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Determination of the Absolute Configuration of the Chiral Biaryl System in the Streptonigrin Antibiotics by Exciton Coupled Circular Dichroic Spectroscopy

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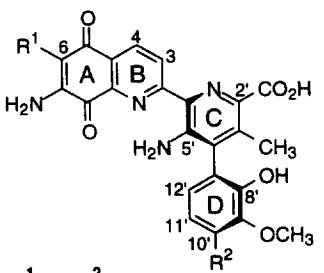
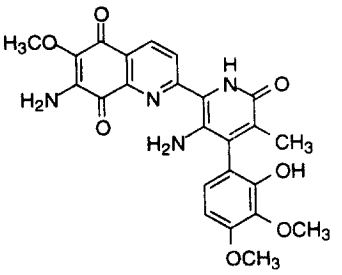
Abstract: The absolute configuration of the chiral phenylpyridyl segment of the Actinomycete antibiotic streptonigrin (**1**) is defined as *R* by analysis of the exciton coupled circular dichroic spectra of selected derivatives. The absolute configuration of the co-occurring 10'-*O*-demethylstreptonigrin (**2**) is assigned as *R* by chiroptical correlation with streptonigrin. Circular dichroic spectroscopy indicates that the related phenylpyridone streptonigrone (**5**) is either achiral or a racemate.

INTRODUCTION

Streptonigrin was first isolated in America in 1959 from cultures of *Streptomyces flocculus*.[1,2] The same dark brown crystalline compound was subsequently isolated from other Actinomycete species in France and the Soviet Union under the names rufochromomycin and bruneomycin. The then unique tetracyclic phenylpyridyl-quinolinequinone structure of streptonigrin (**1**), without regard to configuration, was established by degradative and spectral studies,[3] and was later confirmed by X-ray crystallography.[4] A range of related antibiotics, including the phenylpyridines 10'-*O*-demethylstreptonigrin (**2**),[5] 6-*O*-demethylstreptonigrin (**3**)[6] and 10'-demethoxystreptonigrin (**4**),[7] the phenylpyridone streptonigrone (**5**),[8,9] and the β-carboline lavendamycin[10] have since been isolated. Several syntheses of streptonigrin and its congeners, including streptonigrone, have been published.[11] Streptonigrin itself possesses broad spectrum antibiotic and antitumour activity[12] together with potent cytotoxic and antiviral properties.[13,14,15]



	R¹	R²	
1	OCH_3	OCH_3	Streptonigrin
2	OCH_3	OH	10'-*O*-Demethylstreptonigrin
3	OH	OCH_3	6-*O*-Demethylstreptonigrin
4	OCH_3	H	10'-Demethoxystreptonigrin



5 Streptonigrone

Note: The absolute configurations depicted derive from the present work.

Crystallographic studies[4] revealed that the plane of the phenolic D-ring of streptonigrin (**1**) is almost perpendicular to the ABC-ring system, the pyridyl C-ring being held essentially coplanar with the AB-quinolinequinone by hydrogen bonding between the pyridyl 5'-amino group and the quinoline nitrogen. Thus streptonigrin potentially possessed axial chirality about the phenylpyridyl CD-linkage. The existence of such chirality in solution, reflecting restriction of rotation about this linkage by the three substituents *ortho* to it,

was confirmed by measurement of the circular dichroic (CD) spectrum of streptonigrin.[16] Neither the crystallographic nor the CD study established the absolute configuration of streptonigrin, although the latter authors suggested the *S* configuration without substantive evidence.[17] We now define the absolute configuration of streptonigrin (**1**) and 10'-*O*-demethylstreptonigrin (**2**), and comment on the chirality of 6-*O*-demethylstreptonigrin (**3**), 10'-demethoxystreptonigrin (**4**), and streptonigrone (**5**).

Conventional applications of chiroptical spectroscopy to the determination of the absolute configuration of organic compounds involve empirical correlations of their spectra with those of structurally related reference compounds of known chirality. This approach is not applicable in the case of the streptonigrin antibiotics, since appropriate reference compounds are lacking. Exciton coupled circular dichroic (ECCD) spectroscopy, however, permits the direct determination in solution of the absolute configuration of compounds containing two or more interacting chromophores.[18,19] The process depends upon the intramolecular coupling of close-lying energy levels of two chromophores whose electric transition dipole moments are in a chiral relationship, resulting in split Cotton effects of mutually opposite signs in CD spectra. The signs of the Cotton effects are non-empirically defined by the absolute twist between the electric transition dipole moments, *i.e.* if the twist is clockwise then the Cotton effect at longer wavelength is positive, and *vice versa*.

RESULTS AND DISCUSSION

Streptonigrin (1), 10'-*O*-Demethylstreptonigrin (2) and Streptonigrone (5)

Streptonigrin (**1**), 10'-*O*-demethylstreptonigrin (**2**) and streptonigrone (**5**) were obtained by fermentation of an unidentified *Streptomyces* species (IA-CAS isolate No. 144).[8] The application of ECCD spectroscopy to the determination of their chirality necessitates first some consideration of the electronic absorption spectrum of streptonigrin. In order to obviate possible complications in the electronic and chiroptical spectra of streptonigrin (**1**) and its derivatives due to varying extents of zwitterion formation between the free carboxyl function and the adjacent pyridyl nitrogen, as has been observed for streptonigrin itself in different organic solvents,[20] the antibiotic was converted into its methyl ester (**6**) by treatment with diazomethane (Scheme 1). This esterification also facilitated selective derivatisation and subsequent chromatographic purification of the products.

The electronic spectra of streptonigrin (**1**) and its methyl ester (**6**) in ethanol display similar intense maxima at 247, 295 and 379 nm, with much weaker absorption extending beyond 500 nm (Figs 1 and 2). These spectra represent primarily the dominant absorption of the pyridylquinolinequinone ABC-ring system, upon which is superimposed the lesser absorption of the out-of-plane trioxygenated phenyl D-ring.[21] The planarity of the ABC-ring system, originally observed in the solid state,[4] has recently been shown by NMR studies to be preserved in tetrahydrofuran solution.[20] Although the hydrogen bond which stabilises this conformation would be expected to be of lesser significance in the ethanol solution used for the present spectra than in the non-hydroxylic tetrahydrofuran, there is nevertheless no steric reason for the C-ring not to be in the AB-ring plane, and thus conjugated with the AB-rings. This expectation is confirmed by the bathochromic effects observed upon adding a 2-(2'-pyridyl) substituent to 7-amino-6-methoxy-5,8-quinolinequinone, whereupon even in the absence of any possible hydrogen bond the intense UV absorption maxima alter from 232 and 269 nm to 250, 274 and 315 nm.[22,23] The addition of a 2-{6'-(4'-aryl-2',3'-dimethylpyridyl)} substituent has a similar effect, producing intense maxima at 260 and 317 nm with a weak maximum also being reported at 484 nm.[24] The extra trimethoxyphenyl D-ring in this latter example has negligible effect on the 315 nm maximum, presumably because even with only two substituents *ortho* to the biaryl linkage the C and D-rings prefer a non-planar conformation. The presence of 5'-amino, 2'-carboxy and 3'-methyl substituents on the attached pyridyl ring as in streptonigrin (**1**) and its ester (**6**), however, does result in a further bathochromic shift, the intense maxima now occurring at 247, 295 and 379 nm (Figs 1 and 2).

