CD Exciton Chirality Method. New Red-Shifted Chromophores for Hydroxyl Groups

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Abstract: When hydroxyl groups are derivatized to apply the CD exciton chirality method, the absorption maxima of introduced chromophores should not overlap with that of the substrate, except for cases in which the coupling between the existing and the introduced chromophores are deliberately sought for. Thus, the availability of red-shifted chromophores that do not overlap with the substrate absorption would greatly expand the applicability of this versatile CD method. Four such red-shifted chromophores, chrom-II, -III, -IV, and -V, have been developed to convert hydroxyl groups into esters that absorb strongly in the range 360–410 nm. Using the chromophoric triazole amide, they can readily derivatize hydroxyl groups of the substrates on a microscale. The bischromophoric esters of 1(R),2(R)-cyclohexanediol (14–18) exhibited intense exciton-split CD curves with the signs correctly representing the absolute sense of twist between the two hydroxyl groups. The 1,2-diol moieties of taxinine (2) and chromomycin A3 (3) derivatives, already having strong absorptions at 260–275 nm, were esterified with the new chromophores; this gave rise to strong coupltles isolated from the CD Cotton effects of starting materials, the signs of which were in agreement with the absolute configuration of these two natural products. Thus, O-acylating chromophores should be useful for determinations of absolute configurations and conformations of chiral substrates, including biopolymers; they could also be conveniently used in conjunction with the red-shifted chromophores developed recently for primary amino groups.

Introduction

The CD exciton chirality method is a versatile tool for determining the absolute configuration and/or conformation of molecules in solution.1 The interactions between excited states of chromophores exhibit typical split CD Cotton effects, the signs of which are defined in a nonempirical manner by the chirality of the chromophores and an additivity relation.1,2 Interpretations of the split CD curves are straightforward, the method being particularly useful when a sample is only available in submilligram quantities. However, the absorption maxima at 230–310 nm of the commonly introduced chromophores are frequently overlap with those of the substrates or of biopolymers, including nucleic acids and proteins. Unless one specifically utilizes the coupling between an existing chromophore and the introduced chromophore, the overlap of maxima leads to unnecessary complications in the interpretation of the data. Thus, availability of red-shifted chromophores that can be readily introduced into substrates should contribute greatly to further applications of the exciton chirality method.

Some red-shifted chromophores have already been made. We previously used the p-(dimethylamino)cinnamaldehyde chromophore (chrom-I, Figure 1), \( \lambda_{\text{max}} = 362 \text{ nm, } \epsilon = 31 000 \), to study the absolute configuration of mitomycin C derivative, mitosene.3 The chromophores were introduced by O,N-bisaclylation of the substrate; however, the absorption maximum is not as red-shifted as those described below, and partial overlap with the substrate chromophore was still observed. We also studied the biscyanine derivative, which gave extremely strong UV and CD absorptions in the 480–550-nm region; however, the chromophore, although of great theoretical interest, is unstable and gave rise to split CD curves of signs opposite to those expected from the exciton chirality method.4 Lightner recently reported that dipyrrinone carboxylic acid chromophores reacted with 1(R),2(R)-cyclohexanediol to form the corresponding diester, which shows intense bisignate CD around 380 nm.5

For primary amino functions we have recently developed several red-shifted Schiff base and protonated Schiff base chromophores which are suited for selective microscale derivatizations and exhibit superior exciton chirality properties.6 In this article we describe the preparations, spectral properties, and applications of four new red-shifted chromophores, chrom-II to -V (Figure 1), \( \lambda_{\text{max}} = 360–410 \text{ nm, which can be used for microscale O- and probably N-derivatizations. Spectroscopic data for the bischromophoric derivatives of 1(R),2(R)-cyclohexanediol (1) are also given in Figure 1. To demonstrate the advantage of the red-shifted chromophores, derivatives of the natural products taxinine (2) and chromomycin A3 (3), both with strong UV absorptions, were derivatized directly with one of the new chromophores, upon which exciton-split CD coupltles separated from the substrate CD bands were obtained (Figures 4 and 5).

