C_{2v} symmetric 1,5-glycols can be assigned based on the LECCD spectra of their derived TPP esters and that the method can be extended to 1,7- and 1,9-glycols.[10] To apply the bis(TPP glycol) method to **2**, the C2 carbonyl group was asymmetrically transformed to introduce a stereodefined C2 OH group that would be used to "interrogate" the remote C10 OH configuration. A sample of **1** was methanolyzed under acidic conditions (Scheme 1, MeOH, HCl, reflux) and the resulting mixture of aglycone and *O*-methyl glycosides separated by silica gel chromatography (7:3 hexanes/EtOAc) to afford pure **2**.[11] A convenient UV chromophore (2-naphthoic acid: $\lambda = 234$ nm, ε 58000 $M^{-1}cm^{-1}$) was first attached at the C10 hydroxy group to assist detection of intermediates during each of the following conversions. Esterification of **2** (≈0.6 mg, *N*-2-naphthoylimidazole, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), CH_3CN) gave **4** that was reduced ($NaBH_4$, EtOH) to a mixture of C2 epimeric glycol mononaphthoates (**4a**,**b**). The mixture was resolved by enzyme-catalyzed acetylation (Novozym 435, vinyl acetate, CH_3CN, 45°C) to give the product (2*R*)-*O*-acetate ester **5a** (48%) and residual (2*S*)-alcohol **4b** (45%), which were easily separated by silica chromatography. The configurations at C2 of **5a** and **4b** followed from the enantiopreference of Novozyme 435 for (2*R*)-methyl carbinols[12] and were supported by Mosher ester analysis of synthetic homologues (see below).[13]

The configuration of the natural product-derived glycol diester **5a** corresponds to one of the family of stereoisomers **6a–d** depicted in Figure 1. As expected, the relative stereo relationship of the known C2 center to C10 in **5a** or **4b** could not be determined at this stage owing to the large distance between the two OH groups, however, the correct natural product configuration was successfully elucidated as follows: The ester groups in **5a** were removed (NH_3, MeOH, 15 min, 97%) to give the 1,9-glycol **6a**. Glycol **6a** was converted into the purple TPP ester **7a** (Scheme 1; λ_{max} 416 nm, ε428000, HRFABMS *m*/*z* 1582.9874, $[MH]^+$) via the mixed TPP anhydride (*O*-pivaloyl TPP, CH_2Cl_2, 8 h, Et_3N) followed by HPLC purification (74%). CD measurement of a sample of **7a** that had been constituted into liposomes (DPC 2 mg mL^{-1}, average diameter $\varnothing \approx 30$ nm, $c = 0.65$ μM based on the extinction of TPP, ε 428000) showed strong exciton coupling (Figure 4) characterized by a high intensity positive bisignate CD signal ($A = +22$), however, the CD of **7a** in MeOH showed only baseline as expected from conformational averaging of the lipid chains of **7a** in isotropic solution.

We propose that the lipid chains are partially oriented in the extended conformation by hydrophobic packing with the fatty acyl chains of DPC. Assuming that the liposomal bilayer



Scheme 1. Conversion of **1** into nonadecane-1,10-diol TPP-carboxylate diester (**7a**).

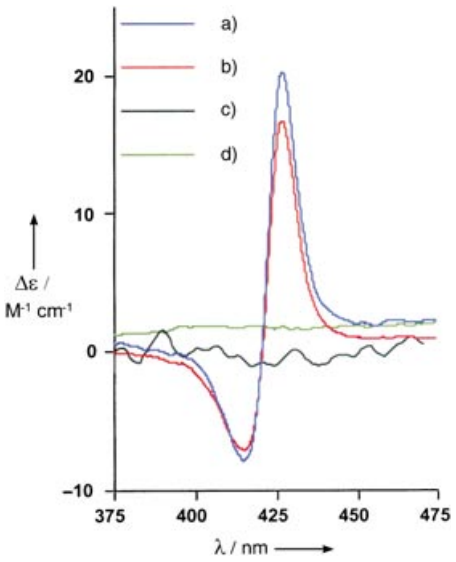


Figure 4. CD spectra of TPP esters formulated in MeOH or DPC liposomes (2 mg mL^{-1}, 1,2-distearoyl-*sn*-glycero-3-phosphocholine), $T = 22\,^{\circ}C$. a) "pseudo-C_{2v}" **9a** in liposomes. b) **7a** in liposomes. c) "pseudo-*meso*" **9b** in liposomes. d) **7a** in MeOH.