In these 2-pyridyl-substituted 5,8-quinolinequinones the additional substituent in the heterocyclic ring has compensated for the electronegativity of the quinolinequinone nitrogen, and their electronic spectra now

resemble those of 1,4-naphthaquinones in possessing an intense absorption band above 300 nm. Similar effects have been observed with alkyl substitution in the heterocyclic ring of 5,8-quinolinequinones.[25] This intense long wavelength absorption band at 328 nm of 1,4-naphthaquinone[26] is known to arise from the longitudinal electric transition moment directed along the long axis of the chromophore. The corresponding bands in these 2-pyridyl-substituted 5,8-quinolinequinones, and in particular the 379 nm absorption band in streptonigrin (**1**) and its ester (**6**), must similarly arise from electric transition moments directed along the long axis of the extended planar ABC-ring system.

The CD spectrum of streptonigrin (**1**) in ethanol in our hands displays a series of weak extrema at 237 ($\Delta\varepsilon$ 1.5), 252 (4.7), 277 (1.3), 302 (3.1), and 362 nm (0.2) (Fig. 1).[27] This spectrum differs substantially from that of Fiallo and Garnier-Suillerot[28] recorded between 300-600 nm in aqueous potassium chloride containing HEPES buffer, possibly because of the major solvent change. More surprising was the significant divergence from that of Dholakia and Gillard,[16] which was recorded in the same solvent, ethanol. The cause of this latter difference is unclear, but may reflect the known difficulty in obtaining satisfactory chiroptical data from solutes with intense UV absorption, and/or the presence of different ionisation states resulting from different pH of the solvent (see above). Our CD spectrum of streptonigrin methyl ester (**6**) (Fig. 2) was similar to that of streptonigrin (**1**). Direct analysis of the possible exciton couplings in these spectra in order to determine absolute configuration was deemed inadvisable, however, due to the relatively weak nature of the Cotton effects and the lack of understanding of which electric transition moments are interacting. Derivatisation with dominant chromophores having defined electric transition moments was therefore necessary, and under conditions which would avoid the known tendency of the chiral CD-ring system of streptonigrin to racemise on heating.[29]

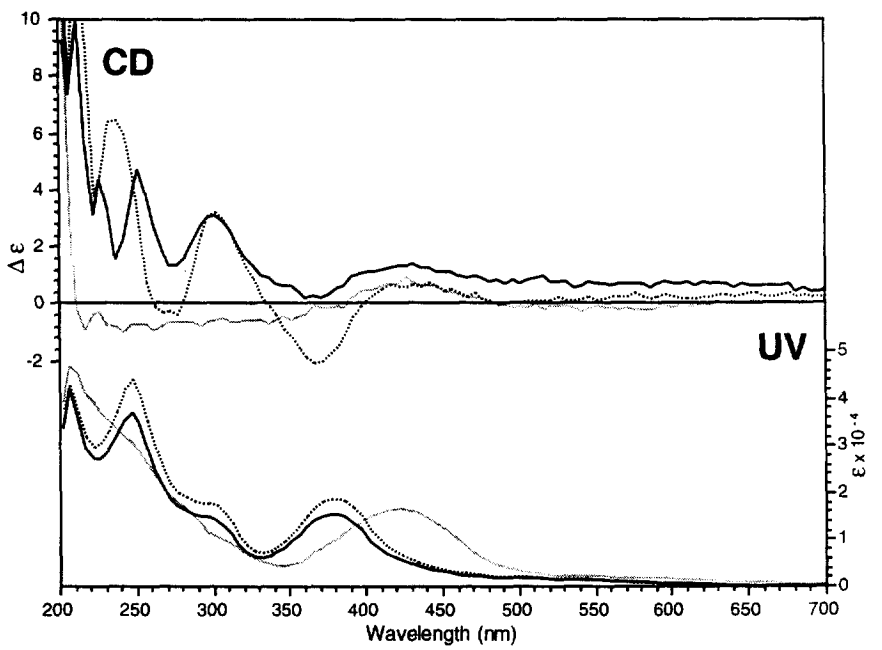


Fig. 1. Electronic and CD spectra of streptonigrin (**1** —), 10'-*O*-demethylstreptonigrin (**2** ·····) and streptonigrone (**5** – –).

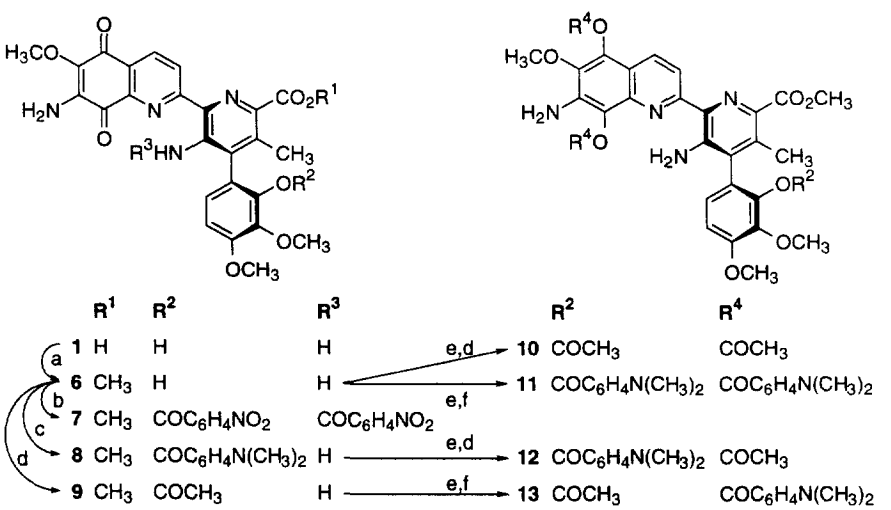
10'-*O*-Demethylstreptonigrin (**2**) co-occurs with streptonigrin (**1**),[5] suggesting a common biosynthetic pathway and absolute configuration. This is confirmed by comparison of their CD spectra, which reveals close

similarity above 250 nm (Fig. 1). The validity of this comparison is contingent upon the absence of the 10'-*O*-methyl group, as in demethylstreptonigrin (**2**), not significantly altering the D-ring chromophore of streptonigrin (**1**), an expectation which is supported by the correspondence of their electronic absorption spectra (Fig. 1). The configuration defined for streptonigrin will thus apply also to 10'-*O*-demethylstreptonigrin.

Streptonigrone (**5**) also co-ccurs with streptonigrin (**1**),[8,9] and differs structurally only in that the C-ring is now a 2-pyridone rather than a pyridine carboxylic acid. The CD-ring system of streptonigrone is thus no longer a biaryl, and whether the rotational energy barrier about the linking bond, which still bears three "ortho" substituents, is sufficient to confer axial chirality depends upon the planarity and flexibility of the pyridone ring. Circular dichroic spectroscopy shows that streptonigrone is optically inactive (Fig. 1). This indicates that streptonigrone (**5**) is either inherently achiral or a racemate, alternatives which differ only in the size of the rotational energy barrier.