Synthesis of Red-Shifted Chromophores

Since chromophoric interactions in exciton coupling are approximately linearly proportional to the absorption coefficients of the chromophores,1,7 we designed and synthesized a series of aromatic polyme chromophores (Figure 1, chrom-II to -V) having intense absorptions (\( \epsilon = 31 000–58 000 \)) in the region 360–410 nm. Chrom-I is commercially available, while chrom-II was obtained by condensation of p-(dimethylamino)cinnamaldehyde (5) with triethyl phosphonoacetate (Scheme 1). Scheme II outlines the
CD Exciton Chirality Method

The red-shifted chromophores were first tested with 1(R),2(R)-cyclohexanediol (1). 1(R),2(R)-Cyclohexanediol bischromophoric derivatives (14-18) were prepared as shown in Figure 1. The chromophores were initially introduced by acylation of the substrate with chromophoric acid chloride2 but these procedures were found to be difficult to work with in microgram scale. A search for improved conditions showed that the derivatizations could be performed on a microscale using the more active imidazole or triazole amide as acylating reagents, the latter being more reactive (Figure 1).

The reaction of 1(R),2(R)-cyclohexanediol (1) with 3-[4-(dimethylamino)phenyl]-2-propenyl imidazole (reaction time, 4 h) was faster than that of 1 with 3-[4-(dimethylamino)phenyl]-2-propenyl imidazole (18 h). Thus, the bischromophoric derivatives (14-18) were best prepared by treatment of 1 with an excess amount of triazole amide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 43-89% yields. The advantages of using triazole amide as acylating reagents, the latter being more reactive (Figure 1). For example, the reaction of 1(R),2(R)-cyclohexanediol (1) with 3-[4-(dimethylamino)phenyl]-2-propenyl imidazole (18 h) was faster than that of 1 with 3-[4-(dimethylamino)phenyl]-2-propenyl imidazole (18 h). Thus, the bischromophoric derivatives (14-18) were best prepared by treatment of 1 with an excess amount of triazole amide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 43-89% yields. The advantages of using triazole amide as acylating reagents, the latter being more reactive (Figure 1). For example, the reaction of 1(R),2(R)-cyclohexanediol (1) with 3-[4-(dimethylamino)phenyl]-2-propenyl imidazole (18 h) was faster than that of 1 with 3-[4-(dimethylamino)phenyl]-2-propenyl imidazole (18 h). Thus, the bischromophoric derivatives (14-18) were best prepared by treatment of 1 with an excess amount of triazole amide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), 43-89% yields. The advantages of using triazole amide as acylating reagents, the latter being more reactive (Figure 1).

Reagents and conditions: (a) 1,1'-carbonyldi(1,2,4-triazole), CH2Cl2. Tr, triazolyl.

Scheme Ia

Reagents and conditions: (a) (EtO)2POCHzCOzEt, NaH, benzene, room temperature. (b) LiOH, MeOH/H2O/DME, room temperature. (c) (EtO)2POCzCl, DMF, 80-100 °C. (b) (EtO)2POCHzCOzEt, NaH, benzene, room temperature. (c) (EtO)2POCHzCOzEt, LiN(TMS)2, THF, room temperature. (d) (EtO)2POCHzCOzEt, LiN(TMS)2, THF, room temperature. (e) 1,1'-carbonyldi(1,2,4-triazole), CH2Cl2. Tr, triazolyl.

