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Absolute Stereochemistry of the Strevertenes

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ABSTRACT The CD exciton chirality method was applied to determine the absolute stereochemistry of the strevertenes, antifungal pentaene macrolides produced by *Streptoverticillium* sp. LL-30F848. The CD difference spectrum of strevertene A methyl ester 15-dimethylaminobenzoate showed a positive couplet between the dimethylaminobenzoate and the pentaene chromophores, and therefore established the 15*R* configuration. Thus, by considering the relative configurations of the remaining stereogenic centers as derived from X-ray crystallography and ROESY experiments, the absolute stereochemistry of the strevertenes is established as *2R, 3S, 5S, 7S, 11R, 13R, 14R, 15R, 26S* and *27R. Chirality 12:43–51, 2000.* © 2000 Wiley-Liss, Inc.

KEY WORDS: polyene macrolides; polyene; CD exciton chirality method; allylic alcohols; circular dichroism; absolute configurations

Recently, we reported the isolation and structure determination of the strevertene antibiotics,[1] a new series of polyene macrolides produced by *Streptoverticillium* sp. LL-30F848. Members of this family differ from each other in the substitution pattern at three chiral centers (Fig. 1).

Polyene macrolides,[2] a large group of natural products, are characterized by: 1) a 16- to 44-membered lactone ring, 2) a polyene chain of 3 to 8 conjugated double bonds, and 3) a polyol chain, typically 1,3-diols, that may include 1,2 and/or 1,4-diols. In addition, many polyene macrolides bear a sugar moiety, usually D-mycosamine, in β-glycosidic linkage. Knowledge of the absolute stereochemistry of the polyene macrolides is essential for clarifying structure–activity relationships and for developing antifungal agents with increased therapeutic index. However, of the more than 200 polyene macrolides listed in a 1984 review,[2] ca. 40 were with known relative configurations, and the full structure was established only for amphotericin B.[2] Largely due to improvements in spectroscopy and chemical synthesis, the relative and absolute configurations of about a dozen polyene macrolides have since been reported. The notorious difficulties involved in complete structure elucidation of this class of compounds are attributed to their nature. Namely, the polyene macrolides typically occur as complex mixtures that cannot be readily purified because of poor solubility in organic solvents. Moreover, crystallization of the molecules is often hampered by instability of solutions to light and oxygen.

The methods employed for structure determination of polyene macrolides fall into three groups:[3,4]

1) Structures derived from X-ray analysis. The relative and absolute configuration of amphotericin B[5] and the relative stereochemistry of roxaticin[6] were determined by X-ray analysis of their derivatives.

2) Structures determined by a combination of NMR analysis and chemical methods. a) Rychnovsky and coworkers[7,8] determined the relative stereochemistry of roflamycoin,[9] filipin III,[3,10,11] and dermostatin A and B[8] using a 1,3-diol ^{13}C acetonide method that they had developed. The absolute stereochemistry of these compounds was subsequently determined by a modified Mosher NMR method and/or by degradation studies. b) The configuration of mycoticin A and B was determined by Schreiber et al.,[12–14] who applied a two-directional chain synthesis strategy to prepare all four tetraacetonide stereoisomers. c) The circular dichroic (CD) exciton chirality method was utilized to determine the absolute configuration of several chiral centers of lienomycin.[15] The reported structure determinations of nystatin A_1[16,17] and pentamycin[18] also fall into this group.

3) Structures elucidated by NMR analysis. Configurational assignment of pimaricin relied primarily on NMR analysis,[19,20] in which proton–proton through-space correlations with the sugar D-mycosamine were utilized to deduce the absolute configuration from the known stereochemistry of this internal chiral probe. The configurations in candidin[21] and vacidin A[22] were determined in a similar manner.

As the polyol chain is a major segment in polyene macrolides and most of the chiral centers are located in this portion of the molecule, absolute configurational studies have been performed on this polyol moiety, including application of the CD exciton chirality method.[23,24] Harada et al.[25] performed detailed CD studies on 1,3-diols, while we devised a bichromophoric exciton chirality method,[26–28]

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strevertene	R_1	R_2	R_3
A	CH_3	COOH	CH_3
B	CH_2CH_3	COOH	CH_3
C	CH_3	COOH	CH_2CH_3
D	CH_2CH_3	COOH	CH_2CH_3
E	CH_3	COOH	$CH(CH_3)_2$
F	CH_2CH_3	COOH	$CH(CH_3)_2$
G	CH_3	CH_2OH	CH_3

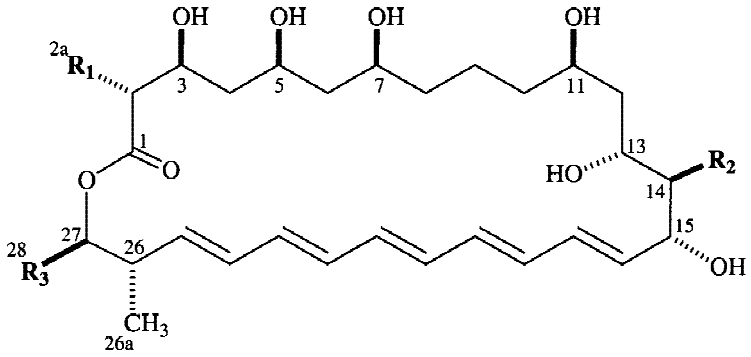


Fig. 1. Structure and relative configurations of strevertenes A–G produced by *Streptoverticillium* LL-30F848.

and Mori and coworkers developed a differential CD method for acyclic polyols.[29–31] Application of these methods usually requires removal of the polyene moiety followed by CD studies of the remaining polyol chain. In contrast, the protocol described here requires no degradation.

RESULTS AND DISCUSSION

The following presents the X-ray structure of strevertene G, which confirms the relative stereochemistry of the strevertenes A–F reported earlier,[1] and reports the absolute configuration of strevertene A as determined by the exciton chirality method. The relative stereochemistry of the strevertenes, as determined by ROESY measurements and depicted in Figure 1,[1] has now been confirmed by X-ray crystallographic studies of strevertene G, which was obtained as yellow rod-shaped crystals from methanol.† A crystal having the approximate dimensions 0.08 × 0.08 × 0.25 mm was selected for X-ray diffraction. Crystal survey, unit cell determination, and data collection were performed using copper radiation at 23 ± 1°C. The structure of strevertene G (Fig. 2) was solved by direct methods and refined by full-matrix least-squares and difference Fourier methods. Unit cell constants and an orientation matrix for data collections, obtained from a least-squares refinement using the setting angles of 24 carefully centered reflections in the range $40.83 < 2\Theta < 49.86°$) corresponded to a primitive monoclinic cell with dimensions of: a = 8.160 (2) Å, b = 7.847 (1) Å, c = 25.727 (1) Å, β = 92.439(9)°, and V = 1645.8 (3) Å^3. For Z = 2 and F.W. = 566.73, the calculated density is 1.144 g/cm^3. A statistical analysis of intensity distribution determined the space group to be $P2_1$. Of the collected 2,836 reflections, 2,629 were unique ($R_{int.}$ = 0.032). The intensities of the three representative reflections were measured after every 150 reflections. Over the course of data collection, the standards increased by 1.5%. A linear correlation factor was applied to the data to account for this phenomenon.

