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Unique UV–Vis Absorption and Circular Dichroic Exciton-Split Spectra of a Chiral Biscyanine Dye: Origin and Nature

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Abstract: The exciton coupling between two cyanine dyes, characterized by intense and sharp absorption bands in the visible region, has been studied by UV–vis and CD spectroscopy because it provides a unique system of theoretical and applicational interests. Biscyanine dye **4b** prepared from (1*S*,2*S*)-(+)-*trans*-1,2-cyclohexanediamine (**1**) shows two well-separated and strong electronic absorption bands at λ_{max} 550 (ϵ 182 000) and 480 nm (ϵ 191 000). These visible bands are accompanied by strong exciton split CD Cotton effects, λ_{ext} 546 ($\Delta\epsilon$ −232) and 475 nm ($\Delta\epsilon$ +231); however, the signs of the split Cotton effects are opposite those of the bis *p*-methoxycinnamide) **2** which exhibits positive first and negative second Cotton effects. The stable conformation UV–VIS and CD spectra of the model biscyanine (1*S*,2*S*)-**5** as calculated by molecular mechanics and the π-electron SCF-CI-DV MO method yielded typical exciton-split CD curves with nearly equal negative and positive band areas ("regional sum rule"): visible, λ_{max} 550 (ϵ 157 900) and 474 nm (ϵ 255 200); CD, λ_{ext} 550 ($\Delta\epsilon$ −140) and 474 nm ($\Delta\epsilon$ +136). The calculation indicated that the sign reversal in the bisignate CD of **4b** in comparison with that of the bis(*p*-methoxycinnamide) **2** was due to a unique conformation of the two cyanine dye side chains; furthermore, it indicated that the large separation of the two bands in the visible and CD spectra was a result of strong exciton coupling between two cyanine dye electronic transitions characterized by their narrow bandwidths and location at long wavelength region. This is the first example in which exciton-split electronic absorption and CD bands appear as widely separated distinct peaks as large as 70 nm in solution.

Introduction

The exciton chirality method is a versatile microscale method for determining absolute configurations and conformations of molecules with two or more chromophores in solution without reference compounds.[2,3] The interacting or coupled chromophores give rise to circular dichroic curves (CD) with "split" Cotton effects, the signs of which represent the chirality of the chromophoric electric transition moments. The interacting chromophores can be present in the original substrate or can be introduced by derivatization. Sometimes, the introduced chromophore can be selected so that it couples with the existing chromophore. When two identical chromophores interact, the amplitude (or *A* value) of the split CD is maximal at a chromophoric projection angle of 70°, proportional to the square of the UV–vis ϵ value, and is inversely proportional to the square of the interchromophoric distance. In the case of different chromophores, coupling is still observed when the λ_{max} values are separated by 100 nm. A crucial aspect of the exciton chirality method is the additivity relation. Namely, the exciton-split CD curve can be approximated by the summation of each interacting basis pair, i.e., when the chromophores are identical it is the *A* value,[4,5] whereas when they are dissimilar it is the entire CD curve[6] that can be approximated by this pairwise additivity relation.

In contrast to the cases in which the derivatization is performed so that the λ_{max} of the newly introduced chromophore is selected to maximally overlap with the substrate chromophore, frequently a new chromophore has to be selected to *avoid* the coupling with the existent chromophore. For example, *p*-dimethylaminocinnamate (dma-Cin) with a red-shifted λ_{max} at 362 nm, ϵ 30 400, was used to determine the chirality between the hydroxyl and amino groups in a mitosene derivative with existent strong absorptions at 309 (ϵ 10 000) and 245 nm (ϵ 14 400);[7] the UV maxima of the exciton-coupled chromophores are sufficiently red-shifted from 309 nm that the exciton-split CD of the *O*,*N*-bis-dma-Cin derivative of mitosene appeared as two bands of opposite sign, nearly equal intensities (so-called conservative couplet) and separated from other Cotton effects. Such separation from bands of different origin leads to nonambiguous and clearcut assignments of chirality.

Thus, the concept of the "red-shifted chromophore" meets one of the general and important requirements in the exciton chirality method: Cotton effects arising from exciton coupling to be isolated from other strong Cotton effects. If such chromophores also possess intense ϵ values, this would lead to increased sensitivity, thus permitting microscale structure determination.

The Cyanine Dye Chromophore

The cyanine dyes, characterized by their red-shifted, intense, and narrow maxima,[8] were investigated[9] as potential chro-

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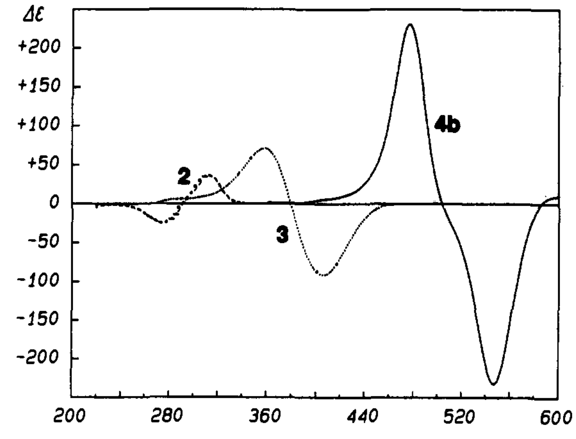


Figure 1. CD spectra of bis-amide (1*S*,2*S*)-**2** in MeCN (dashed line), neutral Schiff base (1*S*,2*S*)-**3** in CH_2Cl_2 (dotted line), and biscation (1*S*,2*S*)-**4b** in CH_2Cl_2 (solid line).