Scheme Ib

Figure 1. The λmax (e) in acetone/toluene of chromophores chrom-1 to -V and UV/vis and CD data for diesters 14-18 in acetone/toluene. The A values indicate the differences in A of the split CD curves. A negative sign shows that the first and second Cotton effects at longer and shorter wavelengths have negative and positive signs, respectively. Reagents and conditions: (a) for 14, 4c, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), CH2Cl2; for 15, 6c, DBU, CH2Cl2; for 16, 9c, DBU, CH2Cl2; for 17, 10c, DBU, CH2Cl2; for 18, 13c, DBU, CH2Cl2.
amides prepared in advance rather than in situ were that (i) the reactions can be conveniently performed on a microgram scale in high yield and (ii) the triazole amides can be stored and used whenever needed. In all cases, the bichromophoric derivatives of diol I with chrom-I to -V were also prepared on a 100–500-µg scale. In general, a small reaction vial was flame-dried and cooled under vacuum immediately prior to use. The reaction mixture of diol I and the triazole amide reagent was dried under vacuum for 2 h, and the solvent was added under argon. After concentration, the product was isolated through TLC and HPLC. If necessary, the yield was calculated from the UV/vis ε value. To avoid photoisomerization of the chromophore in the light, the reaction flasks were covered with aluminum foil, and the UV/vis and CD spectra were recorded immediately after preparation of the solution. It should be noted that, except for chrom-III, the UV and CD intensities of other chromophores are reduced from 40 to 60% when the dilute solutions used for measurements are left in the light for over 5 h. If for any reason experiments require the UV and CD solutions to be exposed to light for a long period, chrom-III should be employed, which according to sensitivity studies12 was shown to be stable to light. The stability of chrom-III to light can be ascribed to the steric bulk of the julolidine moiety which disfavors the C=C double bond to adopt a cisoid structure.

**UV and CD Spectral Data**

The spectral properties of the diesters of 1(R),2(R)-cyclo-hexanediol (14–18) are listed in Figure 1. The exciton couplings of these bichromophoric derivatives give rise to bisignate CD curves with intense A values in the range −78 to −119 which correctly represent the absolute sense of twist between the two hydroxy groups before derivatization.

Of the chromophores listed in Figure 1, p-(dimethylamino)cinnamate (chrom-I1) is convenient in the sense that it is commercially available; however, it is the least red-shifted. Although milligram quantities of the substrate are required when its acid chloride is used, this can be scaled down to the microgram scale upon usage of the triazole amide. Extension of the conjugation by one double bond leading to chrom-II results in a 20–30-nm bathochromic shift; this chromophore has been used in derivatizing the taxinime and chromomycin derivatives. The julolidine type chromophores (chrom-III and -IV) are similar to the N,N-dimethylaniline type chromophores (chrom-I and -II). However, the nitrogen atom is more in-plane with the aromatic ring in julolidine than in N,N-dimethylaniline,13 and this increase in hybridization results in the bathochromic and hyperchromic shifts in the former type of chromophore. Thus, the λmax positions of these two types of chromophores are in the order chrom-III > chrom-I (Figure 2) and chrom-IV > chrom-II (Figure 3), chrom-IV being the most red-shifted.

Benzothiazole chromophore (chrom-V) is another red-shifted chromophore with a strong CT band at 358 nm (ε 58 000).14 As can be seen in Figure 2, the A value of diester 18 is the largest of the chromophores shown in Figure 1; since an approximately linear relation exists between the A value and the UV ε and since the A value is inversely proportional to the square of the 

(12) Exposure to light of an NMR solution of bis(dimethylamino)cinnamate 14 for a long period, i.e., 24 h, led to partial double bond isomerization as evidenced by the appearance of cis coupling (J = 12.8 Hz) in addition to the trans coupling (J = 16.0 Hz) of the olefinic protons. The same isomerization was observed for the higher homologue 15 upon UV irradiation (see Experimental Section).


Scheme IV

Reagents and conditions: (a) Reference 16. (b) For 22a, 4c (excess), DBU (excess), MeCN, 12 h; for 22b, 6c (excess), DBU (excess), MeCN, 24 h; for 22c, 1c (excess), DBU (excess), MeCN, 24 h.