As is evident from the X-ray data, the polyene chain in strevertene G exists in a linear elongated form, as was noted for other polyene macrolides. The heptaene chain in N-iodoacetylamphotericin B has been characterized as having a "fully extended" planar trans configuration.[32] The geometry of the polyene chain of roxaticin was also shown to be all-trans according an X-ray analysis of its derivative, hepta-O-acetylroxaticin.[6] In the case of the strevertenes, ROESY correlations and vicinal coupling constants of the olefinic protons suggest that, in solution, the pentaene chain have also an all-trans geometry.[1] Similar NMR studies revealed that the conformations of the polyene chain in vacidin A,[33] pimaricin,[19] filipin III,[34] and amphotericin B methyl ester[35] adopt the all-trans geometry in solution as well. The UV spectra of the strevertenes exhibit fine structures with λ_{max} at 317, 332, and 350 nm, characteristic of a rigid, all-trans pentaene chromophore. The IR double bond stretching frequency ca. 1,500 cm^{-1} provides further support for the existence of a rigid polyene chain.

In order to determine the absolute configuration of the strevertenes by the exciton chirality method, we considered using the coupling between the pentaene system and a second chromophore introduced at one of the six hydroxyls groups. Of these groups, the 15-OH appeared to be the most suitable for the following reasons. A chromophore introduced at C-15 is in "allylic" position to the pentaene chain, and the two moieties would thereby provide a 1,2-bichromophoric system suitable for application of the CD method. Actually, this exciton coupling approach is an ex-

†Crystallographic data (excluding structure factors) for the structures in this article have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 135449. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033 or E-mail: deposit@ccdc.cam.ac.uk).

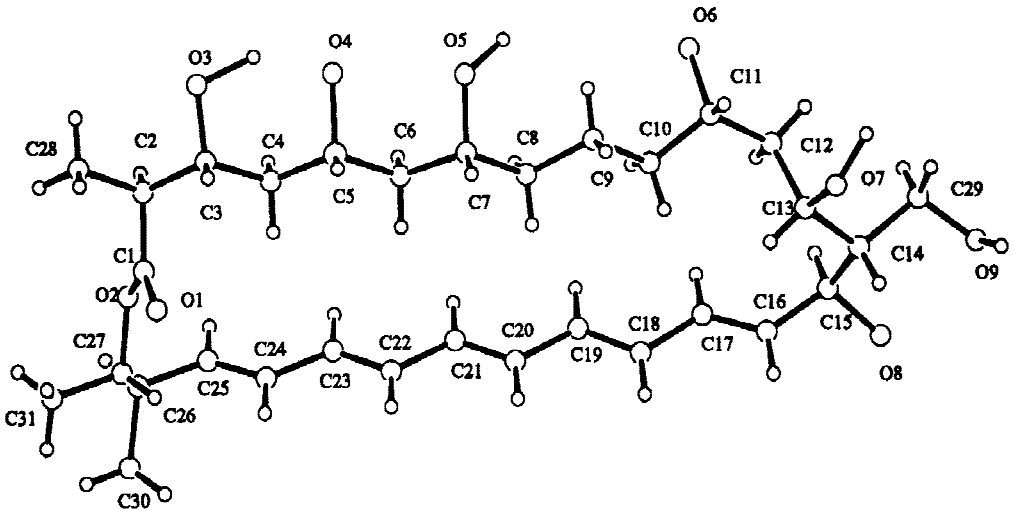
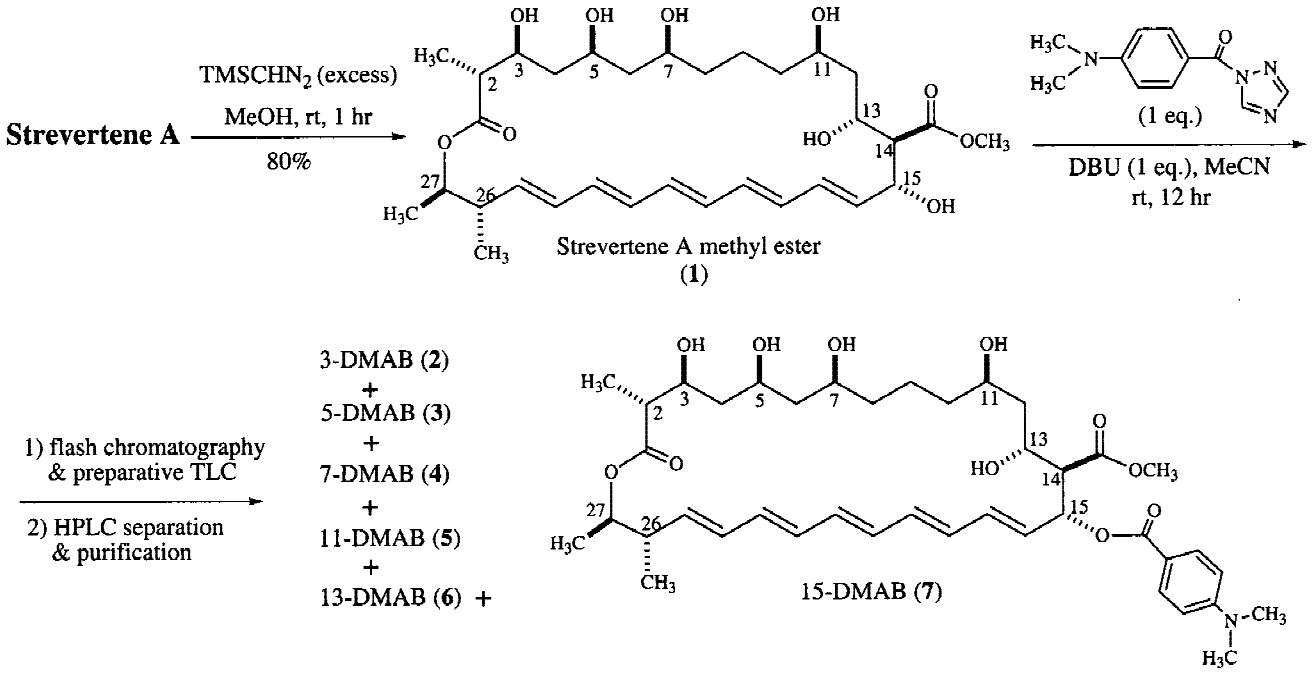


Fig. 2. The X-ray structure of strevertene G.

tension of the allylic alcohol strategy, where coupling of the introduced benzoate with the adjacent double bond leads to a straightforward determination of the absolute configuration.[36,37] A similar case was encountered during studies of 14-hydroxy-4,14-*retro*-retinol, where coupling between the *p*-methoxycinnamoyl chromophore introduced at 14-OH and the pentaene led to establishment of the 14-hydroxyl absolute configuration.[38] In the present case, 4-dimethylaminobenzoate (DMAB) was chosen as the chromophore, because its absorption maximum, λ_{max} = 311 nm, ε = 30,400 in EtOH,[39] was close to that of the pentaene chromophore, λ_{max} ca. 300–350 nm.

The experiments were performed on strevertene A, the most abundant of the strevertenes. We initially attempted to differentiate the 15-OH from the remaining hydroxyls by using phenylboronic ester[40–42] or acetonide to react selectively with the 1,3-diols. However, the results were unsatisfactory as phenylboronic acid caused lactone formation between the 14-carboxyl and the 11-OH groups of strevertene A, thus giving rise to a macrolide skeleton unsuited for further studies. Acetonide formation under different conditions led to either decomposition or a mixture of various acetonides. In view of these results and the noted instability of polyene macrolides, a new protocol, as shown in Scheme 1, was adopted.