5',8'-Di-*N,O*-*p*-nitrobenzoyl Derivative 7

The 8'-phenolic and 5'-amino groups of streptonigrin methyl ester (**6**) are ideally located about the phenylpyridyl CD-linkage for acylation with chromophoric aromatic acids suitable for ECCD spectroscopy. Furthermore, it is known that selective acylation of these groups, initially of the 8'-phenol under mild conditions then subsequently of the 5'-amino group under forcing conditions, can be achieved in the presence of the less nucleophilic 7-amino group.[30] Accordingly the 5',8'-di-*N,O*-*p*-nitrobenzoyl derivative **7** was prepared (Scheme 1), and its structure confirmed by HRMS and NMR spectroscopy. In particular, acylation of the 8'-hydroxyl group was verified by the significant downfield shifts of the resonances of the two D-ring protons,[31] and of the 5'-amino group by the continued presence of the two protons of the 7-amino group at δ 5.22. These latter protons in the parent methyl ester (**6**) resonate at δ 5.08, and are identifiable as such by a 3-bond correlation with the C8-carbonyl carbon visible in a long-range HETCOR spectrum. As expected, the di-*p*-nitrobenzoyl derivative **7** showed enhanced electronic absorption in the vicinity of 260 nm arising from the *p*-nitrobenzoyl chromophores, together with broad absorption between 300-340 nm reflecting the hypsochromic effect of N-acylation upon the ABC-ring chromophore of the methyl ester **6** (Fig. 2).



Scheme 1. Reagents and conditions: a) CH_2N_2, CH_2Cl_2, 0 °C to r.t., 87%; b) *p*-$NO_2C_6H_4COCl$, DMAP, CH_2Cl_2, reflux, 46%; c) *p*-$(CH_3)_2NC_6H_4CO_2C_6H_3(NO_2)_2$, DMAP, CH_2Cl_2, reflux, 93%; d) Ac_2O, DMAP, CH_2Cl_2, r.t., **9** 69%, **10** 37% (2 steps), **12** 55% (2 steps); e) H_2, Pd/C (10%), acetone, r.t.; f) *p*-$(CH_3)_2NC_6H_4CO_2C_6H_3(NO_2)_2$, DMAP, CH_2Cl_2, **11** 70% (2 steps), **13** 37% (2 steps).

The CD spectrum of the di-*p*-nitrobenzoyl derivative **7** displays weak to moderate intensity extrema at 232 (Δε -3.4), 245 (5.1), 271 (-15.6), and 340 nm (7.5) (Fig. 2). Exciton coupling of the two *p*-nitrobenzoyl chromophores would be expected to result in Cotton effects centred around ~260 nm, and the adjacent positive and negative extrema at 245 and 271 nm may reflect this interaction. If this is true, the electric transition moments of the longitudinal axis of the *p*-nitrobenzoyl chromophores should be related by an anticlockwise twist (*cf.* moments X and Z in Fig. 2), leading to the conclusion that the absolute configuration of the biaryl CD-ring linkage of streptonigrin (**1**) is *R*. This conclusion is at best tentative, however, due to the possible superimposition of the absorption maxima of the *p*-nitrobenzoyl chromophores upon maxima of the AB-ring system, resulting in possible contributions from unknown exciton interactions.

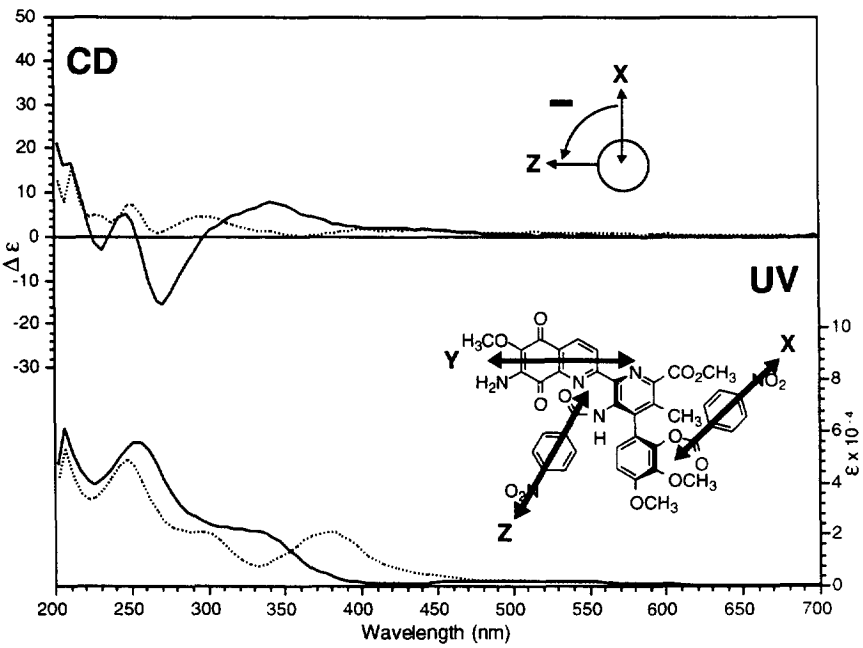


Fig. 2. Electronic and CD spectra of streptonigrin methyl ester (**6** ·····) and its 5',8'-di-*N,O*-*p*-nitrobenzoyl derivative **7** (—).

8'-*p*-Dimethylaminobenzoate 8 and 8'-Acetate 9

In comparison to the *p*-nitrobenzoate chromophore, where the electric transition moment along the long axis of the chromophore gives rise to an absorption maximum at 260 nm with ε_{max} 15,100, the corresponding transition of the *p*-dimethylaminobenzoate group occurs at longer wavelength, λ_{max} 310 nm, with double the intensity, ε_{max} 30,400.[18] A similar relationship holds for the corresponding *p*-nitro- and *p*-dimethylaminobenzamide chromophores. These characteristics make both the electronic absorption and the related exciton coupling arising from *p*-dimethylaminobenzoyl derivatives more readily identifiable than those from *p*-nitrobenzoyl derivatives, particularly against the background of a complex chromophoric system as in streptonigrin. Attempts to *p*-dimethylaminobenzoylate both the 5'-amino and 8'-phenolic groups of streptonigrin methyl ester (**6**) using 1,3-dicyclohexylcarbodiimide, 4-dimethylaminopyridine (DMAP) and *p*-dimethylaminobenzoic acid at 40 °C, however, yielded only the impure 8'-*p*-dimethylaminobenzoate **8**. Although this was not the desired *p*-dimethylaminobenzoyl analogue of the di-*p*-nitrobenzoyl derivative **7**, its chiroptical properties were nevertheless of interest. This *p*-dimethylaminobenzoate **8** was better prepared by

treatment of the ester **6** with 2,4-dinitrophenyl *p*-dimethylaminobenzoate and DMAP at 40 °C (Scheme 1). Its structure was confirmed by the downfield NMR shifts of the C11' and C12' aryl protons, consistent with phenolic acylation.[31] The corresponding 8'-acetate **9** (Scheme 1) showed similar acylation shifts.

The electronic spectra of the *p*-dimethylaminobenzoate **8** and acetate **9** both display major absorption bands at 244 and 378 nm (Fig. 3). The latter maximum corresponds accurately to the 379 band in unacylated streptonigrin (**1**) and its methyl ester (**6**), being derived from the longitudinal electric transition moment of the unaltered ABC-ring system (see above). The two spectra differ in the 300 nm region, where the shoulder at 285 nm in the acetate **9** is dominated by the expected intense maximum at 315 nm in the *p*-dimethylaminobenzoate **8**. The key feature of the CD spectrum of the *p*-dimethylaminobenzoate **8** is the pair of intense Cotton effects of opposite sign at 321 ($\Delta\varepsilon$ 39.3) and 361 nm (-14.3) (Fig. 3). These extrema ($\Delta\Delta\varepsilon$ = 53.6) centred around 341 nm can be assigned to exciton coupling between the longitudinal transitions of the *p*-dimethylaminobenzoate and ABC-ring chromophores absorbing at 315 and 378 nm. Since the sign of the longer wavelength extremum is negative and of the shorter wavelength extremum positive, then the electric transition moments of the coupled chromophores are related by an anticlockwise twist (moments X and Y in Fig. 3). ECCD spectroscopy thus defines the absolute configuration about the phenylpyridyl CD-ring linkage of streptonigrin methyl ester 8'-*p*-dimethylaminobenzoate (**8**), and thus of streptonigrin (**1**) itself, as *R*. This assignment of the observed Cotton effects and the resulting configurational conclusion assume that there is no significant exciton interaction between the esterified D-ring and the pyridylquinolinequinone ABC-ring system. This was confirmed by the CD spectrum of the acetate **9** which would display such an interaction if present, but which shows no significant Cotton effects in the 300-400 nm range (Fig. 3).