Application of Red-Shifted Chromophores to Taxinine and Chromomycin A3

A demonstration of the utility of these red-shifted chromophores is provided by derivatives of taxinine (2) and chromomycin A3 (3). Taxinine (2) (Scheme IV), the major component of the Japanese yew tree,15 belongs to the taxoid group of diterpenes,15 where taxol16a and taxotere16b are prominent members attracting great interest because of their antitumor activities. The highly strained enone moiety of taxinine shows a strong Cotton effect at 262 nm arising from a $\pi-\pi^*$ transition, and a weaker Cotton effect at 354 nm from an $n-\pi^*$ transition (Table I, Figure 4), the 262-nm Cotton effect overlapping with the bands of conventional chromophores. Chromomycin A3 (3), which was previously used as a clinical antitumor antibiotic, contains a naphthalene moiety as a clinical antitumor antibiotic, contains a naphthalene moiety absorbing at 270 nm. If one were to determine the absolute configuration of the taxane skeleton, the exciton chirality method was applied to 9,10-desacetyltetrahydrotaxinine by converting the 9,10-glycol moiety into the bis(benzote). However, interpretation of the CD data was not necessarily straightforward because of the interaction between the enone of the substrate ($\lambda_{\text{max}}$ 274 nm) and benzoate chromophores ($\lambda_{\text{max}}$ 230 nm); a conclusion from the CD interpretation was subsequently confirmed by X-ray crystallography.19 To obtain the bischromophoric derivatives of taxinine (22a-c), 9,10-dihydroxy taxinine 2a was first prepared by hydrolysis of taxinine (2),20 and then acylated with red-shifted chromophores of chrom-I (22a), -II (22b), and -IV (22c). Their UV and CD data are listed in Table I. All derivatizations were carried out on a microgram scale with triazole amide as the acylating agent in the presence of DBU. From the nonoverlapping negative CD coupling at the longer wavelengths, the stereochemistry at C-9 and C-10 of the diol can be unambiguously assigned as $R$ (Figure 4), in agreement with previous results.

The absolute configuration of chromomycin A3 was determined in 1979.17b Because of the difficulties in introducing two benzoate chromophores at C-1' and C-2' of a chromomycin derivative by conventional methods, the skeletal absolute configuration was determined by the exciton chirality method where the exciton coupling between the naphthalenoid absorption and a $p$-methoxybenzate chromophore introduced at C-1' of isochromomycinone derivatives (3n, 23a-d) was interpreted. In the present case, it was possible to derive both C-1' and C-2' of 3a with the triazole amides of chrom-I and chrom-V in microscale under very mild conditions. The UV and positively split CD curves, a conclusion from the CD data, clearly show that the chirality between the 1'/2' substituents is positive. The monodervative 23a, resulting from a short acylation period of 3a, showed an

Table I. UV/Vis and CD in Acetonitrile for Taxinine (2), Taxinine Derivatives (22a-c), and Isochromomycinone Derivatives (3a, 23a–d)*

<table>
<thead>
<tr>
<th>compd</th>
<th>UV: $\lambda_{\text{max}}$ (nm)</th>
<th>CD: $\Delta A$ (nm)</th>
<th>$A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>274 (26 000)</td>
<td>354 (52 000)</td>
<td>227 (12.4)</td>
</tr>
<tr>
<td>22a</td>
<td>364 (54 000)</td>
<td>388 (52 90)</td>
<td>209 (4.8)</td>
</tr>
<tr>
<td>22b</td>
<td>382 (55 400)</td>
<td>418 (52 80)</td>
<td>252 (3.5)</td>
</tr>
<tr>
<td>22c</td>
<td>413 (63 000)</td>
<td>455 (52 80)</td>
<td>227 (12.4)</td>
</tr>
<tr>
<td>3a</td>
<td>267 (54 000)</td>
<td>370 (53 000)</td>
<td>230 (3.5)</td>
</tr>
<tr>
<td>3b</td>
<td>387 (34 000)</td>
<td>365 (52 000)</td>
<td>390 (4.8)</td>
</tr>
<tr>
<td>3c</td>
<td>385 (55 400)</td>
<td>426 (55 40)</td>
<td>252 (3.5)</td>
</tr>
<tr>
<td>3d</td>
<td>357 (53 600)</td>
<td>390 (47 20)</td>
<td>209 (4.8)</td>
</tr>
</tbody>
</table>

* The $A$ values indicate the differences in $\Delta A$ between the two extrema of the split CD curves. A negative sign shows that the first and second Cotton effects at longer and shorter wavelengths have negative and positive signs, respectively, and vice versa. For 2 and 3a, only the main absorption bands before derivatization are given, whereas for the rest (22 and 23), the maxima of the introduced chromophores are given.