First, the carboxylic acid group of strevertene A was



Scheme 1. Derivatization of strevertene A methyl ester (1) into dimethylaminobenzoates **2–7**.

TABLE 1. ^{1}H-NMR chemical shifts and the COSY crosspeaks of strevertene A methyl ester (1) in methanol-d_4

H	δ (ppm)	show COSY crosspeaks with proton	H	δ (ppm)	show COSY crosspeaks with proton
2	2.35	2a, 3	17–23	6.37–6.20	16, 24
3	3.83	2, 4	24	6.15	25, 26
5	3.96	4, 6	25	5.91	24, 26
7	3.67	6, 8	26	2.35	26a, 27
11	3.65	10, 12	27	4.80	26, 28
13	3.60	12, 14	28	1.24	27
14	2.80	13, 15	2a	1.15	2
15	4.32	14, 16	26a	1.06	26
16	5.58	15, 17	OCH_3	3.70	

converted to its methyl ester (**1**) with $TMSCHN_2$[43] and then derivatized to its DMAB ester with triazole/DBU.[44] In order to minimize the amounts of possible multiple substitution products and to also generate a mixture of monobenzoates, strevertene A methyl ester (**1**) was reacted with only one equivalent of 4-dimethylaminobenzoyltriazole and one equivalent of DBU. The reaction mixture was submitted to flash chromatography and preparative TLC to recover the starting material and to also remove the reagents and bisbenzoates. The mono-benzoate mixture was then subjected to reversed phase HPLC separation and purification. Six mono-DMABs: 3-DMAB (**2**), 5-DMAB (**3**), 7-DMAB (**4**), 11-DMAB (**5**), 13-DMAB (**6**), and 15-DMAB (**7**) were isolated in about 10% total yield. The direct benzoylation of ester (**1**) had the advantage of avoiding the cumbersome protection/deprotection steps that would have diminished the overall yield considerably.

Structural assignment of the six mono-DMABs was based on ^{1}H-NMR and COSY spectra. First, all proton resonances of strevertene A methyl ester (**1**) were assigned unambiguously based on magnitude COSY spectra and diagnostic peaks, as indicated in Table 1; introduction of a DMAB group resulted in the expected downfield shift of the carbinyl proton (Table 2).

15-DMAB (**7**), along with 3-DMAB (**2**), 5-DMAB (**3**), 7-DMAB (**4**), 11-DMAB (**5**), 13-DMAB (**6**), and strevertene A methyl ester (**1**), was submitted to UV/VIS and CD measurements. The CD spectra of all mono-DMABs are presented as CD difference spectra by subtracting the CD spectrum of strevertene A methyl ester (**1**) from that of the respective mono-DMAB spectrum. Any couplet observed in the range between 280 nm and 370 nm in these difference CDs thus reflects the net exciton coupling between the pentaene and DMAB chromophores.

The UV/VIS and CD spectra of strevertene A methyl ester(**1**) in methanol are shown in Figure 3. The fine structured UV/VIS band at 350–280 nm with maxima at 318 (ε 50,570), 332 (ε 82,700), and 350 nm (ε 84,012) arises from the all-trans pentaene chromophore. This chromophore is expected to interact through space with the introduced DMAB chromophore, thus giving rise to a split CD, provided that the two chromophores are spatially close and the projection angle between their transition moments is not 0° or 180°. The CD spectrum of strevertene A methyl ester (**1**) exhibited negative peaks between 285 nm and 360 nm, i.e., 313 nm (–4.2), 329 nm (–6.0), and 349 nm (–4.4), arising from the pentaene, whereas the positive peak around 238 nm (+7.7) most likely is associated with the ester carbonyl.

The UV/VIS and the difference CD spectra of 15-DMAB (**7**) in methanol are depicted in Figure 4. The UV spectrum of 15-DMAB (**7**) shows λ_{max} at 320 nm (ε 77,950), 336 nm (ε 92,810), 353 nm (ε 84,000), and a shoulder at 308 nm (ε 55,690), while the CD spectrum showed positive peaks at 336 nm (+37.6), 354 nm (shoulder, +18.8), and a negative peak at 303 nm (–21.4). Since the DMAB chromophore absorbs at 311 nm, it was concluded that these peaks were due to the exciton coupling between the pentaene and 15-DMAB chromophores. The ^{1}H-NMR coupling constant $J_{15,16}$ of 9.2 Hz indicates that 15-H and 16-H adopt a *trans*-antiperiplanar relation, i.e, 15-H is eclipsed by the pentaene chain, as shown in the Newman projection (Fig. 4). The lowest energy conformation of 15-DMAB (**7**) from MacroModel V6.0 studies (Fig. 4) supported the conclusion drawn from NMR J_{vic} values; it is also in agreement with the lowest energy conformation of allylic alcohols, in which the allylic proton and the double bond are eclipsed, and the observed J_{vic} between the olefinic and carbinyl protons was 5.2–9.2 Hz.[36,37] The analysis with MacroModel V6.0 further revealed that the interchromophoric distance between the pentaene and the DMAB chromophore is ca. 10 Å, with a projection angle of about +145°, as shown in the depicted absolute configuration. Considering all experimental data, the first positive Cotton effect observed at longer wavelengths, followed by a negative Cotton effect at shorter wavelengths, is assigned to a positive exciton coupling between the polyene and DMAB chromophores in 15-DMAB (**7**). Consequently, this positive exciton chirality leads to *R*-absolute configuration at C-15. This assignment, in conjunction with the known relative configurations already determined by NMR and X-ray data, also establishes the absolute configurations for the remaining nine stereogenic centers. It is therefore established that the absolute stereochemistry of the strevertenes is *2R, 3S, 5S, 7S, 11R, 13R, 14R, 15R, 26S* and *27R* (Fig. 5).

It should be noted that while the difference CD spectrum

TABLE 2. Comparison of ^{1}H-NMR chemical shifts (ppm) of characteristic protons in strevertene A methyl ester (1) and DMAB derivatives 2–7, in methanol-d_4

H	Chemical shifts (ppm) in strevertene A methyl ester **(1)**	DMAB derivatives	Chemical shifts (ppm) in DMAB derivatives
3	3.83	3-DMAB **(2)**	5.25
5	3.96	5-DMAB **(3)**	5.25
7	3.67	7-DMAB **(4)**	5.14
11	3.65	11-DMAB **(5)**	5.13
13	3.60	13-DMAB **(6)**	5.01
15	4.32	15-DMAB **(7)**	5.63