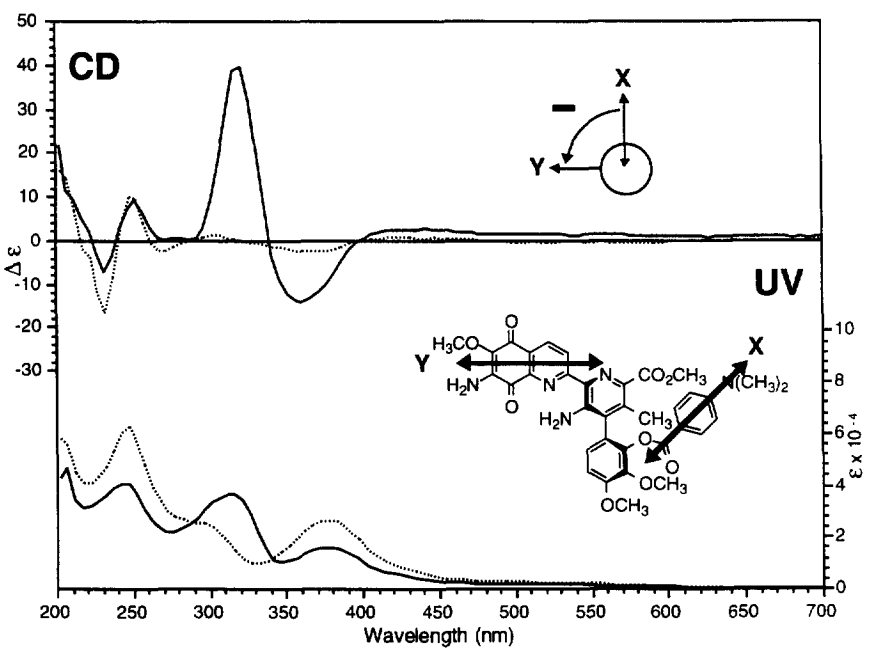


Fig. 3. Electronic and CD spectra of the 8'-*p*-dimethylaminobenzoate **8** (—) and 8'-acetate **9** (·····).

This assignment of the *R* configuration to streptonigrin (**1**), based upon electronic interaction between the introduced aroyl substituent and the ABC-ring system in the 8'-*p*-dimethylaminobenzoyl methyl ester **8**, is in agreement with the tentative conclusion drawn from consideration of the mutual aroyl interactions in the

5',8'-di-*N,O*-*p*-nitrobenzoyl methyl ester **7**. Confirmation of this configurational conclusion was derived from analysis of further derivatives of streptonigrin.

Dihydro-5,8-diacetate-8'-*p*-dimethylaminobenzoate 12 and Dihydro-5,8,8'-triacetate 10

The use of a quinolinequinone chromophore in ECCD analysis, as in the *p*-dimethylaminobenzoate **8** above, is to the authors' knowledge unprecedented. In contrast, the quinoline chromophore has been used in several applications of ECCD analysis,[18] and the intense long wavelength absorption band of the parent heterocycle at 315 nm is known to originate in the longitudinal electric transition moment directed along the long axis of the chromophore.[32] Our attention was thus drawn to the possible reduction of the pyridyl-substituted quinolinequinone ABC-ring system to provide a pyridylquinoline chromophore that could interact with a C8'-*p*-dimethylaminobenzoate ester. Such reduction was achieved by treatment of the *p*-dimethylaminobenzoate **8** with hydrogen over palladised charcoal, followed by immediate acetylation of the air sensitive 5,8-quinolinehydroquinone to afforded the dihydro-5,8-diacetate-8'-*p*-dimethylaminobenzoate **12** (Scheme 1). The structure of the diacetate **12** was established by FABMS, and in particular by ^{1}H NMR spectroscopy which indicated the presence of two acetate methyl groups at δ 2.48 and 2.47 and the two-proton signal of the 7-amino group at δ 4.25, confirming *O,O*-diacetylation of the 5,8-hydroquinone. The corresponding dihydro-5,8,8'-triacetate **10** was prepared by similar reductive acetylation of streptonigrin methyl ester (**6**) (Scheme 1).

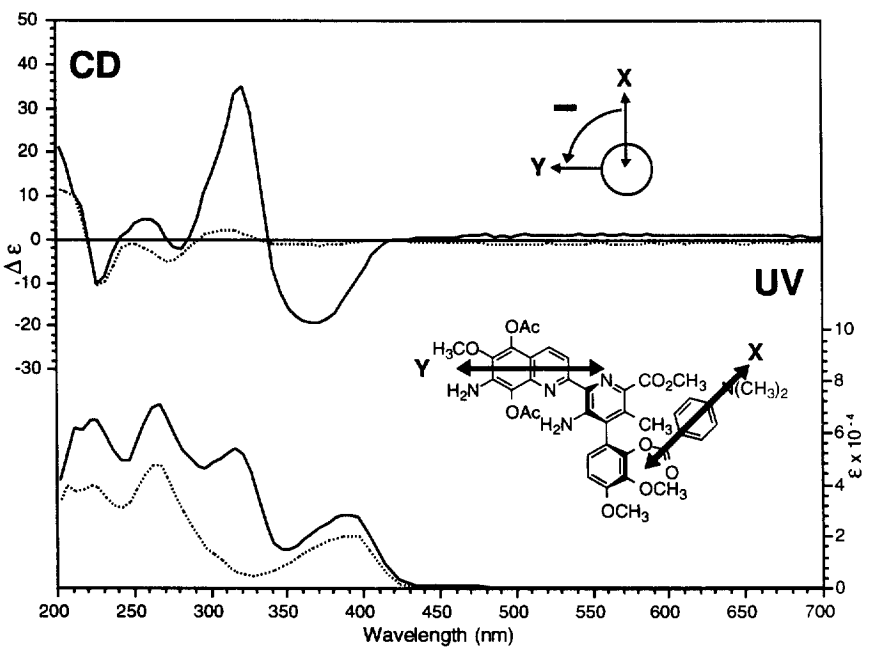


Fig. 4. Electronic and CD spectra of the dihydro-5,8-diacetate-8'-*p*-dimethylaminobenzoate **12** (—) and the dihydro-5,8,8'-triacetate **10** (·····).

The electronic spectra of the triacetate **10** and the diacetate **12** show similar intense absorption maxima near 265 and 390 nm, the latter band arising from the longitudinal electric transition moment of the pyridylquinoline ABC-ring system (Fig. 4). The diacetate **12,** however, shows an additional strong band at 317 nm due to the presence of the *p*-dimethylaminobenzoate chromophore. As anticipated, the CD spectrum of the diacetate **12** displays a pair of intense Cotton effects of opposite sign at 321 (Δε 34.9) and 370 nm (-19.3) (ΔΔε 54.2), reflecting exciton coupling between the longitudinal transitions of the 8'-*p*-dimethylaminobenzoate and

ABC-ring chromophores (Fig. 4). The lack of significant Cotton effects in this region in the CD spectrum of the triacetate **10** verifies the absence of interfering exciton interactions and confirms this interpretation. The longer wavelength extremum is again negative, establishing that an anticlockwise twist exists between the transition moments of the exciton coupled chromophores (moments X and Y in Fig. 4). Hence ECCD spectroscopy unambiguously determines the absolute configuration of the diacetate **12** to be *R*, in agreement with the previous assignments for streptonigrin (**1**).