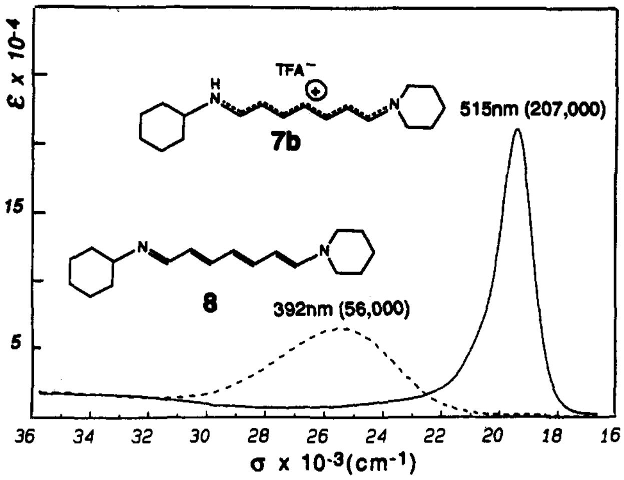
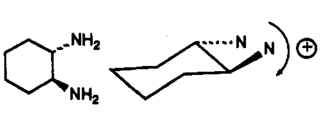
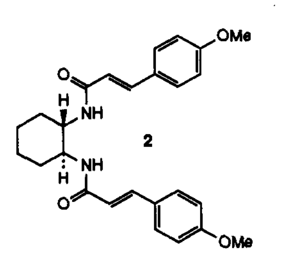


Figure 2. UV–vis spectra in CH_2Cl_2 of monocation **7b** (solid line) and mono-Schiff base **8** (dashed line).



(1S,2S)-1



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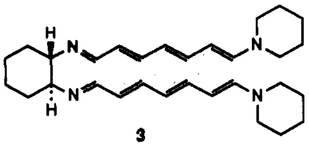

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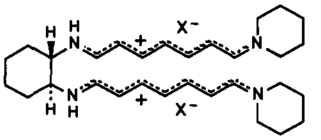
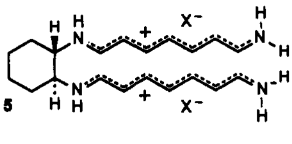


4a X⁻ : Cl⁻
4b X⁻ : TFA⁻



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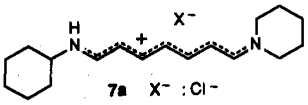
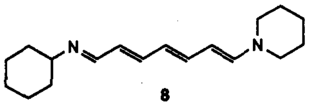


7a X⁻ : Cl⁻
7b X⁻ : TFA⁻



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mophores for various CD studies. The dibenzamide and bis(*p*-methoxycinnamide) of (1*S*,2*S*)-(+)-*trans*-1,2-cyclohexanediamine (**1**) exhibit positive first and negative second Cotton effects, in agreement with the positive exciton chirality (Figure 1): dibenzamide derivative,[10] λ_{max} 225.5 nm (ϵ 20 000), λ_{ext} 239 ($\Delta\epsilon$ + 13.0) and 220 nm ($\Delta\epsilon$ −9.4); bis(*p*-methoxycinnamide) **2**,[11] in MeCN, 305 nm (shoulder, ϵ 32 000), λ_{max} 287 nm (ϵ 41 000), λ_{ext} 312 ($\Delta\epsilon$ +36) and 274 nm ($\Delta\epsilon$ −25). Recently a sign reversal in exciton coupling has also been reported.[12] For example, such a sign reversal occurred for (1*R*,2*R*)-cyclohexanediol dipyrrinone bisester when the solvent was changed from CH_2Cl_2 to DMSO; inversion from the expected sign in the latter solvent was attributed to chromophoric reorientation induced by hydrogen bonding.[12a]

The corresponding neutral Schiff base **3** and especially its protonated form, the cyanine dye **4**, exhibit exciton couplets with remarkable properties: (1) the absorption maxima (Figure 3 below) and CD extrema (Figure 6 below) are located in the visible region, removed from most other chromophores; (2) the couplets show very large ϵ and $\Delta\epsilon$ values, especially those of **4** resulting from high degree of positive charge delocalization; (3) the sign of the exciton-split Cotton effects in both **3** and **4** are opposite that of bisamide **2**, demonstrating that the chromophores adopt a unique conformation; and (4) the intense couplet of biscyanine dye **4** suggests that such chromophores could be useful when dealing with chiral samples available in very limited amounts or when the functional groups to be derivatized are quite remote (of the order of 20–30 Å). The cyanine dye **4** with intense and sharp absorption bands in the UV–vis region around 515 nm (Figure 2) could provide a powerful new tool for CD studies, for example, of biopolymer conformations in solution.

The chiral biscyanine dye **4b**, prepared during an exploratory search for such chromophores, shows two distinct and widely separated visible bands at 550 (ϵ 182 000) and 480 nm (ϵ 191 000)-(Figure 3).[9] These two visible absorption bands, flanking the absorption band of monomeric cyanine dye at 515 nm, are separated by as much as 70 nm. Moreover, the CD spectrum of biscyanine dye **4b** exhibits two CD Cotton effects opposite in sign and of almost equal intensities: λ_{ext} 546 ($\Delta\epsilon$ −232) and 475 nm ($\Delta\epsilon$ +231) (Figure 4-IIe). Therefore, the large separation of the two visible and CD bands may be interpreted as arising from the exciton interaction between the two side-chain cyanine chromophores. However, such large separation of two UV–vis peaks due to exciton coupling has only been encountered in anthracene crystals.[13] Much smaller separation has been observed only in a few cases in solution.[14] An alternative explanation for the appearance of the two absorption bands is the existence of two chemical species which absorb at 550 and 480 nm, respectively.