orients the long-chain bis(TPP ester) **7a** in an extended conformation and that the *O*-TPP groups subtend torsional angles as shown in Figure 5, the observed positive bisignate

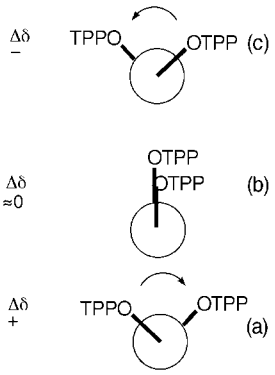
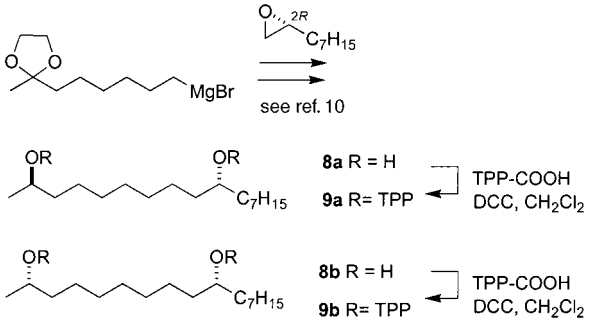


Figure 5. Predicted sign of exciton coupling CD for extended conformation of long-chain 1,*n*-glycol TTP diesters ($n = 7,9$). a) *anti* conformation, positive helicity ("pseudo-C_{2v}" symmetric)→positive split CD. b) synperiplanar ("pseudo-*meso*")→zero signal; c) *anti* conformation, negative helicity ("pseudo-C_2" symmetric)→negative split CD.

signal would be expected for the (2*R*,10*R*)-**7a** where the two TPP groups are oriented *anti* with respect to the plane of the extended chain conformation (Figure 5a). Conversely, a negative signal would be expected for the *anti*-oriented TPP groups of the enantiomeric (2*S*,10*S*)-**7c** (Figure 5c) and a weak or baseline signal would be observed for the synperiplanar TPP groups of the remaining "pseudo-*meso*" stereomers, (2*S*,10*R*)-**7b** and (2*R*,10*S*)-**7d** (Figure 5b). Indeed, the configurational assignment of **7a** was entirely supported by measurements of the corresponding TPP esters of synthetic homologues **8a** and **8b**, each prepared from (*R*)-1,2-epoxynonane (Scheme 2).[10,13] The CD spectrum of liposomal formulations of the corresponding TPP ester (2*R*,10*R*)-**9a** (Scheme 2) was virtually identical to that of the natural product derived **7a**, but the synthetic epimer, (2*S*,10*R*)-**9b**, gave only baseline signal.



Scheme 2. Synthesis of model 1,9-diols.

In conclusion, the C10 configuration of caminoside A is assigned as *R*, which completes the entire configurational assignment of **1**.[14] The advantage of LECCD lies in partial ordering of the lipid chains that induces a non-averaged net ECCD signal in 1,9-glycol TPP diesters that communicates the relative configuration of the 1,9-glycol, derived from aglycone **2** in a stereodefined manner. Since the C2 configuration of **6a** is securely established, the absolute configuration at C10 is communicated by the relative stereochemistry revealed from LECCD measurements of the corresponding 1,9-glycol TPP diesters **7a** and **7b**. In principle, the method maybe extended to other suitably derivatized acyclic hydroxy-substituted long-chain lipids that defy conventional methods for stereochemical interrogation because they contain remotely located stereocenters, such as the antiviral glycolipids, fattiviracin[15] from *Streptomyces microflavus* No. 2445 and cycloviracins from *Kibdelosporangium albatum* so. nov. (R761-7).[16] We have estimated, by extrapolation of the A magnitudes from LECCD spectra of 1,*n*-glycol TPP esters ($n = 7, 9$), that a measurable LECCD signal should be observed up to $n = 13$,[10] however, refinements of methods for ordering of long-chain esters in liposomes may extend transmission of stereochemical information beyond this limit.

Experimental Section

All the new compounds were purified by HPLC and gave satisfactory high-resolution mass spectra (HRMS) and ^{1}H NMR spectra.