Dihydro-5,8,8'-tri-*p*-dimethylaminobenzoate 11 and Dihydro-8'-acetate-5,8-di-*p*-dimethylaminobenzoate 13

In all the aroyl derivatives of streptonigrin (**1**) prepared so far, the longitudinal axes of the interacting chromophores used for ECCD analysis have been related in space by an anticlockwise twist. We sought to design a derivative in which this twist was clockwise, as evidenced by the longer wavelength extremum of the resulting split Cotton effects being positive rather than negative, and recognised that *p*-dimethylaminobenzoylation of streptonigrin 5,8-hydroquinone, in the presence of an 8'-*p*-dimethylaminobenzoate chromophore, would accomplish this goal. Accordingly, streptonigrin methyl ester (**6**) was hydrogenated and the 5,8-hydroquinone and 8'-hydroxyl groups were simultaneously benzoylated to give the dihydro-5,8,8'-tri-*p*-dimethylaminobenzoate **11** (Scheme 1), the structure of which was confirmed by FABMS and ^{1}H and ^{13}C NMR spectroscopy. Similar reductive benzoylation of the 8'-acetate **9** afforded the dihydro-8'-acetate-5,8-di-*p*-dimethylaminobenzoate **13**.

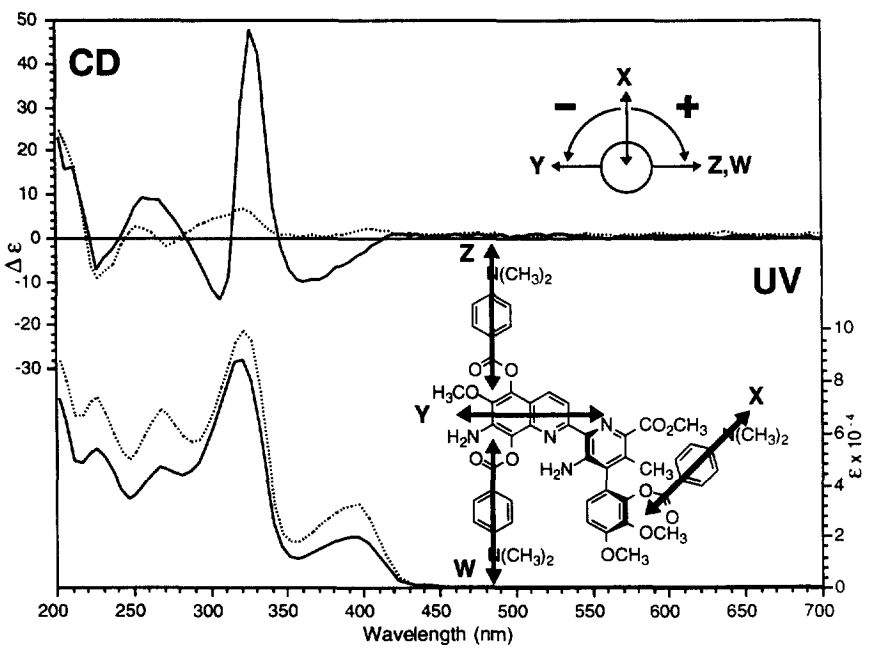


Fig. 5. Electronic and CD spectra of the dihydro-5,8,8'-tri-*p*-dimethylaminobenzoate **11** (——) and dihydro-8'-acetate-5,8-di-*p*-dimethylaminobenzoate **13** (·····).

The electronic spectra of the 5,8,8'-tri-*p*-dimethylaminobenzoate **11** and the corresponding 8'-acetate-5,8-di-*p*-dimethylaminobenzoate **13** are similar, being dominated by the intense 318 nm bands arising principally from the longitudinal electric transition moments of the *p*-dimethylaminobenzoate chromophores (Fig. 5). Also notable are the maxima at 399 nm reflecting the longitudinal moments of the

aroylated pyridylquinoline ABC-ring systems. The CD spectrum of the tri-*p*-dimethylaminobenzoate **11** (Fig. 5) displays a series of extrema of alternating sign at 225 ($\Delta\varepsilon$ -7.4), 257 (9.1), 308 (-14.4), 327 (47.9), and 362 nm (-10.3). These extrema must reflect, *inter alia*, exciton coupling between the longitudinal moment of the 8'-*p*-dimethylaminobenzoate chromophore and the longitudinal moments of the pyridylquinoline ABC-ring system (moments X and Y in Fig. 5) and of the 5- and 8-*p*-dimethylaminobenzoate chromophores (moments X and W, Z in Fig. 5). The former coupling will give rise to the negative long wavelength extremum at 362 nm, the split Cotton effect partner of which will contribute to the intense positive extremum at 327 nm. This interaction parallels that seen clearly in the 5,8-diacetate-8'-*p*-dimethylaminobenzoate **12.** Coupling between the *p*-dimethylaminobenzoate chromophores themselves must contribute to the 327 nm extremum, and is probably also reflected in the negative extremum at 308 nm. No exciton coupling would be expected, however, between the 5- and 8-*p*-dimethylaminobenzoate chromophores, or between these chromophores and the pyridylquinoline ABC-ring chromophore, due to their co-planarity. The validity of this analysis is established by the lack of intense Cotton effects in the 300-400 nm region of the CD spectrum of the 8'-acetate-5,8-di-*p*-dimethylaminobenzoate **13**, showing that *all* the relevant Cotton effects in the spectrum of the tri-*p*-dimethylaminobenzoate **11** derive from interactions with the 8'-*p*-dimethylaminobenzoate chromophore. As with the 8'-*p*-dimethylaminobenzoate **8** and the 5,8-diacetate-8'-*p*-dimethylaminobenzoate **12**, interactions involving the esterified D-ring do not interfere with the ECCD analysis.

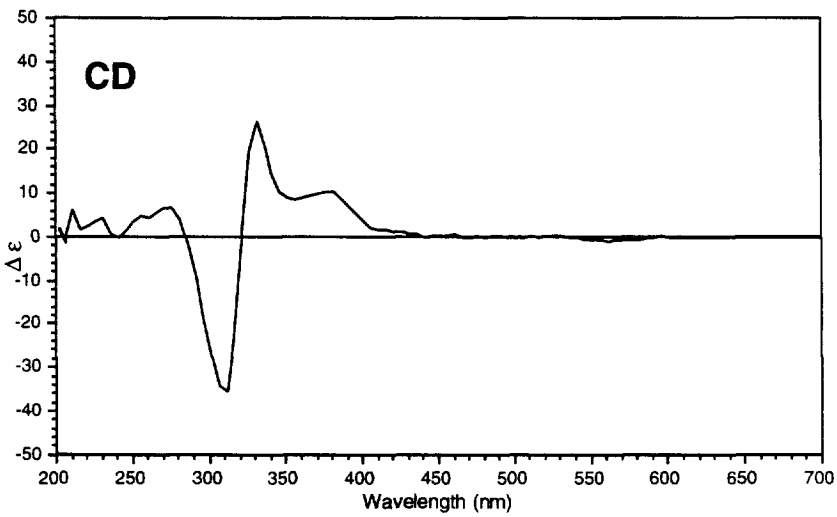


Fig. 6. CD difference spectrum of the dihydro-5,8-diacetate-8'-*p*-dimethylaminobenzoate **12** subtracted from that of the dihydro-5,8,8'-tri-*p*-dimethylaminobenzoate **11**.