If these widely separated CD Cotton effects result from exciton coupling, positive first and negative second Cotton effects should be expected because the two amino groups of (1*S*,2*S*)-(+)-*trans*-1,2-cyclohexanediamine (**1**) constitute a clockwise screw. However, the signs of observed Cotton effects are opposite the expectation: negative first Cotton effect at longer wavelengths and positive second Cotton effect at shorter wavelengths.

To clarify the origin and mechanism of the two observed CD and UV–vis bands with such large separation and the observed sign reversal in CD Cotton effects, theoretical calculations of the CD and UV–vis spectra of the model biscyanine biscation (1*S*,2*S*)-**5** were performed by application of the π-electron SCF-CI-DV MO method.[2] In the cases of natural products and synthetic chiral compounds with twisted π-electron chromophores, the absolute stereochemistry of such compounds has been theoretically determined by calculation of CD and UV–vis spectra

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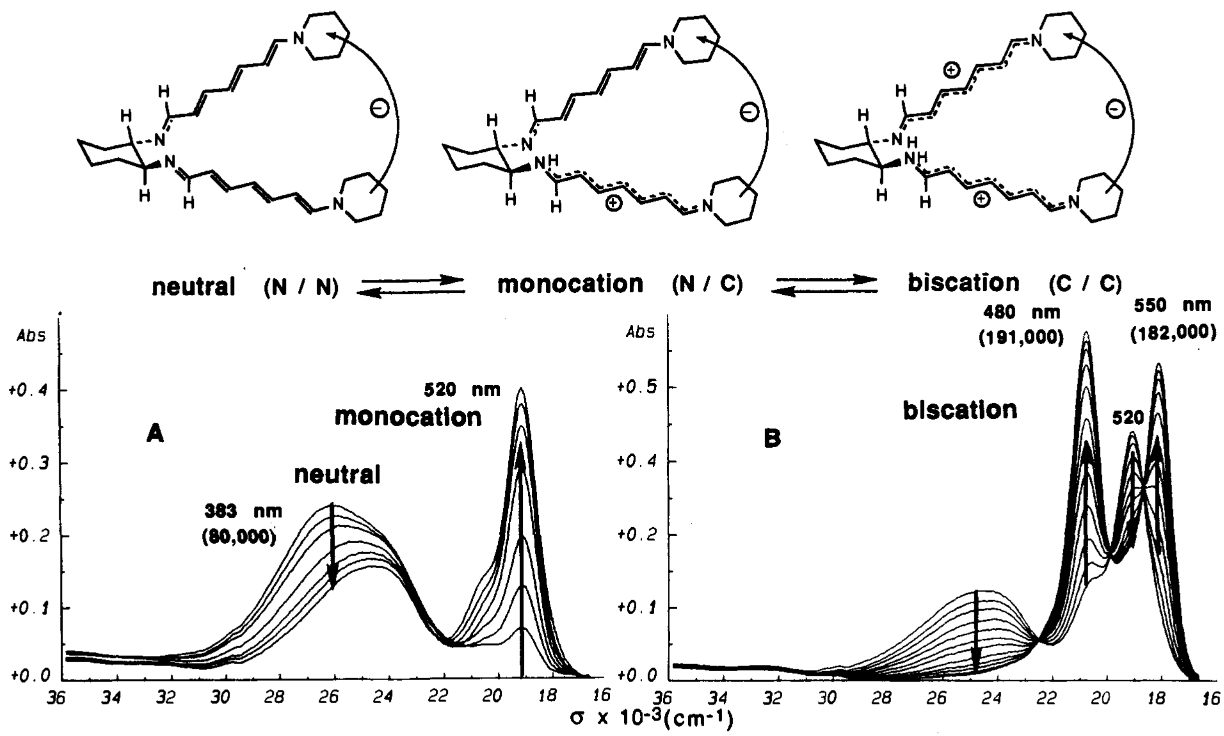

Figure 3. Changes in UV–vis spectra upon stepwise addition of a CH_2Cl_2 solution of TFA (5.0×10^{-4} M) to a CH_2Cl_2 solution of bis-Schiff base **3** (3×10^{-6} M). Arrows on the spectra denote direction of changes. (A) Equilibrium between neutral Schiff base **3** and monocation. (B) Equilibrium between monocation and biscation **4b**.

using the above MO method,[15] and their absolute stereostructures have been experimentally verified by total synthesis, chemical correlation, and/or X-ray structure determination.[15a,b,16] The calculated CD and UV–vis spectral data of (1*S*,2*S*)-**5** agree well with the observed spectral data of (1*S*,2*S*)-**4b**. Analysis of calculation results clarifies that the separation of the two CD and UV–vis bands is due to the exciton coupling between two cyanine chromophores. The unprotonated bis-Schiff base **3** also exhibits similar bisignate CD Cotton effects in the region of 330–450 nm. In the following, we account for this unique behavior of biscyanine dyes.

Results and Discussion

Synthesis of Biscyanine Dyes 4a and 4b and Change of UV–Vis **Spectra upon Titration of a Solution of Bis-Schiff Base (1*S*,2*S*)-3 with Trifluoroacetic Acid.** Reaction of (1*S*,2*S*)-(+)-*trans*-1,2-cyclohexanediamine (**1**) and 7-piperidinohepta-2,4,6-trienal (**6**, merocyanine)[17] followed by flash chromatography yielded biscation **4a**; the reaction was carried out in milligram scale under argon in the dark.[18] Deprotonation of **4a** to bis-Schiff base **3** was performed in a UV cell; subsequent titration of a solution of **3** by addition of trifluoroacetic acid led to the spectral changes shown in Figures 3 and 4.