LECCD: General procedures for liposome preparation and characterization of the materials by TEM are described in detail, elsewhere.[10] Briefly, measured aliquots of DPC and TPP ester **7a** were dissolved in $CHCl_3$ and "shell-evaporated" in a round bottom flask. Water (2 mL) was added and the mixture sonicated (ultrasound bath). The crude liposomes were annealed by thermal cycling (60→25 °C→60 °C, ×3) before repeated passage through a porous nylon membrane (100 nm pore, Liposofast, Avestin). CD spectra were on purified material were measured at room temperature (23 °C) in a 1-

mm path-length cell (Jasco 810 spectropolarimeter). No smoothing or noise reduction algorithms were applied to the spectral data.

4a and **4b**: A solution of **2** (0.6 mg, 2 μmol), *N*-2-naphthoylimidazole (1.0 mg, 4.48 μmol), and DBU (0.5 mg, 3.3 μmol) in CH_3CN (0.5 mL) was heated to 65 °C. After 8 h the mixture was cooled to room temperature and the volatiles were removed under a stream of N_2. The residue was purified on a short SiO_2 column (pipette, 5:95 EtOAc/*n*-hexane) to obtain a yellow oil of crude naphthoate esters (0.78 mg, 87 %). The ester mixture was dissolved in EtOH and treated with $NaBH_4$ (1 mg, 26 μmol) at 0 °C. After stirring for 20 minutes, the mixture was quenched with NH_4Cl (sat. aq) and extracted with CH_2Cl_2 (2 × 2 mL). The volatiles were removed, and the residue purified through a short plug of silica (1:4 EtOAc/*n*-hexane) to provide an inseparable mixture of **4a** and **4b** (0.71 mg, 84 % yield over 2 steps) that was used immediately in the next step. ^{1}H NMR ($CDCl_3$): $\delta = 7.28$–7.51 (m, 7H), 4.02 (m, 1H), 3.34 (m, 1H), 1.57 (m, 4H), 1.19–1.47 (m, 26H), 0.90 (t, $J = 6.8$ Hz, 3H).

5a: Novozym 435 (1 mg) was added to a solution of **4a** and **4b** (0.7 mg, 1.6 μmol) in CH_3CN (1 mL) followed by the addition of a solution of vinyl acetate (2.7 μmol) in CH_3CN (100 μL). The mixture was heated to 45 °C with vigorous stirring for 17 h, cooled to room temperature and filtered through a plug of cotton. Purification of the product on a short SiO_2 column (4 × 10 mm, 1:99 EtOAc/*n*-hexane) gave acetate **5a** (0.36 mg, 48 %). FAB HRMS *m*/*z* $[M+H]^+$ found 497.3634; calcd for $C_{32}H_{49}O_4$, 497.3631.

6a: A methanol solution of acetate **5a** (0.34 mg, 0.7 μmol) was treated with saturated solution of NH_3 in MeOH (0.5 mL), and the mixture allowed to stir at 0 °C for 15 minutes before neutralization with 1M HCl (aq). The mixture was concentrated, extracted with CH_2Cl_2 (2 × 1 mL) and the volatiles removed to provide **6a** (0.2 mg, 97 %). Desorption chemical ionization (DCI) HRMS *m*/*z* $[M+H]^+$ found 301.3015; calcd for $C_{19}H_{41}O_2$, 301.3017.

7a: The mixed anhydride (4 mg, 6.1 μmol), prepared from 5-(4′-carboxyphenyl)-5,10,15,20-tetraphenylporphyrin and pivalic acid, in a solution of **6a** (0.19 mg, 0.7 μmol) in CH_2Cl_2 (1 mL) was treated with Et_3N (4 μL). After stirring for 8 h, the mixture was washed with H_2O, dried over Na_2SO_4, and concentrated to give a purple film. Purification of the product by normal phase HPLC (SiO_2, 5 μ, 4.6 × 250, 2:98 *i*PrOH:*n*-hexane) gave **7a** (0.92 mg, 74 %). FAB HRMS *m*/*z* $[M+H]^+$ found: 1582.9874; calcd for $C_{109}H_{97}N_8O_4$: 1582.9877. UV (MeOH) λ645 (ε4700), 511 (16300), 416 nm (428000).

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[14] Three other analogues from *C. sphaeroconia* (caminosides B–D) are homologues of **1** that differ only by replacement or interchange of *O*-acetyl or butanoyl groups at C3 and C4 of

the second glucose residue.[11] Since the constitution of the aglycone is the same in each of the caminosides A–D, we assume that the configuration is also the same (10*R*).

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