Figure 4. UV/vis and CD in acetonitrile of taxinine (2) (dashed) and bischromophoric derivative 22c (solid).

interchromophoric distance, chromophores such as chrom-V could be useful in derivatizing hydroxyl groups which are remote.1
The derivatization can be performed on a microscale with chromophoric triazole amide as acylation agent. These chromophores have been introduced into the taxinine and chromomycin skeletons, both of which have strong absorptions which would interact with those of conventional chromophores used in the exciton chirality method; the red-shifted chromophores give clear-cut couplings unperturbed by the substrate absorptions, and thus lead to unambiguous assignment of absolute configurations. The chromophores described above should also prove to be useful when used in conjunction with the red-shifted chromophores recently developed for the microscale derivatization of primary amino groups. Further applications of these chromophores in the field of biopolymers are under study.

Experimental Section

General Procedures. Solvents employed were reagent grade. Anhydrous solvents were dried and distilled (THF and benzene from Na/benzophenone; CH2Cl2 from CaH2). Acetonitrile was dried over molecular sieves (4 Å). Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Moisture-sensitive reactions were performed in flame-dried glassware under argon. Reactions were followed by thin-layer chromatography (TLC) using Analtech silica gel GHLF (250 nm thick).

Chromatography solvents were HPLC grade. Flash chromatography was performed using ICN silica gel (32-63 mesh). HPLC purifications were performed using a Rainin HPLC system equipped with a Rainin Dynamax Model UV-D detector.

1H NMR spectra were obtained on a Varian VXR400, VXR300, or VXR200 and are reported in parts per million (δ) relative to CHCl3 (7.24 ppm) as an internal reference, with coupling constants (J) reported in hertz (Hz). Low-resolution and high-resolution FAB mass spectra were measured on a JEOL JMS-DX303 HF mass spectrometer using glycerol matrix and Xe ionizing gas. CI mass spectra were measured on a NER MAG R10-10 spectrometer with CH3I or NH3 as ionizing gas. UV/vis and CD spectra were recorded as acetonitrile solutions. Smoothing and other manipulation of spectra were carried out with software developed in house: DFT (discrete Fourier transform) procedure for smoothing. The concentration of natural product derivatives (22a-c and 23a-d) in acetonitrile used for the measurements of UV/vis and CD spectra was determined from the experimental ε value.

Representative Procedure for the Preparation of Chromophoric Triazole Amide (Method A): 1-[1-Oxo-3-[4-(dimethylamino)phenyl]-2-propenyl]-1H-1,2,4-triazole (4c). A solution of (dimethylamino)cinnamic acid (400 mg, 2.6 mmol) and 1,1′-carbonyldi(1,2,4-triazole) (480 mg, 2.9 mmol) in CH2Cl2 (50 mL) and acetonitrile (10 mL) was stirred at room temperature for 4 h. The solution was then washed with saturated aqueous NaHCO3 (3 × 20 mL) and brine (20 mL) and dried (Na2SO4). The organic layer was concentrated under reduced pressure to give 4c as a yellow solid (484 mg, 81%). 1H NMR (400 MHz, CDCl3) δ 3.08 (s, 6 H, N(CH3)2), 6.59 (d, J = 8.9 Hz, 2 H, Ar), 7.42 (d, J = 16.0 Hz, 1 H, H-2), 7.60 (d, J = 8.9 Hz, 2 H, Ar), 8.07 (d, J = 16.0 Hz, 1 H, H-3), 8.06 (s, 1 H, triazole), 9.01 (s, 1 H, triazole).