As described above, there are two possible mechanisms for explanation of the appearance of two intense absorption bands separated by as much as 70 nm in visible spectra of biscation **4b**: (1) the exciton coupling mechanism and (2) the existence of two chemical species which absorb at 550 and 480 nm, respectively. However, from the titration curves shown in Figure 4-I, the second mechanism can be excluded. The titration curves 3 and 4 in Figure 4-I plotted against 480 and 550 nm, respectively, show a similar pattern to each other, indicating that the two absorption bands with λ_{max} 480 and 550 nm are due to a single chemical species.

Stable Conformation of the Model Biscation (1*S*,2*S*)-5. The molecular framework and conformation of the model (1*S*,2*S*)-**5** biscation were calculated by molecular mechanics (MMPI and MMP2),[19] where the parameters of the nitrogen atom were modified. In view of the nature of the delocalized cyanine cation, bond alternation in the imine polyene chromophores was eliminated. The stable conformation obtained is illustrated in Figure 5. The two protons H_a and H_b are in an almost *syn* relation, and therefore the side chains of the two cyanine chromophores constitute a counterclockwise screw sense. The cyanine chromophore moieties are almost flat. The right half of Figure 5 shows a projection from the top of the molecule, where the angle between the two long axes of the cyanine chromophores is ca. 70°.

Theoretical Calculation of CD and UV–Vis Spectra of the Model Compound Biscyanine Biscation (1*S*,2*S*)-5. Numerical calculations were carried out on the NEC ACOS 2000 computer at the Computer Center of Tohoku University. The CD and UV–vis spectral curves of (1*S*,2*S*)-**5** were calculated by the π-electron SCF-CI-DV MO method.[2,20,21] In the dipole velocity method, the rotational strength R_{ba} and dipole strength D_{ba} are expressed as follows:

$$R_{ba} = 2(\varphi_a|\nabla|\varphi_b)(\varphi_a|r \times \nabla|\varphi_b)\beta_M{}^2/(\pi\sigma_{ba}) \qquad (1)$$

$$D_{ba} = 2(\varphi_a|\nabla|\varphi_b)^2\,\beta_M{}^2/(\pi\sigma_{ba})^2 \qquad (2)$$

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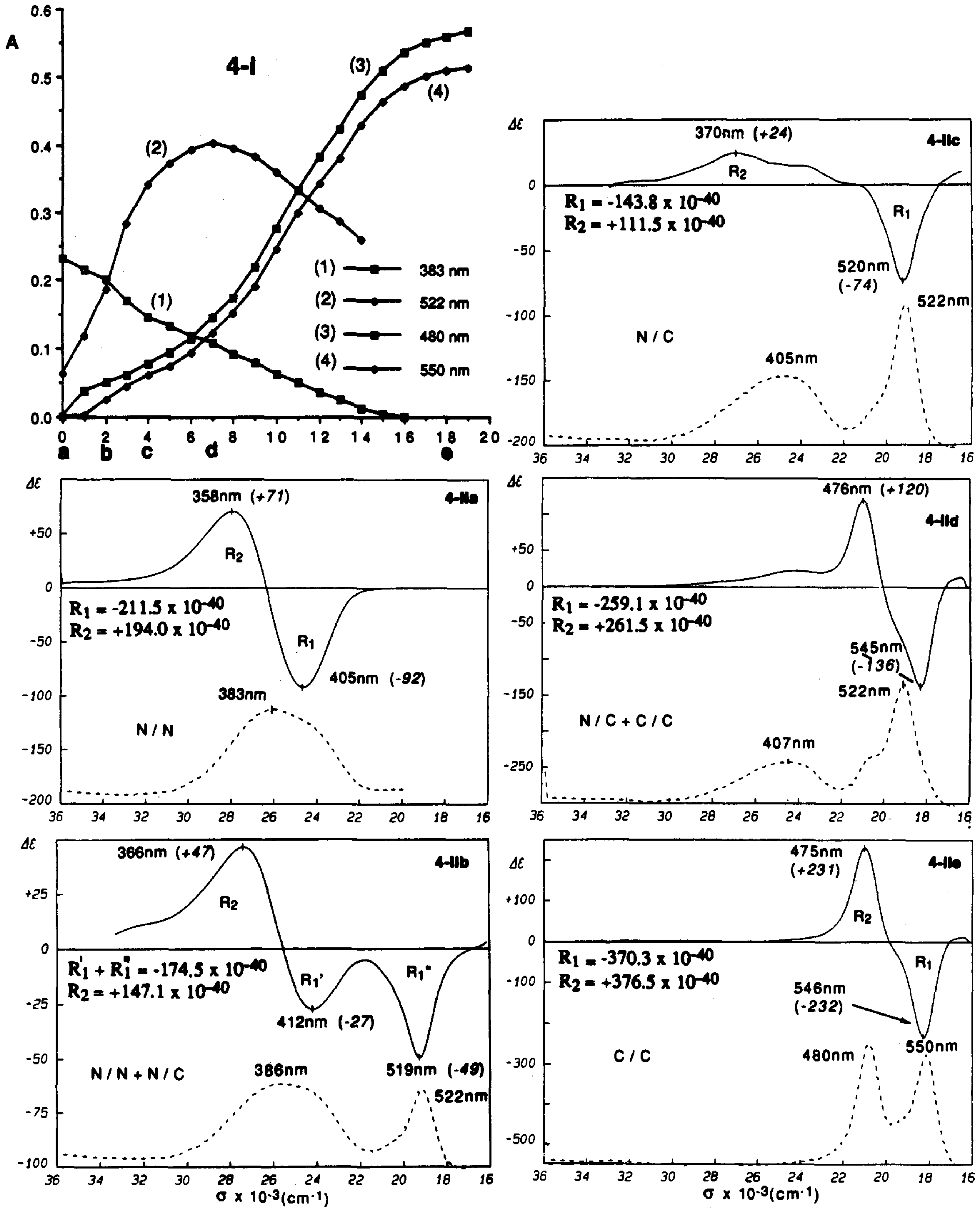
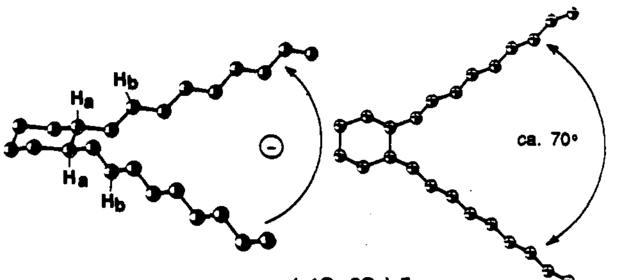