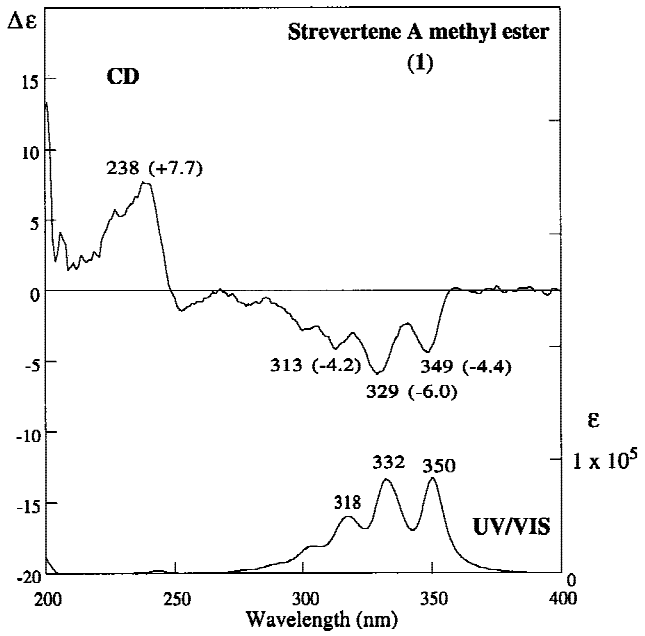


Fig. 3. The CD and UV/VIS of strevertene A methyl ester (**1**) in methanol.

of 11-DMAB (**5**) also showed a strong positive coupling (Fig. 6), the spectra of 3-DMAB (**2**), 5-DMAB (**3**), and 13-DMAB (**6**) exhibited negative exciton couplings. The difference CD spectrum of 7-DMAB (**4**) did not show any coupling pattern between 260 and 370 nm.

Extensive conformational studies of the polyol moieties, which have been well documented in our previous publication[1] and also in the literature,[19,33–35,45] allow us to also rationalize the observed through-space-couplings of the other DMAB derivatives, when the now established absolute stereochemistry of strevertene A (Fig. 5) is being considered. Modeling studies on 3-DMAB (**2**), 5-DMAB (**3**), 11-DMAB (**5**), and 13-DMAB (**6**) predicted the projection angle between the pentaene and the DMAB chromophores to be about –124°, –117° (Fig. 7), +84° and –96°, respectively, which agreed with the observed exciton couplings. The model of 7-DMAB (**4**) (Fig. 7) clearly showed that DMAB bisected the pentaene chromophore and hence was unsuitable for exciton split CD, while 3-DMAB (**2**), 5-DMAB (**3**), 11-DMAB (**5**), 13-DMAB (**6**) all exhibited exciton couplings. However, since the exact spatial orientation between the DMAB and the pentaene chromophores in these mono-DMABs could not be determined unequivocally, these derivatives were not used for configurational assignments.

CONCLUSION

The absolute configuration of strevertene A has been determined by application of the exciton chirality method to its 15-DMAB derivative, where the existing pentaene chromophore served as coupling partner of the introduced dimethylaminobenzoate chromophore. Based on the relative configurations of the 10 stereogenic centers, as established by X-ray crystallography and NMR ROESY measurements,[1] the full structure of strevertene A, including the

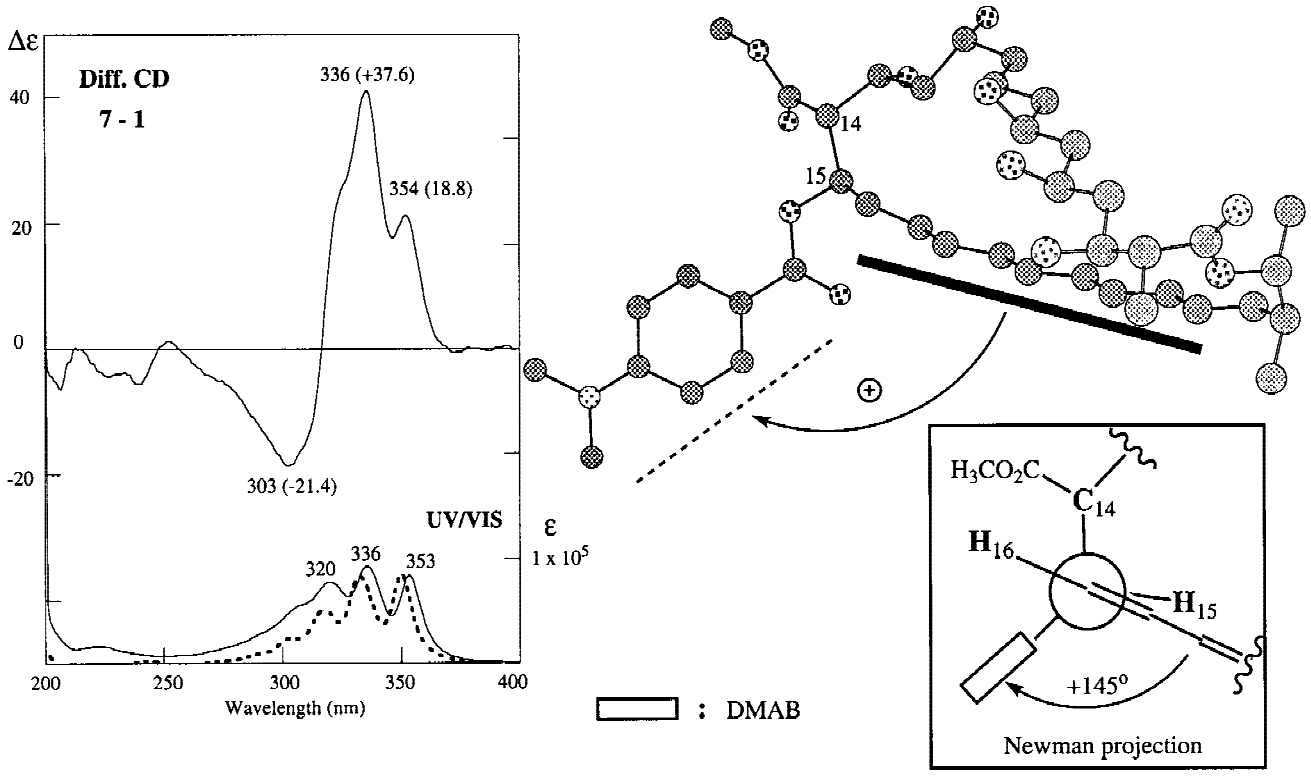


Fig. 4. UV/VIS of 15-DMAB (**7**) (solid line) and of strevertene A methyl ester (**1**) (dashed line), and difference CD (**7 – 1**), and lowest energy conformer of 15-DMAB (**7**), in methanol. The thick and dotted lines denote transition moments of the pentaene and DMAB chromophores, respectively. Reference value for 4-$(CH_3)_2$N-C_6H_4-CO_2CH_3 in EtOH: λ_{max} 311 nm (ε 30,400) (see Ref 39).