5-[4-(Dimethylamino)phenyl]-2,4-pentadienoic Acid, Ethyl Ester (6a). To a solution of triethyl phosphonoacetate (1.012 g, 4.50 mmol) in anhydrous THF (50 mL) under argon was added dropwise a solution of dimethyl bis(trimethyloxysilyl)amide (4.32 mL, 1 M in THF) over 30 min at -78 °C. The mixture was stirred at this temperature for 1 h, and a solution of (dimethylamino)cinnamaldehyde (0.525 g, 3.0 mmol) in anhydrous THF (3 mL) was added. The reaction mixture was stirred at -78 °C under argon for ca. 4 h and was allowed to warm to room temperature. The reaction was quenched with acetic acid at ca. 0 °C adjusting to pH 7. The mixture was extracted with ether (3 × 20 mL), and the organic layers were combined, washed with brine, dried (Na2SO4), and purified by flash chromatography (silica gel, 20%-30% ethyl acetate/hexane) to afford the ester 6a (0.638 g, 87%) as a yellow solid: 1H NMR (400 MHz, CDCl3) δ 1.30 (t, J = 7.2 Hz, 3 H, OEt), 2.99 (s, 6 H, N(CH3)2), 4.20 (q, J = 7.2 Hz, 2 H, OEt), 5.86 (d, J = 15.2 Hz, 1 H, H-2), 6.66 (d, J = 8.8 Hz, 2 H, m-Ar), 6.85 (d, J = 11.2 Hz, 1 H, H-4), 6.82 (d, J = 15.6 Hz, 1 H, H-5), 7.35 (d, J = 8.8 Hz, 2 H, o-Ar), 7.43 (dd, J = 10-13 and 256 nm) (Figure 5). This negative couplet at a position corresponding to 267 nm, could tentatively be assigned to an intermolecular coupling between two naphthalene rings due to strong hydrogen bonds involving the 2'-OH groups; upon further acetylation of 2'-OH, this characteristic couplet disappears (Figure 5). However, other interpretations are possible, and additional studies would be necessary to clarify the origin of this "couplet".

Conclusion

Intense bisignate CD curves are seen for bischromophoric esters of 1(R,2(R)-cyclohexanediol (14-18) with the red-shifted chromophores chrom-1-V in the region 360-410 nm. The derivatization can be performed on a microscale with chromophoric triazole amide as acylation agent. These chromophores have been introduced into the taxinine and chromomycin skeletons, both of which have strong absorptions which would interact with those of conventional chromophores used in the exciton chirality method; the red-shifted chromophores give clear-cut couplings unperturbed by the substrate absorptions, and thus lead to unambiguous assignment of absolute configurations. The chromophores described above should also prove to be useful when used in conjunction with the red-shifted chromophores recently developed for the microscale derivatization of primary amino groups. Further applications of these chromophores in the field of biopolymers are under study.
temperature and was stirred for 24 h, concentrated under reduced pressure, and the residue was purified by flash chromatography (10-20% ethyl acetate/hexane) to afford the ester (10b) as a white solid: 1H NMR (300 MHz, CDCl3) δ 1.31 (t, J = 7.0 Hz, 3 H, OEt), 3.68 (q, J = 6.8 Hz, 2 H, Ar), 7.33 (m, 2 H, H-2, H-6), 7.46 (m, 2 H, Ar), 8.07 (d, J = 8.0 Hz, 1 H, Ar), 8.03 (d, J = 7.8 Hz, 1 H, Ar), 7.99 (d, J = 8.0 Hz, 1 H, Ar); EI-MS (NH3) m/z 258 (M+ 1)*.

2-(2-Benzothiazolylmethyl)phosphonate (11b). To a solution of lithium diisopropylamide (3 mL, 2.0 M in heptane/THF) in THF (3 mL) was added dropwise a solution of aldehyde 8 (0.99 mmol) in THF (2 mL) at -78 °C. After being stirred for 1 h under argon, the solution was warmed to 0 °C and quenched with saturated ammonium chloride. The mixture was poured into water, extracted with ether (3 x 20 mL), dried (NazSO4), and concentrated under reduced pressure. The residue was purified by flash chromatography (10-20% ethyl acetate/hexane) to afford the ester (11b) as a yellow solid: 1H NMR (200 MHz, CDCl3) δ 1.31 (t, J = 7.0 Hz, 3 H, OEt), 3.68 (q, J = 6.8 Hz, 2 H, Ar), 7.33 (m, 2 H, H-2, H-6), 7.46 (m, 2 H, Ar), 8.07 (d, J = 8.0 Hz, 1 H, Ar), 8.03 (d, J = 7.8 Hz, 1 H, Ar), 7.99 (d, J = 8.0 Hz, 1 H, Ar); EI-MS (NH3) m/z 258 (M+ 1)*.
Derivatives of Dihydroxy Taxinine (22b). To a solution of dihydroxy taxinine 2a using the corresponding chromophoric triazole amides 6c. The product was purified by TLC overnight. It was then diluted with water (0.5 mL) and extracted with the presence of the starting material 3a. The main product was isolated by preparative TLC (silica gel, 1:1:2 ethyl acetate/dichloromethane/hexane; CI-MS (NH3) \(m/z \) 688 (100) (M + 1)\(^+\); FAB-HRMS for C\(_{39}\)H\(_{46}\)N\(_2\)O\(_{12}\), calcd 878.3043, found 878.3046.}