Figure 4. I. Titration curves of neutral Schiff base **3** with trifluoroacetic acid (TFA), both in CH_2Cl_2. Horizontal axis: each aliquot represents 1.56 × 10^{-3} μmol of TFA (5.0 × 10^{-4} M solution) added to 1.3 mL of a 3.00 × 10^{-6} M solution of **3**. Points a–e show some relevant stages of the titration with trifluoroacetic acid. Vertical axis: absorbance at λ_{max}. (1) Neutral, 383 nm. (2) Monocation, 522 nm. (3) Biscation, 480 nm. (4) Biscation, 550 nm. II. Changes in CD (solid line) and UV–vis (dashed line) spectra of neutral Schiff base **3** accompanying the titration with TFA, both in CH_2Cl_2. Plots a–e correspond to points a–e in I: (a) neutral Schiff base **3**; (b) mixture of neutral Schiff base and monocation species; (c) monocation; (d) mixture of monocation and biscation; (e) biscation species **4b**.

where ∇ is the del operator, **r** is a distance vector, β_M is the Bohr magneton, and σ_{ba} is the excitation wavenumber of the transition a → b. The z-axis component of the electric and magnetic transition moments are expressed, respectively, as[2,21]



(1S, 2S)-5

Figure 5. Stereoscopic view of the stable conformation of biscyanine biscation (1*S*,2*S*)-**5**. (a) Protons H_a and H_b are almost syn; the two cyanine side chains constitute a counterclockwise screw or negative exciton chirality. (b) Top view of the stable conformation; the calculated angle between two side chains is ca. 70°.

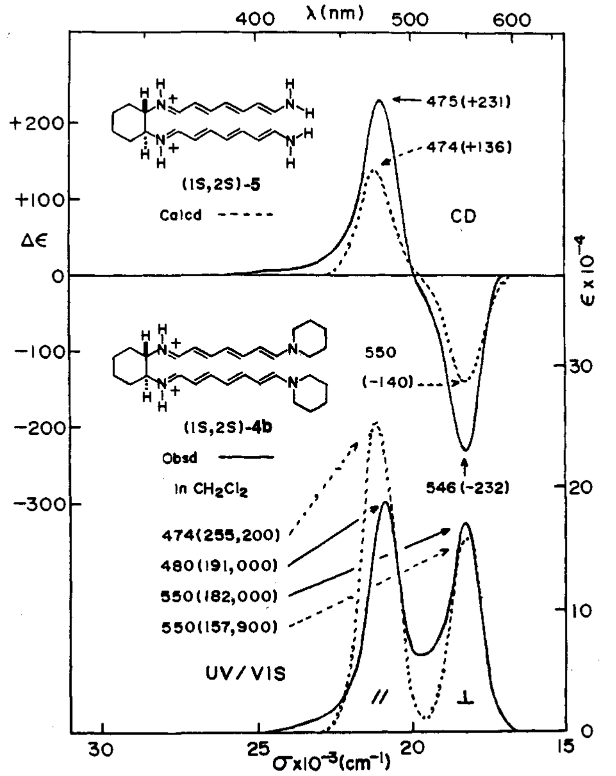


Figure 6. Observed CD and UV–vis spectra (solid lines) of biscation (1*S*,2*S*)-**4b** in CH_2Cl_2 and calculated CD and UV–vis spectra (dashed lines) of model compound (1*S*,2*S*)-**5**.

$$(\varphi_a|\nabla|\varphi_b)_z = \sum\nolimits_{\text{bonds}}(C_{ra}C_{sb} - C_{sa}C_{rb})\langle\nabla_{rs}\rangle \cos Z_{rs} \quad (3)$$