Precise ECCD analysis of the 300-400 nm region of the CD spectrum of the 5,8,8'-tri-*p*-dimethylaminobenzoate **11** is hindered by the overlapping exciton interactions referred to above. The negative extremum at 308 nm, however, suggests that a positive split Cotton effect partner is contributing to the intense positive extremum at 327 nm. In order to expose this possible exciton coupling between the 8' and the 5- and 8-*p*-dimethylaminobenzoate chromophores (moments X and W, Z in Fig. 5), it is necessary to remove the contribution to the 327 band arising from the interaction of the 8'-*p*-dimethylaminobenzoate with the pyridylquinoline ABC-ring system (moments X and Y in Fig. 5). The previously measured CD spectrum (Fig. 4) of the 5,8-diacetate-8'-*p*-dimethylaminobenzoate **12** above 300 nm essentially corresponds to just this interaction, with the proviso that the acyl groups esterifying the 5 and 8-positions are acetate rather than

p-dimethylaminobenzoate. Accordingly, this CD spectrum of the 5,8-diacetate-8'-*p*-dimethylaminobenzoate **12** was subtracted from that of the 5,8,8'-tri-*p*-dimethylaminobenzoate **11** (Fig. 5), to give the difference spectrum shown in Fig. 6. Intense split Cotton effects of opposite sign ($\Delta\Delta\varepsilon$ 61.7) are now clearly visible at 310 ($\Delta\varepsilon$ -35.7) and 332 nm (26.0), the longer wavelength extremum now being positive as predicted. The signs of these Cotton effects indicate that the longitudinal moment of the 8'-*p*-dimethylaminobenzoate chromophore in the derivative **11** is related to those of the 5- and 8-*p*-dimethylaminobenzoate chromophores by clockwise twists (moments X and W, Z in Fig. 5), and that the derivative accordingly has the *R* configuration.[33] This application of ECCD difference spectroscopy further supports the absolute configuration of streptonigrin itself (**1**) as *R*.

Conclusion

The present ECCD studies with chromophoric aroyl derivatives of streptonigrin (**1**) establish the absolute configuration about the phenylpyridyl CD-ring linkage of the antibiotic as *R*. The absolute configuration of natural 10'-*O*-demethylstreptonigrin (**2**) was determined to be *R* by chiroptical correlation with streptonigrin itself. It is probable on biogenetic grounds that other phenylpyridyl-quinolinequinones which co-occur with streptonigrin in various Actinomycete species, such as 6-*O*-demethylstreptonigrin (**3**)[6] and 10'-demethoxystreptonigrin (**4**),[7] share this *R* configuration. In contrast to these phenylpyridines, circular dichroic spectroscopy demonstrates that the phenylpyridone streptonigrone (**5**) is either achiral or a racemate.

EXPERIMENTAL

General. Flash chromatography[34] was carried out using 230-400 mesh silica gel. Buffered silica refers to silica gel stirred in a pH 6.8 phosphate buffer, filtered, then dried at 105-110 °C. ^1H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz respectively on Varian Gemini-300 or VXR-300 spectrometers with TMS or the solvent signal as the internal reference. $CDCl_3$ was stored over K_2CO_3 prior to use. Electron impact mass spectra (MS) were obtained on a VG Micromass 7070F double-focussing mass spectrometer operating at 70 eV, high resolution electron impact mass spectra (HRMS) on an AEI MS 902 instrument. Fast atom bombardment mass spectra (FABMS) were recorded on a ZAB2-SEQ spectrometer using 3-nitrobenzyl alcohol as the matrix.

Uncorrected CD spectra were measured on a Carey 61 spectrophotometer connected to an IBM PC clone and the ΔA values stored as an ASCII text file. The spectra were recorded over the range 200-800 nm at 22 °C in distilled spectroscopic grade EtOH using a 0.5 cm cell. CD measurements were collected at 1 nm intervals, averaging 200 readings at each wavelength over a 20 second period. Corrected electronic spectra were measured over the range 200-800 nm at 22 °C in distilled spectroscopic grade ethanol using a 1 cm cell with a Hewlett-Packard 8450A UV/VIS spectrophotometer connected to a Vaxserver 3100 and the A values stored as an ASCII text file. The CD and electronic spectra were transferred to an Apple Macintosh® and the CD spectra corrected by subtraction of a recently collected blank (solvent only). Then the CD and electronic ΔA and A data were transformed into $\Delta\varepsilon$ and ε values (l mol^{-1} cm^{-1}) respectively, smoothed by averaging over an interval of 5 nm and graphed.

Streptonigrin (1), 10'-*O*-demethylstreptonigrin (2) and streptonigrone (5). Fermentation of the *Streptomyces* species (IA-CAS isolate No. 144) was carried out as described previously.[8] The culture (3 l) was adjusted to pH 6.5, centrifuged and the supernatant extracted with EtOAc (3 x 3 l). The extract was dried over Na_2SO_4 and evaporated to give a black crude residue (1.2 g). The residue was partitioned between CH_2Cl_2 (100 mL) and ice-cold 2% aqueous $NaHCO_3$ (3 x 100 mL). The bicarbonate extracts were acidified to pH 6.5 with 2M HCl, extracted with CH_2Cl_2 (3 x 200 mL), and the extracts washed with water, dried, and evaporated. The residue (200 mg) was twice chromatographed on Sephadex LH-20, eluting with 1% MeOH/$CHCl_3$, affording streptonigrin (**1**) (110 mg): ^1H NMR (CD_2Cl_2) δ 11.40 (bs, 1H, CO_2H), 8.74 (d, J = 8.5 Hz, 1H, C(3)*H*), 8.48 (d, J = 8.5 Hz, 1H, C(4)*H*), 6.79 (AB_q, J = 8.4 Hz, $\Delta\upsilon$ = 28.2 Hz, 2H, C(12')*H*, C(11')*H*),

6.06 (bs, 1H, O*H*), 5.20 (bs, 2H, C(7)NH_2), 4.08, 4.00, 3.97 (s, each 3H, OCH_3), 2.45 (s, 3H, ArCH_3); MS *m/z* 506 (M^+).

The pH 6.5 aqueous layer remaining was readjusted to pH 3 and the CH_2Cl_2 (3 x 200 mL) extraction and Sephadex LH-20 chromatography procedure repeated to give 10'-*O*-demethylstreptonigrin (**2**) (15 mg): ^{1}H NMR (CD_3OD) δ 9.01 (d, *J* = 8.1 Hz, 1H, C(3)*H*), 8.45 (d, *J* = 8.1 Hz, 1H, C(4)*H*), 6.65 (AB_q, *J* = 8.6 Hz, Δυ = 17.1 Hz, 2H, C(12')*H*, C(11')*H*), 3.84, 3.79 (s, each 3H, OCH_3), 2.26 (s, 3H, ArCH_3).