**Isocrosochromomycinone Bis Derivative 23a.** A solution of the isocrosochromomycinone derivative 3a\(^{12}\) (0.80 mg, 1.6 \(\mu\)mol), triazole amide 6c (0.46 mg, 1.6 \(\mu\)mol), and DBU (0.5 mL 0.01 M solution in acetonitrile) in anhydrous acetonitrile was left to stand at room temperature for 30 min. TLC (1:1:2 ethyl acetate/dichloromethane/hexane) of the reaction mixture showed the presence of one main product and only traces of the starting material 3a. The main product was isolated by preparative TLC (silica gel, 1:1:2 ethyl acetate/dichloromethane/hexane) and additionally purified by HPLC (5-\(\mu\)M YMC silica gel, 20:80 methanol/dichloromethane/hexane; CI-MS (NH\(_3\)) \(m/z \) 688 (100) (M + 1)\(^+\); FAB-MS for C\(_{39}\)H\(_{46}\)N\(_2\)O\(_{12}\), calcd 878.3043, found 878.3054.

**Isocrosochromomycinone Bis Derivative 23c.** A solution of 3a (0.30 mg, 0.5 \(\mu\)mol), triazole amide 6c (12.0 mg, 0.045 mmol), and DBU (10 drops, 0.1 M solution in acetonitrile) in anhydrous acetonitrile (1 mL) was stirred for 7 days at room temperature in the dark. After evaporation of the solvent, the product was purified by preparative TLC (silica gel, 1:1:2 ethyl acetate/dichloromethane/hexane) and by HPLC (5-\(\mu\)M YMC silica gel, 3\% methanol/dichloromethane; CI-MS (NH\(_3\)) \(m/z \) 730 (100) (M + 1)\(^+\); FAB-HRMS for C\(_{41}\)H\(_{47}\)N\(_2\)O\(_{13}\), calcd 929.3149, found 929.3163.

**Isocrosochromomycinone Bis Derivative 23d.** A solution of 3a (0.1 mg, 0.2 \(\mu\)mol), triazole amide 6c (1.0 mg, 3.75 \(\mu\)mol), and DBU (1 drop, 0.01 M solution in acetonitrile) in anhydrous acetonitrile (0.2 mL) was stirred for 4 days at room temperature in the dark. After evaporation of the solvent, the product was purified by preparative TLC (silica gel, 40\% ethyl acetate/hexane) and by HPLC (3-\(\mu\)M YMC silica gel, 40\% ethyl acetate/hexane; FAB-MS 967 (M\(^+\)); FAB-HRMS for C\(_{41}\)H\(_{47}\)N\(_2\)O\(_{13}\), calcd 967.2935, found 967.2964.

**Irradiation of 15.** A solution of 15 (3.00 mg, 0.006 mmol) in acetonitrile (3 mL) was irradiated for 40 min with 1000-W high-pressure mercury lamp behind a Pyrex glass. HPLC analysis (5-\(\mu\)M YMC silica gel, 4:2:20 ethyl acetate/dichloromethane/hexane) of the reaction mixture showed the presence of the starting material and two new products. The products were separated from the starting material as a mixture by HPLC (the same conditions as above). The \(^1\)H NMR (400 MHz, CDCl\(_3\)) spectrum of this mixture shows the presence of an isomer with a cis double bond between C-4 and C-5 (6 at 5.53 ppm, \(J = 11.2 \text{ Hz} \) for H-2).

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