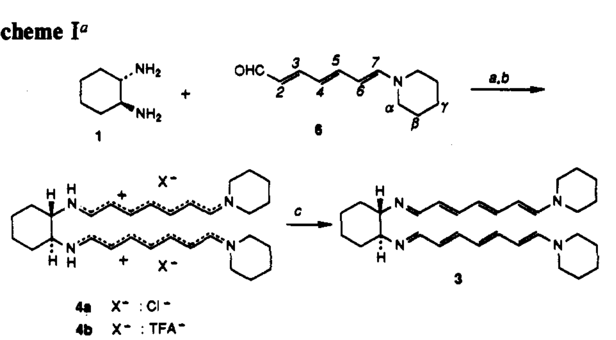
$$(\varphi_a|\mathbf{r} \times \nabla|\varphi_b)_z = \sum\nolimits_{\text{bonds}}(C_{ra}C_{sb} - C_{sa}C_{rb})\langle\nabla_{rs}\rangle(X_{rs} \cos Y_{rs} - Y_{rs} \cos X_{rs}) \quad (4)$$

$$\cos Z_{rs} = (Z_r - Z_s)/r_{rs} \quad (5)$$

$$X_{rs} = (X_r + X_s)/2 \quad (6)$$

where C_{ra} is the coefficient of atomic orbital r in the wave function φ_a, $\langle\nabla_{rs}\rangle$ is the expectation value of a dipole velocity vector ∇_{rs} which is directed along the bond rs in the direction r → s, X_r, Y_r, and Z_r are the *x*, *y*, and *z* coordinates of atom r, respectively, and r_{rs} is the interatomic distance between atoms r and s. In a similar way, the *x* and *y* components of the electric and magnetic transition moments were calculated.

Scheme I[a]



[a] (a) Dry MeOH, 60–65 °C, 4 h. (b) SiO_2 flash chromatography, MeOH/$CHCl_3$/1 M HCl (20:80:0.4). (c) CH_2Cl_2/NaOH.

In the π-electron SCF-CI-DV MO calculation, the following values of atomic orbital parameters were employed: for sp^2 carbon, $Z(C) = 1.0$, $W(C) = -11.16$ eV, $(rr|rr)(C) = 11.13$ eV, β(C–C, 1.388 Å) = −2.18 eV, $\langle\nabla\rangle$(C–C, 1.388 Å) = 4.70 × 10^7 cm^{-1}; for nitrogen, $Z(N) = 2.0$, $W(N) = -27.70$ eV, $(rr|rr)(N) = 17.44$ eV, β(C–N) = −1.899 eV, $\langle\nabla\rangle$(C–N) = 5.50 × 10^7 cm^{-1}. The electric repulsion integral (rr|ss) was estimated by the Nishimoto–Mataga equation. The resonance integral and del value were calculated by employing the following equations, respectively:

$$\beta = [S/S(1.388\ \text{Å})]\ \beta(1.388\ \text{Å}) \cos\theta \quad (7)$$

$$\langle\nabla\rangle = [\langle\nabla\rangle(\text{empir}, 1.388\ \text{Å})/\langle\nabla\rangle(\text{theor}, 1.388\ \text{Å})] \times \langle\nabla\rangle(\text{theor}) \cos\theta \quad (8)$$

where θ is a dihedral angle. The overlap integral *S* and $\langle\nabla\rangle$(theor) were calculated on the basis of the Slater orbitals. The configuration interaction between 80 singly excited states of lower energy were included.

The curves of the component CD and UV bands were approximated by the Gaussian distribution:

$$\Delta\epsilon(\sigma) = \sum \Delta\epsilon_k \exp[-((\sigma - \sigma_k)/\Delta\sigma)^2] \quad (9)$$

$$\epsilon(\sigma) = \sum \epsilon_k \exp[-((\sigma - \sigma_k)/\Delta\sigma)^2] \quad (10)$$

where $2\Delta\sigma$ is the 1/*e* bandwidth. The $2\Delta\sigma$ value of 1524.2 cm^{-1} was adopted for biscation (1*S*,2*S*)-**5**.

The CD and UV-vis spectral curves of (1*S*,2*S*)-**5** were calculated by the π-electron SCF-CI-DV MO method[2,20,21] using atomic coordinates obtained by molecular mechanics[19] calculation. When the MO parameters were evaluated, four nitrogen atoms were treated as being identical; doubly charged cores were assigned to them. The calculated CD and UV–vis spectral curves are shown in Figure 6. The UV–vis absorption spectral curve of (1*S*,2*S*)-**5** exhibits two intense π → π* bands at λ_{max} 550 (ε 157 900) and 474 nm (ε 255 200). These calculated values agree well with the observed UV–vis data of (1*S*,2*S*)-**4b**: λ_{max} 550 (ε 182 000) and 480 nm (ε 191 000). The calculated band separation of 76 nm between the first and second bands agrees with the observed band separation of 70 nm. Although the calculated intensity of the second absorption band is somewhat larger than that of the observed band, the basic pattern of the UV–vis spectral curve of biscyanine biscation (1*S*,2*S*)-**4b** is well reproduced by the theoretical calculation.

The CD spectral curve of (1*S*,2*S*)-**5** was similarly calculated to give typical exciton-split CD Cotton effects as shown in Figure 6. In the region of the first visible absorption band, there was obtained a negative CD Cotton effect at λ_{ext} 550 nm (Δε −140), while in the second absorption band region, a positive extremum at λ_{ext} 474 nm (Δε +136) was observed. Although these CD Cotton effects obtained by theoretical calculation were weaker than the observed ones, the calculated CD values were in good agreement with those of observed CD data, including sign, position, and shape of Cotton effects (Figure 6). Observed data of (1*S*,2*S*)-**4b**: λ_{ext} 546 (Δε −232) and 475 (+231). Since the calculation was carried out for the enantiomer of (1*S*,2*S*) absolute configuration and the cyanine biscation **4b** was prepared starting from (1*S*,2*S*)-(+)-*trans*-1,2-cyclohaxanediamine, these results