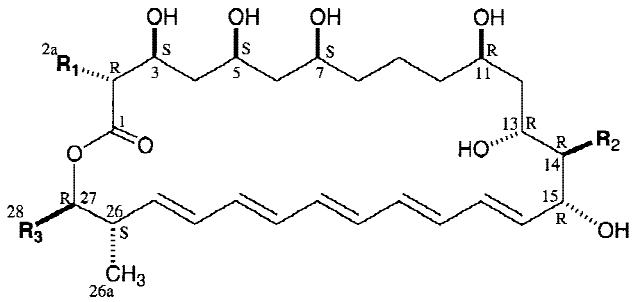


Fig. 5. The absolute configuration of the strevertenes A–G.

absolute configurations of all 10 stereogenic centers, can be represented by the structure in Figure 5. We expect that application of the current approach, where no cleavage reactions of the polyene skeleton are involved, can be extended to determine the absolute stereochemistry of other polyene macrolides. Of the over 200 polyene macrolides reported, many carry "allylic" hydroxyl function, e.g., amphotericin B, candicidin, candidin, partricins, perimycins, filipin III, pentamycin, lucensomycin, rimocidin, and tetrins. For the various polyenes with different UV-absorption maxima (Table 3),[46] suitable chromophores with absorption maxima matching those of the polyenes should be selected to achieve an optimal exciton coupling.[44]

EXPERIMENTAL

General

All chemicals were purchased from Aldrich (Milwaukee, WI). Methanol, used for reactions was anhydrous grade, that for UV and CD analyses was spectrophotometric grade. Analytical and preparative TLC was run on precoated silica-gel plates (Analtech, 20 × 20 cm, 250 or 500 microns). The final separation and purification of the compounds were performed using the following HPLC equipment and method: A Perkin-Elmer (Oak Brook, IL) Model series 4 liquid chromatography instrument with a UV detection at 310 nm and a Supelco Hypersil ODS reversed phase HPLC column (250 × 4.6 mm, 5 μm). NMR data were collected on a Bruker (Fremont, CA) DMX 500 (500 MHz) NMR spectrometer employing standard pulse sequences. Chemical shifts of ^{1}H NMR signals were determined in ppm relative to the solvent signals of residual CH_2DOD at δ_H 3.30 ppm. FAB mass spectra were recorded using a JEOL (Peabody, MA) JMS-HX 110HF/HX 110HF tandem mass spectrometer. ESI mass spectra were obtained on a JEOL JMS-LCmate mass spectrometer. UV spectra were taken on a Perkin-Elmer Lambda 6 model. CD spectra were measured on a Jasco J-720 spectropolarimeter. The concentrations of 3-DMAB (**2**), 5-DMAB (**3**), 7-DMAB (**4**), 11-DMAB (**5**), and 13-DMAB (**6**) were estimated using the ε value of strevertene A methyl ester (**1**), ε_{350} = 84,000, since the absorption of the DMAB chromophore at ca. 350 nm is almost zero. The accepted reference value, ε_{350} = 84,000, corroborate with the computed sum UV/VIS spectrum of strevertene A methyl ester (**1**) and dimethylaminobenzoate, which shows at 353 nm ε = 84,082. All molecular mechanics calculations were carried out with MacroModel V6.0 on Silicon Graphics 3D workstation at the Department of Chemistry, Columbia University. The Monte Carlo conformational search was carried out by using a modified MM2 as the force field, water as the solvent, and 1,000 structures were generated. The X-ray analysis was performed by Molecular Structure Corp. on a Rigaku AFC6R diffractometer supplied by a nickel-filtered Cu-Kα radiation and a 12 kW rotating anode generator.

Preparation of Strevertene A Methyl Ester (1)

To a solution of strevertene A (15 mg, 0.026 mmol) in 0.5 ml methanol was added 1 ml of 1 M $TMSCHN_2$ hexanes solution. The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the resulting material was purified by column chromatography (silica gel, 19/1 chloroform/methanol) to give strevertene A methyl ester (**1**) in 80% yield. ^{1}H-NMR (500 MHz, CD_3OD) δ 6.37-6.20 (m, 7H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 6.15 (dd, 1H, J = 9.2 Hz, 15.5 Hz, H-24), 5.91 (dd, 1H, J = 7.0 Hz, 15.4 Hz, H-25), 5.58 (dd, 1H, J = 8.9 Hz, 14.1 Hz, H-16), 4.80 (1H,

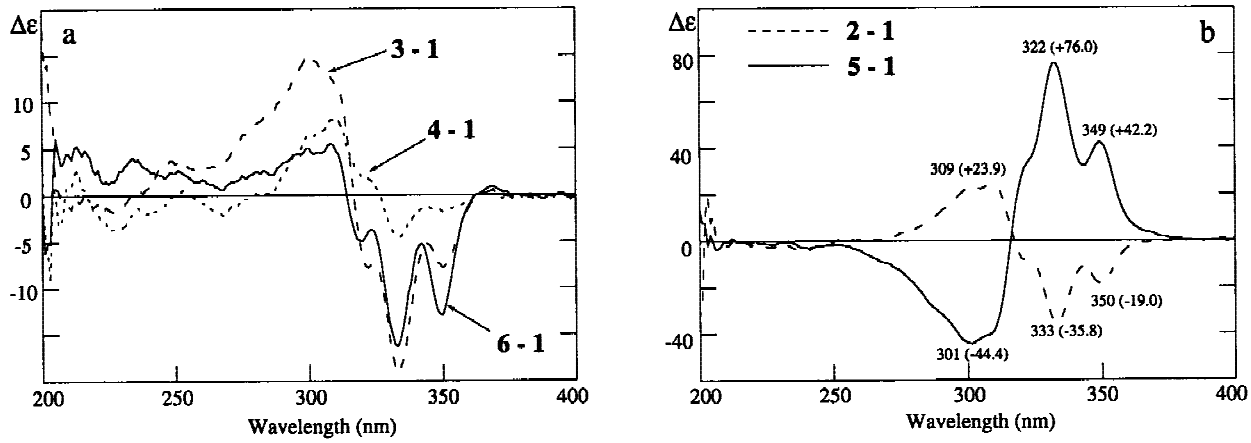


Fig. 6. Difference CD between dimethylaminobenzoates 3-DMAB (**2**), 5-DMAB (**3**), 7-DMAB (**4**), 11-DMAB (**5**), 13-DMAB (**6**), and methyl ester (**1**), in methanol: (**a**) **3 – 1**, **4 – 1**, and **6 – 1**; (**b**) **2 – 1** and **5 – 1**.

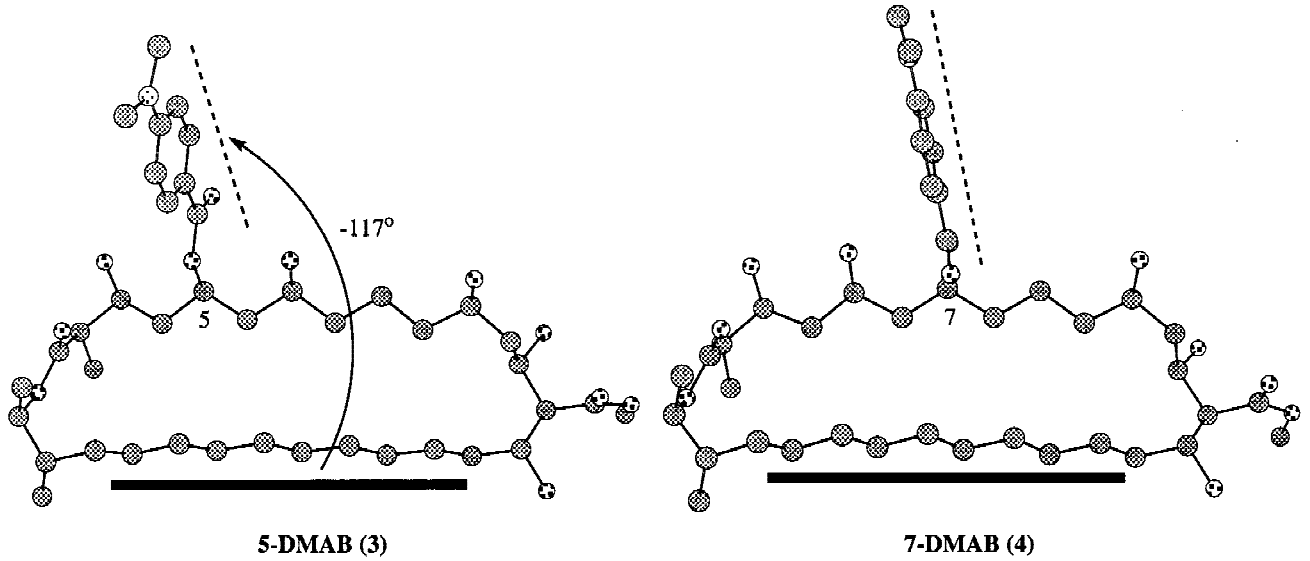


Fig. 7. The 5-DMAB (**3**) and 7-DMAB (**4**) derivatives. The thick and dotted lines denote, respectively, transition moments of the pentaene and the DMAB chromophores.