The bicarbonate-washed CH_2Cl_2 solution was evaporated (200 mg) and chromatographed twice on Sephadex LH-20, elution with 1% MeOH/$CHCl_3$ providing streptonigrone (**5**) (10 mg): ^{1}H NMR ($CDCl_3$) δ 8.36 (AB_q, *J* = 8.4 Hz, Δυ = 21.2 Hz, 2H, C(3)*H*, C(4)*H*), 6.84 (d, *J* = 9.0 Hz, 1H, C(12')*H*), 6.66 (d, *J* = 9.0 Hz, 1H, C(11')*H*), 6.34 (bs, 1H, O*H*), 5.05 (bs, 2H, C(7)NH_2), 4.06, 3.99, 3.95 (s, each 3H, OCH_3), 2.03 (s, 3H, ArCH_3); MS *m/z* 478 (M^+).

Streptonigrin methyl ester (6). Treatment of streptonigrin (**1**) (57.9 mg) in CH_2Cl_2 (5 ml) with diazomethane (*ca* 5 equiv.) for 1 h followed by flash chromatography (2% MeOH / $CHCl_3$, pH 6.8 buffered silica) afforded the methyl ester (**6**) (52.0 mg, 87%) as a deep red amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.96 (d, *J* = 8.4 Hz, 1H, C(3)*H*), 8.39 (d, *J* = 8.4 Hz, 1H, C(4)*H*), 6.74 (AB_q, *J* = 8.6 Hz, Δυ = 45.0 Hz, 2H, C(12')*H*, C(11')*H*), 5.95 (s, 1H, O*H*), 5.08 (bs, 2H, C(7)NH_2), 4.07, 3.98, 3.97, 3.94 (s, each 3H, CO_2CH_3, 3xOCH_3), 2.32 (s, 3H, ArCH_3); ^{13}C NMR ($CDCl_3$) δ 179.9, 177.4, 167.0, 161.3, 152.7, 147.0, 145.6, 143.9, 138.9, 137.7, 137.2, 136.3, 135.4, 133.7, 132.5, 131.0, 126.6, 126.3, 125.1, 113.9, 105.0, 61.2, 60.6, 55.9, 52.1, 17.3; HRMS $C_{26}H_{24}N_4O_8$ (M^+) requires *m/z* 520.1594, found 520.1594.

5',8'-Di-*N,O*-*p*-nitrobenzoyl derivative 7. A solution of the ester (**6**) (5.9 mg, 11 μmol), 4-nitrobenzoyl chloride (16.8 mg, 91 μmol) and DMAP (11.1 mg, 91 μmol) in CH_2Cl_2 (2 ml) was refluxed for 48 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered and then concentrated *in vacuo*. Purification of the product by flash chromatography ($CHCl_3$, pH 6.8 buffered silica) afforded the 5',8'-di-*N,O*-*p*-nitrobenzoyl derivative **7** (4.3 mg, 46%) as a purple amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.67 (d, *J* = 8.3 Hz, 1H, C(3)*H*), 8.45 (d, *J* = 8.3 Hz, 1H, C(4)*H*), 8.30-8.07 (m, 8H, Ar*H*), 7.30 (d, *J* = 8.7 Hz, 1H, C(12')*H*), 7.02 (d, *J* = 8.7 Hz, 1H, C(11')*H*), 5.21 (bs, 2H, C(7)NH_2), 4.12, 4.00, 3.90, 3.74 (s, each 3H, CO_2CH_3, 3xOCH_3), 2.43 (s, 3H, ArCH_3); ^{13}C NMR ($CDCl_3$) δ 179.5, 176.3, 166.5, 162.5, 162.3, 158.9, 153.6, 150.7, 149.9, 146.5, 144.5, 143.9, 143.3, 141.2, 140.2, 139.0, 138.6, 137.8, 135.7, 135.5, 134.5, 134.1, 131.2, 129.2, 128.2, 128.2, 126.6, 123.7, 123.6, 122.0, 110.4, 60.6, 60.7, 56.0, 52.9, 17.3; HRMS $C_{40}H_{30}N_6O_{14}$ (M^+) requires *m/z* 818.1820, found 818.1824.

8'-*p*-Dimethylaminobenzoate 8. A solution of the ester (**6**) (19.2 mg, 37 μmol), 2,4-dinitrophenyl *p*-dimethylaminobenzoate (48.9 mg, 148 μmol) and DMAP (18.0 mg, 148 μmol) in CH_2Cl_2 (5 ml) was refluxed for 18 h. Work-up as for the derivative **7** followed by flash chromatography (1% MeOH / $CHCl_3$, pH 6.8 buffered silica) afforded the 8'-*p*-dimethylaminobenzoate **8** (22.9 mg, 93%) as a deep red amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.88 (d, *J* = 8.3 Hz, 1H, C(3)*H*), 8.36 (d, *J* = 8.3 Hz, 1H, C(4)*H*), 7.79 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.97 (AB_q, *J* = 8.7 Hz, Δυ = 15.4 Hz, 2H, C(12')*H*, C(11')*H*), 6.51 (d, *J* = 9.0 Hz, 2H, Ar*H*), 5.14 (bs, 2H, C(7)NH_2), 4.04, 3.97, 3.94, 3.88 (s, each 3H, CO_2CH_3, 3xOCH_3), 2.97 (s, 6H, N(CH_3)$_2$), 2.38 (s, 3H, ArCH_3); ^{13}C NMR ($CDCl_3$) δ 179.8, 177.5, 166.8, 164.7, 161.2, 154.0, 153.6, 145.9, 144.0, 143.3, 142.7, 139.0, 137.7, 137.1, 134.7, 133.6, 132.2, 131.9, 131.0, 126.6, 126.2, 124.6, 121.7, 114.9, 110.8, 110.6, 60.9, 60.5, 56.1, 52.1, 40.0, 17.5; HRMS $C_{35}H_{33}N_5O_9$ (M^+) requires *m/z* 667.2278, found 667.2276.

8'-Acetate 9. A solution of the ester (**6**) (7.8 mg, 15 μmol), acetic anhydride (4.6 mg, 45 μmol) and DMAP (5.5 mg, 45 μmol) in CH_2Cl_2 (1 ml) was stirred at 0 °C for 1 h. Work-up and chromatography as for the *p*-dimethylaminobenzoate **8** afforded the 8'-acetate **9** (5.8 mg, 69%) as a red amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.98 (d, *J* = 8.5 Hz, 1H, C(3)*H*), 8.42 (d, *J* = 8.5 Hz, 1H, C(4)*H*), 6.95 (AB_q, *J* = 8.6 Hz, Δυ = 25.3 Hz, 2H, C(12')*H*, C(11')*H*), 5.10 (bs, 2H, C(7)NH_2), 4.06, 3.98, 3.96, 3.90 (s, each 3H, CO_2CH_3, 3xOCH_3), 2.28 (s, 3H, ArCH_3), 2.02 (s, 3H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 179.8, 177.4, 168.8, 166.9, 161.2, 154.0, 145.7, 144.1, 142.6, 142.3, 139.0, 137.5, 137.2, 135.1, 133.8, 131.9, 131.1, 126.8, 126.2, 124.7, 121.1, 111.1, 60.8, 60.6, 56.1, 52.2, 20.4, 17.4; HRMS $C_{28}H_{26}N_4O_9$ (M^+) requires *m/z* 562.1700, found 562.1700.