Table I. Calculated Dipole and Rotational Strength of the Transitions of Biscyanine Biscation (1*S*,2*S*)-**5**

transition no.	wavenumber (σ), cm^{-1}	dipole strength ($10^{36}D$), cgs unit	rotational strength ($10^{40}R$), cgs unit
1	18 210	107.6	−237.6
2	21 100	150.0	+200.1
3	33 547	3.6	+93.3
4	33 598	1.2	−95.9
5	35 998	0.5	+19.0
6	37 117	0.4	−18.6

Table II. Exciton Wave Functions of the First Two Transitions of Biscyanine Biscation (1*S*,2*S*)-**5** Obtained by Configuration Interaction and Direction of Their Electric Transition Moments

transition no.	wavenumber (σ), cm^{-1}	wave function[a]	direction of transition[b]
1	18 210	$0.690\,56(\varphi_9 \rightarrow \varphi_{11}) - 0.690\,56\,(\varphi_{10} \rightarrow \varphi_{12})$	⊥
2	21 100	$0.688\,86(\varphi_9 \rightarrow \varphi_{11}) + 0.688\,86\,(\varphi_{10} \rightarrow \varphi_{12})$	‖

[a] Minor terms are neglected. [b] Symbols ⊥ and ‖ indicate that electric transition moments are perpendicular and parallel to the C_2-symmetrical axis, respectively.

establish that the observed strongly split CD and UV–vis bands are indeed due to the exciton coupling between two cyanine chromophores. Furthermore, results of these calculation indicate that cyanine biscation (1*S*,2*S*)-**4b** adopts the conformation depicted in Figure 5 as the stable conformation.

Exciton Coupling Mechanism in CD and UV–vis Spectra of Biscyanine Biscation. The calculated CD and UV–vis spectral data were analyzed in detail in order to clarify the origin of these CD and UV–vis properties. As shown in Table I, there are six $\pi \rightarrow \pi^*$ transitions above 245 nm. The electric transitions 1 and 2 are electronically allowed transitions, because their dipole strengths are as large as 107.6×10^{-36} and 150.0×10^{-36} cgs unit, respectively. On the other hand, the remaining four transitions at 298–270 nm are weak and quite separated from transitions 1 and 2 at 550 and 474 nm, respectively. Therefore, transitions 1 and 2 can consist of the excitation of almost pure exciton coupling mechanism. In fact, as shown in Table II, their wave functions are almost those of typical exciton coupling as expressed as $0.690\,56(\varphi_9 \rightarrow \varphi_{11}) - 0.690\,56(\varphi_{10} \rightarrow \varphi_{12})$ and $0.688\,86(\varphi_9 \rightarrow \varphi_{11}) + 0.688\,86(\varphi_{10} \rightarrow \varphi_{12})$, respectively, where φ_9 and φ_{10} are HOMOs of cyanine side-chain chromophores and φ_{11} and φ_{12} are LUMOs of the chromophores.[22] Since transition 1 is out-of-phase, the transition is polarized perpendicular to the C_2-symmetrical axis of the molecule. On the other hand, the electric transition moment of transition 2 is parallel to the C_2 axis because of the in-phase of wave function.

In the exciton coupling system, the ratio of the dipole strengths of two exciton absorption bands is formulated as follows:

$$D^\alpha / D^\beta = (1 - \cos\theta)(1 + \cos\theta) \qquad (11)$$

where D^α and D^β are dipole strengths of the out-of-phase and in-phase excitations, respectively, and θ is the angle between two transition moments.[23] When the angle θ is larger than 90°, the first absorption band at longer wavelengths is stronger than the second band at shorter wavelengths. When $\theta = 90°$, the intensities of two exciton bands are equal to each other because $D^\alpha = D^\beta$. When the angle θ is less than 90°, the second absorption band at shorter wavelengths is stronger than the first one at longer wavelengths. In the case of biscyanine biscation (1*S*,2*S*)-**4b**, the intensity (ϵ 191 000) of the second band at 480 nm is larger than that (ϵ 182 000) of the first band at 550 nm (Figure 6), indicating that the angle between the two transition moments is less than 90°. In fact, the angle between two cyanine side chains of the model biscyanine biscation (1*S*,2*S*)-**5** was calculated to be ca. 70° by molecular mechanics (Figure 5).

The following observed and calculated results of CD spectra of biscyanine biscation (1*S*,2*S*)-**4b** and its model compound (1*S*,2*S*)-**5**, respectively, also support that the exciton coupling mechanism is operating in the experimental large separation of the two bands. As expected from the exciton theory, the first CD Cotton effect at longer wavelengths is opposite in sign that of the second CD Cotton effect at shorter wavelengths, but their absolute values of $\Delta\epsilon$ are almost the same: first Cotton effect, λ_{ext} 546 nm ($\Delta\epsilon$ −232); second Cotton effect, λ_{ext} 475 nm ($\Delta\epsilon$ +231). Furthermore, the rotational strengths of these observed Cotton effects obtained by integration of peak area have almost equal absolute values but opposite are in sign: $R(\text{first}) = -370.3 \times 10^{-40}$ and $R(\text{second}) = +376.5 \times 10^{-40}$ cgs unit. These observed values are close to the theoretically calculated rotational strength values listed in Table I: $R(\text{first}) = -237.6 \times 10^{-40}$ and $R(\text{second}) = +200.1 \times 10^{-40}$ cgs unit.