H-27, covered by the solvent peak), 4.32 (t, 1H, J = 9.6 Hz, H-15), 3.96 (m, 1H, H-5), 3.83 (t, 1H, J = 7.2 Hz, H-3), 3.70 (s, 3H, $-CO_2CH_3$), 3.67 (m, 1H, H-7), 3.65 (m, 1H, H-13), 3.60 (m, 1H, H-11), 2.80 (dd, 1H, J = 4.0 Hz, 10.0 Hz, H-14), 2.35 (m, 2H, H-2, H-26), 1.24 (d, 3H, J = 6.2 Hz, H-28), 1.15 (d, 3H, J = 7.0 Hz, H-2a), 1.06 (d, 3H, J = 6.9 Hz, H-26a). HRFABMS calcd for $C_{32}H_{50}O_{10}Na$ 617.3302, found 617.3276. UV/VIS spectrum: 318 nm (ε 50,570), 332 nm (ε 82,700), and 350 nm (ε 84,012).

Dimethylaminobenzoylation of Strevertene A Methyl Ester (1)

To a flask which contained **1** (6 mg, 0.01 mmol) and 2.2 mg (0.01 mmol) dimethylaminobenzoyltriazole was added 1.5 ml of anhydrous MeCN; 1.52 mg (0.01 mmol) of DBU was transferred to the reaction flask. The reaction mixture was stirred at room temperature overnight. The solvent was then removed under reduced pressure, and the resulting material was chromatographed on a silica gel column (chloroform to 19/1 chloroform/methanol). The appropriate fractions were applied to plate chromatography (500 microns, silica gel, 19/1 chloroform/methanol) to get six bands, which were further separated by RP-HPLC. Six mono-dimethylaminobenzoates were isolated (yield about 10%): 3-DMAB (**2**), 5-DMAB (**3**), 7-DMAB (**4**), 11-DMAB (**5**), 13-DMAB (**6**), and 15-DMAB (**7**) (**2**:**3**:**4**:**5**:**6**:**7** = 1: 1.5:1:2:5.2:1.3). The gradient HPLC condition was: mobile phase started with 50% CH_3CN in H_2O, after 10 min, was linearly changed to 100% CH_3CN in 10 min and maintained for 5 min, then was changed back to 50% CH_3CN in H_2O in 5 min. The flow rate was 1 ml/min. The retention times of 3-DMAB (**2**), 5-DMAB (**3**), 7-DMAB (**4**), 11-DMAB (**5**), 13-DMAB (**6**), and 15-DMAB (**7**) were 17.2, 10.8, 15.9, 22.0, 20.0, and 24.9 min, respectively.

3-DMAB (2)

^{1}H-NMR (500 MHz, CD_3OD) δ 7.85 (d, 2H, J = 8.9 Hz, Ar-H), 6.72 (d, J = 8.9 Hz, Ar-H), 6.39-6.15 (m, 8H, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24), 5.96 (dd, 1H, J = 7.3 Hz, 15.8 Hz, H-25), 5.56 (dd, 1H, J = 8.8 Hz, 14.3 Hz, H-16), 5.25 (t, 1H, J = 9.2 Hz, H-3), 4.88 (1H, H-27, covered by the solvent peak), 4.31 (t, 1H, J = 9.2 Hz, H-15), 3.81 (m, 1H, H-5), 3.77-3.53 (m, 3H, H-7, H-13, H-11), 3.70 (s, 3H, $-CO_2CH_3$), 3.05 (s, 6H, $-N(CH_3)_2$), 2.79 (m, 2H, H-2, H-14), 2.35 (m, 1H, H-26), 1.24 (d, 3H, J = 6.2 Hz, H-28), 1.15 (d, 3H, J = 7.1 Hz, H-2a), 1.04 (d, 3H, J = 6.9 Hz, H-26a). MS (ESI pos.) $(M + Na)^+$ 764.2.

5-DMAB (3)

^{1}H-NMR (500 MHz, CD_3OD) δ 7.88 (d, 2H, J = 8.9 Hz, Ar-H), 6.73 (d, J = 9.0 Hz, Ar-H), 6.43-6.18 (m, 7H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 6.14 (dd, J = 9.2 Hz, 15.5 Hz, H-24), 5.81 (dd, 1H, J = 7.4 Hz, 15.3 Hz, H-25), 5.59 (dd, 1H, J = 8.7 Hz, 14.1 Hz, H-16), 5.25 (m, 1H, H-5), 4.80 (1H, H-27, covered by the solvent peak), 4.32 (t, 1H, J = 9.2 Hz, H-15), 3.87 (t, 1H, J = 6.4 Hz, H-3), 3.80-3.59 (m, 2H, H-7, H-13), 3.69 (s, 3H, $-CO_2CH_3$), 3.58 (m, 1H, H-11), 3.05 (s, 6H, $-N(CH_3)_2$), 2.80 (dd, 1H, J = 4.3 Hz, 9.8 Hz, H-14), 2.36 (m, 2H, H-2, H-26), 1.23 (d, 3H, J = 6.2 Hz, H-28), 1.11 (d, 3H, J = 7.0 Hz, H-2a), 1.04 (d, 3H, J = 6.9 Hz, H-26a). MS (ESI pos.) $(M + Na)^+$ 764.3.

TABLE 3. UV/VIS absorption maxima of different polyene macrolides*

Polyene macrolides	Maxima (nm)		
Trienes	260–262	270–272	282–285
Tetraenes	290–293	300–305	317–322
Pentaenes	317–320	330–335	348–353
Methylpentaenes	320–324	338–342	355–360
Hexaenes	336–340	355–359	375–380
Heptaenes	358–366	377–388	399–410
Octaenes	372–378	395–405	420–425

*Reference 46.