Dihydro-5,8,8'-triacetate 10. The ester (**6**) (7.0 mg, 13 μmol) in acetone (1 ml) was hydrogenated over 10% palladium on charcoal at r.t. and pressure for 1 h during which the red solution turned pale green. The solution was transferred through a plug of Celite, using positive H_2 pressure and teflon tubing, to a second flask flushed with argon. The solvent was evaporated by a stream of argon, replaced by CH_2Cl_2 (1 ml) followed by cooling to 0 °C, then acetic anhydride (13.7 mg, 134 μmol) and DMAP (2.5 mg, 20 μmol) were added. The stirred mixture was warmed to r.t. over 1 h, worked up as for the derivative **7**, and the crude product purified by flash chromatography (1% MeOH / $CHCl_3$, pH 6.8 buffered silica) then gel filtration ($CHCl_3$, Sephadex LH-20) to afford the dihydro-5,8,8'-triacetate **10** (3.2 mg, 37%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.62 (d, *J* = 9.0 Hz, 1H, C(3)*H*), 8.05 (d, *J* = 9.0 Hz, 1H, C(4)*H*), 6.98 (AB_q, *J* = 8.6 Hz, Δυ = 25.8 Hz, 2H, C(12')*H*, C(11')*H*), 4.28 (bs, 2H, C(7)NH_2), 3.97, 3.96, 3.91 (s, 12H, CO_2CH_3, 3xOCH_3), 2.50, 2.41, 2.26, 2.04 (s, each 3H, ArCH_3, 3x$COCH_3$); ^{13}C NMR ($CDCl_3$) δ 169.0, 168.7, 168.4, 167.2, 158.1, 153.8, 145.1, 142.5, 142.1, 139.9, 136.9, 136.7, 135.2, 135.1, 134.8, 133.3, 131.2, 129.6, 126.3, 125.0, 121.4, 118.3, 114.8, 111.0, 60.8, 56.1, 52.1, 29.7, 20.8, 20.6, 20.4, 17.3.

Dihydro-5,8,8'-tri-*p*-dimethylaminobenzoate 11. The ester (**6**) (11.6 mg, 22 μmol) in acetone (1 ml) was hydrogenated as above and then reacted with 2,4-dinitrophenyl *p*-dimethylaminobenzoate (73.8 mg, 223 μmol) and DMAP (27.2 mg, 223 μmol) for 18 h at r.t. Work-up and chromatography as for the triacetate **10** afforded the dihydro-5,8,8'-tri-*p*-dimethylaminobenzoate **11** (15.0 mg, 70%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.51 (d, *J* = 9.0 Hz, 1H, C(3)*H*), 8.20 (d, *J* = 9.0 Hz, 2H, Ar*H*), 8.18 (d, *J* = 9.0 Hz, 2H, Ar*H*), 8.07 (d, *J* = 9.0 Hz, 1H, C(4)*H*), 7.69 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.84 (AB_q, *J* = 8.6 Hz, Δυ = 30.6 Hz, 2H, C(12')*H*, C(11')*H*), 6.77 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.65 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.49 (d, *J* = 9.0 Hz, 2H, Ar*H*), 4.38 (bs, 2H, C(7)NH_2), 3.99, 3.95, 3.86, 3.80 (s, each 3H, CO_2CH_3, 3xOCH_3), 3.13, 3.04, 2.94 (s, each 6H, N(CH_3)$_2$), 2.28 (s, 3H, ArCH_3); ^{13}C NMR ($CDCl_3$) δ 167.1, 164.8, 164.6, 164.4, 157.8, 153.9, 153.8, 153.6, 153.4, 145.2, 143.1, 142.5, 140.2, 137.0, 136.7, 135.5, 135.1, 132.8, 132.3, 131.9, 130.9, 129.7, 128.7, 125.0, 122.2, 117.7, 115.4, 115.2, 115.0, 114.9, 110.9, 110.7, 110.2, 61.0, 60.7, 56.0, 51.8, 40.1, 40.0, 39.9, 29.7, 17.5; FABMS *m/z* 964 (MH^+).

Dihydro-5,8-diacetate-8'-*p*-dimethylaminobenzoate 12. The *p*-dimethylaminobenzoate **8** (6.0 mg, 9 μmol) in acetone (1 ml) was hydrogenated as above and then treated with acetic anhydride (2.1 mg, 20 μmol) and DMAP (2.5 mg, 20 μmol) for 1 h at r.t. Isolation and purification as for the triacetate **10** afforded the dihydro-5,8-diacetate-8'-*p*-dimethylaminobenzoate **12** (3.7 mg, 55%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.53 (d, *J* = 9.0 Hz, 1H, C(3)*H*), 7.98 (d, *J* = 9.0 Hz, 1H, C(4)*H*), 7.79 (d, *J* = 9.0 Hz, 2H, Ar*H*), 6.97 (AB_q, *J* = 8.6 Hz, Δυ = 21.2 Hz, 2H, C(12')*H*, C(11')*H*), 6.51 (d, *J* = 9.0 Hz, 2H, Ar*H*), 4.25 (bs, 2H, C(7)NH_2), 3.98, 3.95, 3.93, 3.89 (s, each 3H, CO_2CH_3, 3xOCH_3), 2.96 (s, 6H, N(CH_3)$_2$), 2.48, 2.47, 2.35 (s, each 3H, ArCH_3, 2x$COCH_3$); FABMS *m/z* 776 (MNa^+), 754 (MH^+).

Dihydro-8'-acetate-5,8-di-*p*-dimethylaminobenzoate 13. The acetate **9** (5.6 mg, 10 μmol) in acetone (1 ml) was hydrogenated as above and then treated with 2,4-dinitrophenyl *p*-dimethylaminobenzoate (33.0 mg, 99 μmol) and DMAP (12.2 mg, 99 μmol) for 18 h at r.t. Work-up and chromatography as for the triacetate **10** afforded the dihydro-8'-acetate-5,8-di-*p*-dimethylaminobenzoate **13** (3.2 mg, 37%) as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 8.61 (d, *J* = 8.9 Hz, 1H, C(3)*H*), 8.23-8.10 (m, 5H, C(4)*H*, 4xAr*H*), 6.89-6.61 (m, 6H, C(12')*H*, C(11')*H*, 4xAr*H*), 4.36 (bs, 2H, C(7)NH_2), 3.99, 3.94, 3.92, 3.84 (s, each 3H, CO_2CH_3, 3xOCH_3), 3.13, 3.03 (s, each 6H, N(CH_3)$_2$), 2.20, 1.96 (s, each 3H, ArCH_3, COCH_3); FABMS *m/z* 881 (MNa^+), 859 (MH^+).

2,4-Dinitrophenyl *p*-dimethylaminobenzoate. A solution of *p*-dimethylaminobenzoic acid (1.65 g, 10 mmol) and sodium hydride (400 mg, 10 mmol) in benzene (10 ml) was refluxed for 4 h, cooled to 0 °C and treated with 2,4-dinitrofluorobenzene (1.86 g, 10 mmol) with stirring for a further 18 h. Filtration, concentration, and repeated recrystallisation (CH_2Cl_2 / hexane) afforded 2,4-dinitrophenyl *p*-dimethylaminobenzoate (318 mg, 10%) as yellow plates (m.p. 180-182 °C): ^{1}H NMR ($CDCl_3$) δ 8.95 (d, *J* = 2.6 Hz, 1H, Ar*H*), 8.52 (dd, *J* = 2.6, 8.9 Hz, 1H, Ar*H*), 8.03 (d, *J* = 9.1 Hz, 2H, Ar*H*), 7.65 (d, *J* = 8.9 Hz, 1H, Ar*H*), 6.71 (d, *J* = 9.1 Hz, 2H, Ar*H*), 3.11 (s, 6H, N(CH_3)$_2$).

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