7-DMAB (4)

^{1}H-NMR (500 MHz, CD_3OD) δ 7.85 (d, 2H, J = 9.0 Hz, Ar-H), 6.73 (d, J = 9.0 Hz, Ar-H), 6.43-6.22 (m, 7H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 6.13 (dd, J = 9.0 Hz, 15.5 Hz, H-24), 5.84 (dd, 1H, J = 7.0 Hz, 15.6 Hz, H-25), 5.59 (dd, 1H, J = 9.2 Hz, 14.3 Hz, H-16), 5.14 (m, 1H, H-7), 4.80 (1H, H-27, covered by the solvent peak), 4.31 (t, 1H, J = 9.8 Hz, H-15), 3.86 (m, 1H, H-5), 3.81 (t, 1H, J = 9.5 Hz, H-3), 3.69 (s, 3H, $-CO_2CH_3$), 3.63 (m, 1H, H-13), 3.56 (m, 1H, H-11), 3.05 (s, 6H, $-N(CH_3)_2$), 2.80 (dd, 1H, J = 4.2 Hz, 9.9 Hz, H-14), 2.32 (m, 2H, H-2, H-26), 1.23 (d, 3H, J = 6.2 Hz, H-28), 1.11 (d, 3H, J = 7.1 Hz, H-2a), 1.04 (d, 3H, J = 6.9 Hz, H-26a). MS (ESI pos.) $(M + Na)^+$ 764.2.

11-DMAB (5)

^{1}H-NMR (500 MHz, CD_3OD) δ 7.84 (d, 2H, J = 9.0 Hz, Ar-H), 6.71 (d, J = 9.0 Hz, Ar-H), 6.44-6.20 (m, 7H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 6.16 (dd, J = 9.7 Hz, 15.6 Hz, H-24), 5.93 (dd, 1H, J = 7.0 Hz, 15.6 Hz, H-25), 5.61 (dd, 1H, J = 9.0 Hz, 14.6 Hz, H-16), 5.13 (m, 1H, H-11), 4.80 (1H, H-27, covered by the solvent peak), 4.32 (t, 1H, J = 9.7 Hz, H-15), 3.94 (m, 1H, H-5), 3.79 (t, 1H, J = 7.1 Hz, H-3), 3.71-3.51 (m, 2H, H-7, H-13), 3.67 (s, 3H, $-CO_2CH_3$), 3.04 (s, 6H, $-N(CH_3)_2$), 2.84 (dd, 1H, J = 4.3 Hz, 10.4 Hz, H-14), 2.35 (m, 2H, H-2, H-26), 1.24 (d, 3H, J = 6.2 Hz, H-28), 1.14 (d, 3H, J = 7.0 Hz, H-2a), 1.06 (d, 3H, J = 6.9 Hz, H-26a). MS (ESI pos.) $(M + Na)^+$ 764.2.

13-DMAB (6)

^{1}H-NMR (500 MHz, CD_3OD) δ 7.80 (d, 2H, J = 9.0 Hz, Ar-H), 6.71 (d, J = 9.0 Hz, Ar-H), 6.43-6.22 (m, 7H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 6.16 (dd, J = 8.8 Hz, 16.1 Hz, H-24), 5.94 (dd, 1H, J = 6.9 Hz, 15.5 Hz, H-25), 5.68 (dd, 1H, J = 9.4 Hz, 14.8 Hz, H-16), 5.01 (dd, 1H, J = 3.3 Hz, 11.0 Hz, H-13), 4.80 (1H, H-27, covered by the solvent peak), 4.40 (t, 1H, J = 9.8 Hz, H-15), 3.95 (m, 1H, H-5), 3.80 (t, 1H, J = 7.9 Hz, H-3), 3.69 (s, 3H, $-CO_2CH_3$), 3.66 (m, 1H, H-7), 3.39 (m, 1H, H-11), 3.04 (s, 6H, $-N(CH_3)_2$), 2.99 (dd, 1H, J = 4.3 Hz, 10.3 Hz, H-14), 2.35 (m, 2H, H-2, H-26), 1.24 (d, 3H, J = 6.1 Hz, H-28), 1.16 (d, 3H, J = 7.0 Hz, H-2a), 1.07 (d, 3H, J = 7.0 Hz, H-26a). MS (ESI pos.) $(M + Na)^+$ 764.2.

15-DMAB (7)

^{1}H-NMR (500 MHz, CD_3OD) δ 7.77 (d, 2H, J = 9.0 Hz, Ar-H), 6.70 (d, J = 9.0 Hz, Ar-H), 6.53-6.20 (m, 7H, H-17, H-18, H-19, H-20, H-21, H-22, H-23), 6.15 (dd, 1H, J = 10.0 Hz, 15.2 Hz, H-24), 5.90 (dd, 1H, J = 7.0 Hz, 15.5 Hz, H-25), 5.66 (dd, 1H, J = 9.0 Hz, 17.2 Hz, H-16), 5.63 (t, 1H, J = 9.2 Hz, H-15), 4.80 (1H, H-27, covered by the solvent peak), 3.97 (m, 1H, H-13), 3.88-3.81 (m, 2H, H-5, H-3), 3.64 (s, 3H, $-CO_2CH_3$), 3.56 (m, 1H, H-11), 3.15 (dd, 1H, J = 3.7 Hz, 9.9 Hz, H-14), 3.03 (s, 6H, $-N(CH_3)_2$), 2.36 (m, 2H, H-2, H-26), 1.24 (d, 3H, J = 6.2 Hz, H-28), 1.14 (d, 3H, J = 7.0 Hz, H-2a), 1.06 (d, 3H, J = 6.9 Hz, H-26a). MS (ESI pos.) $(M + Na)^+$ 764.3.

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LITERATURE CITED

1. Schlingmann G, Milne L, Borders D, Carter GT. Strevertenes, antifungal pentaene macrolides produced by *Streptoverticillcum* LL-30F848. Tetrahedron 1999;55:5977–5990.
2. Omura S, Tanaka H. Production, structure and antifungal activity of polyene macrolides. In: Omura S, editor. Macrolide antibiotics: chemistry, biology and practice. New York: Academic Press; 1984. p 351–404.
3. Richardson TI, Rychnovsky SD. Filipin III: configuration assignment and confirmation by synthetic correlation. J Org Chem 1996;61:4219–4231.
4. Rychnovsky SD. Oxo polyene macrolide antibiotics. Chem Rev 1995; 95:2021–2040.
5. Mechlinski W, Schaffner CP, Ganis P, Avitabile G. Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. Tetrahedron Lett 1970;44:3873–3876.
6. Maehr H, Yang R, Hong L-N, Liu C-M, Hatada MH, Todaro LJ. Microbial products. 9. Roxaticin, a new oxo pentaene antibiotic. J Org Chem 1989;54:3816–3819.
7. Rychnovsky SD, Rogers BN, Richardson TI. Configurational assignment of polyene macrolide antibiotics using the [^{13}C]acetonide analysis. Acc Chem Res 1998;31:9–17.
8. Rychnovsky SD, Richardson TI, Rogers BN. Two-dimensional NMR analysis of acetonide derivatives in the stereochemical assignment of polyol chains: the absolute configurations of dermostatins A and B. J Org Chem 1997;62:2925–2934.
9. Rychnovsky SD, Griesgraber G, Schlegel R. Stereochemical assignment of roflamycoin by ^{13}C acetonide analysis. J Am Chem Soc 1994; 116:2623–2624.
10. Rychnovsky SD, Richardson TI. Relative and absolute configuration of filipin III. Angew Chem Int Ed Engl 1995;34:1227–1230.
11. Richardson TI, Rychnovsky SD. Total synthesis of filipin III. Tetrahedron 1999;55:8977–8996.
12. Schreiber SL, Goulet MT. Application of the two-directional chain synthesis strategy to the first stereochemical assignment of structure to members of the skipped-polyol polyene macrolide class: mycoticin A and B. J Am Chem Soc 1987;109:8120–8122.
13. Schreiber SL, Goulet MT. Stereochemical studies of the skipped-polyol polyene macrolide class: degradation and partial structure determination of mycoticin A and B. Tetrahedron Lett 1987;28:6001–6004.
14. Schreiber SL, Goulet MT, Sammakia T. Stereochemical studies of the skipped-polyol polyene macrolide class: NMR studies of a tetraformylal derivative of mycoticin A and B. Tetrahedron Lett 1987;28:6005–6008.
15. Pawlak J, Nakanishi K, Iwashita T, Borowski E. Stereochemical studies of polyols from the polyene macrolide lienomycin. J Org Chem 1987;52:2896–2901.
16. Prandi J, Beau J-M. Stereostructure of nystatin A1: a synthetic assignment of the C1-C10 fragment. Tetrahedron Lett 1989;30:4517–4520.
17. Lancelin J-M, Beau J-M. Complete stereostructure of nystatin A1: a proton NMR study. Tetrahedron Lett 1989;30:4521–4524.
18. Oishi T. Studies directed towards the stereoselective synthesis of polyene macrolide antibiotics. Pure Appl Chem 1989;61:427–430.
19. Lancelin J-M, Beau J-M. Stereostructure of pimaricin. J Am Chem Soc 1990;112:4060–4061.
20. Lancelin J-M, Beau J-M. Stereostructure of glycosylated polyene macrolides: the example of pimaricin. Bull Soc Chim Fr 1995;132:215–223.
21. Pawlak J, Sowinski P, Borowski E, Gariboldi P. Stereostructure and NMR characterization of the antibiotic candidin. J Antibiot 1993;46: 1598–1604.
22. Sowinski P, Gariboldi P, Pawlak JK, Borowski E. The structure of vacidin A. an aromatic heptaene macrolide antibiotic. II. Stereochemistry of the antibiotic. J Antibiot 1989;42:1639–1642.
23. Harada N, Nakanishi K. Circular dichroic spectroscopy-exciton coupling in organic stereochemistry. Mill Valley, CA: University Science Books; 1983. p 304–436.
24. Nakanishi K, Berova N. The exciton chirality method. In: Nakanishi K,

Berova N, Woody R, editors. Circular dichroism, principles and applications. New York: VHC Publishers; 1994. p 361–398.

25. Harada N, Saito A, Ono H, Gawronski J, Gawronska K, Sugioka T, Uda H, Kuriki T. A CD method for determination of the absolute stereochemistry of acyclic glycols. 1. Application of the CD exciton chirality method to acyclic 1,3-dibenzoate systems. J Am Chem Soc 1991;113: 3842–3850.
26. Zhao N, Zhou P, Berova N, Nakanishi K. Combined synthetic/CD strategy for the preparation and configurational assignments of model acyclic 1,3-polyols with a 1,2-diol terminal. Chirality 1995;7:636–651.
27. Zhou P, Berova N, Wiesler WT, Nakanishi K. Assignment of relative and absolute configuration of acyclic polyols and aminopolyols by circular dichroism — trends follow Fischer's sugar family tree. Tetrahedron 1993;49:9343–9352.
28. Zhou P, Zhao N, Rele DN, Berova N, Nakanishi K. A chiroptical/chemical strategy for configurational assignments of acyclic 1,3-skipped polyols: model 1,2,4,6-tetrols. J Am Chem Soc 1993;115:9313–9314.
29. Mori Y, Kohchi Y, Suzuki M, Furukawa H. Determination of absolute stereochemistry of acyclic 1,3-polyols by a difference CD method. J Am Chem Soc 1992;114:3557–3559.
30. Mori Y, Furukawa H. A difference CD method for determining absolute stereochemistry of acyclic 1,2,4-triols. Tetrahedron 1995;51:6725–6738.
31. Mori Y, Sawada T, Sasaki N, Furukawa H. A simple strategy for determining the absolute configurations of acyclic 1,2,4,6,8-pentols. J Am Chem Soc 1996;118:1651–1656.
32. Ganis P, Avitabile G, Mechlinski W, Schaffner CP. Polyene macrolide antibiotic amphotericin B. Crystal structure of the N-iodoacetyl derivative. J Am Chem Soc 1971;93:4560–4564.
33. Sowinski P, Gariboldi P, Czerwinski A, Borowski E. The structure of vacidin-A, an aromatic heptaene macrolide antibiotic. 1. Complete assignment of the H-1-NMR spectrum and geometry of the polyene chromophore. J Antibiot 1989;42:1631–1638.
34. Balakrishnan AR, Easwaran KRK. Conformation of polyene antibiotic, filipin III: CD and ^{1}H NMR studies. J Biomol Struct Dyn 1993;11:417–428.
35. Sowinski P, Pawlak J, Borowski E, Gariboldi P. ^{1}H NMR model studies of amphotericin B: comparison of X-ray and NMR stereochemical data. Magn Reson Chem 1992;30:275–279.
36. Humpf H-U, Berova N, Nakanishi K, Jarstfer MB, Poulter CD. Allylic and homoallylic exciton coupled CD: a sensitive method for determining the absolute stereochemistry of natural products. J Org Chem 1995;60:3539–3542.
37. Gonella NC, Nakanishi K, Martin VS, Sharpless KB. General method for determining absolute configurations of acyclic allylic alcohols. J Am Chem Soc 1982;104:3775–3776.
38. Derguini F, Nakanishi K, Hammerling U, Buck J. Intracellular signaling activity of synthetic (14R)-, (14S)-, and (14RS)-14-hydroxy-4,14-retro-retinol. Biochemistry 1994;33:623–628.
39. Humpf H-U, Zhao N, Berova N, Nakanishi K. Absolute stereochemistry of natural 3,4-dihydroxy-β-ionone glycosides by the CD exciton chirality method. J Nat Prod 1994;57:1762–1765.
40. Flores-Parra A, Paredes-Tepox C, Joseph-Nathan P, Contreras R. Preparation of new quinic acid esters in aprotic media. Tetrahedron 1990;46:4137–4148.
41. Frechet JMJ, Nuyens LJ, Seymour E. Use of polymers as protecting groups in organic synthesis. Application of polystyrylboronic acid to the one-pot synthesis of acylated carbohydrate derivatives. J Am Chem Soc 1979;101:432–436.
42. Guedat P, Rehnberg N, Spiess B, Schlewer G. Synthesis of α-trinositol analogues derived from D-(–)-quinic acid as affinity probe precursors. Synlett 1997;5:553–554.
43. Hashimoto N, Aoyama T, Shioiri T. New methods and reagents in organic synthesis. 14. A simple efficient preparation of methyl esters with trimethylsilyldiazomethane (TMSCHN2) and its application to gas chromatographic analysis of fatty acids. Chem Pharm Bull 1981; 29:1475–1478.
44. Cai G, Bozhkova N, Odingo J, Berova N, Nakanishi K. CD exciton chirality method. New red-shifted chromophores for hydroxyl groups. J Am Chem Soc 1993;115:7192–7198.
45. Hoffmann RW, Stenkamp D. Conformation preferences in 2,4,6-trisubstituted heptanes. Tetrahedron 1999;55:7169–7176.
46. Berdy J. CRC handbook of antibiotic compounds. Boca Raton, FL: CRC Press; 1980.