The observed split Cotton effects thus satisfy the "sum rule",[24,25] which is one of the requirements of the exciton coupling mechanism. The general sum rule states that summation of the positive and negative rotational strengths over the entire CD should be 0. In the case of exciton coupling in which the couplets are removed from other bands, the regional sum rule holds: $R(\text{first}) + R(\text{second}) = 0$. The regional sum rule in exciton chirality has been confirmed experimentally in only a few cases, e.g., two polyacene chromophores,[26] and the present case is a further example.

The following points are responsible for the big separation of two strong exciton bands observed in CD and UV–vis spectra of biscyanine biscation (1*S*,2*S*)-**4b**. (i) The energy gap between two exciton bands is as large as 2890 cm^{-1}. Why is the exciton energy split so large? This is because the electrons in a cyanine cation chromophore move as free electrons in the region of the chromophore due to lack of bond alternation; the corresponding electronic transition of cyanine cation chromophore is thus totally allowed and very strong, giving rise to the intense visible absorption band of the monomeric cyanine cation, $\lambda_{\max}$ 515 nm (ϵ 207 000). Since the exciton energy split of the exciton coupling system with two identical chromophores is proportional to the dipole strength of the corresponding transition, the exciton energy split becomes large. (ii) The corresponding visible band of cyanine cation has a very narrow $1/e$ bandwidth of $2\Delta\sigma = 1524$ cm^{-1}; this value is much smaller than those of ordinary UV bands (ca. 5000 cm^{-1}). Why is the bandwidth so narrow? As described above, the corresponding transition is an electronically allowed one. In such cases, in the Franck–Condon potential curves of excited and ground states, equilibrium configurations of ground and excited states are similar to each other. Therefore, the 0–0 vibrational band is the most intense because of efficient overlap between vibrational wave functions, and intensities of other transitions (0–1, 0–2, 0–3, etc.) fall off rapidly. The bandwidth of an intense absorption band thus becomes narrow.

The analysis of observed CD Cotton effects and theoretical calculation results confirm the exciton coupling mechanism being responsible for the large separation in the two bands observed in CD and UV–vis spectra of biscyanine biscation (1*S*,2*S*)-**4b**.

CD and UV-Vis Spectra of Biscyanine Neutral Species (1*S*,2*S*)-3. The UV–vis spectrum of neutral species (1*S*,2*S*)-**3** exhibits a broad absorption band around 383 nm (ϵ 80 000) with an inflection around 410 nm (Figure 4-IIa). The appearance of the shoulder implies that there are two absorption bands due to exciton

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(22) The wave functions of pure exciton coupling between two identical chromophores are expressed as: out-of-phase, $0.707\,11\,(\varphi_{\text{HOMO}} \rightarrow \varphi_{\text{LUMO}}) - 0.707\,11(\varphi_{\text{HOMO}'} \rightarrow \varphi_{\text{LUMO}'})$, and in-phase, $0.707\,11(\varphi_{\text{HOMO}} \rightarrow \varphi_{\text{LUMO}}) + 0.707\,11(\varphi_{\text{HOMO}'} \rightarrow \varphi_{\text{LUMO}'})$, respectively.

(23) Harada, N.; Uda, H. *J. Chem. Soc., Chem. Commun.* **1982**, 230.

(24) Kuhn, W. *Trans. Faraday Soc.* **1930**, *26*, 293.

(25) Reference 2, pp 332–386.

(26) Harada, N.; Takuma, Y.; Uda, H. *J. Am. Chem. Soc.* **1978**, *100*, 4029.

coupling between two neutral cyanine chromophores. This was evidenced by the CD spectrum of neutral species (1*S*,2*S*)-3: the CD spectrum shows typical exciton-split bisignate Cotton effects: λ_{ext} 405 ($\Delta\epsilon$ −92) and 358 nm ($\Delta\epsilon$ +71). As expected from the exciton theory, these two Cotton effects approximately obey the sum rule:[24,25] *R*(first) = -211.5×10^{-40} and *R*(second) = $+194.0 \times 10^{-40}$ cgs unit. Since the sign of the first Cotton effect is negative, the exciton chirality is negative leading to a counterclockwise screw sense of the two transition moments. Therefore, neutral species (1*S*,2*S*)-3 adopts a conformation similar to that of biscation (1*S*,2*S*)-**4b**.

The CD and UV–vis spectral curves of neutral species (1*S*,2*S*)-3 were similarly calculated: UV–vis, λ_{max} 357 nm (ϵ 122,200); CD, λ_{ext} 377 ($\Delta\epsilon$ −86) and 339 nm ($\Delta\epsilon$ +50). The calculated bands are located at shorter wavelengths than the observed bands, but the overall shapes of the UV–vis and CD bands, as well as the Cotton effect signs, are well reproduced by the calculation.

CD and UV–vis Spectra of Biscyanine Monocation Species. During titration of neutral species (1*S*,2*S*)-3 with trifluoroacetic acid, the UV–vis and CD spectra of the monocation species were observed (Figure 4-IIc). The UV–vis spectrum exhibits a sharp band at 522 nm and a broad band at 405 nm arising from the monocationic and neutral chromophores, respectively. The CD spectrum shows a negative Cotton effect at λ_{ext} 520 nm ($\Delta\epsilon$ −74) and a broad positive Cotton effect at λ_{ext} 370 nm ($\Delta\epsilon$ +24). The absolute values of rotational strengths of negative and positive band areas are almost equal: *R*(first) = -143.8×10^{-40} and *R*(second) = $+111.5 \times 10^{-40}$ cgs unit. Therefore, these CD Cotton effects can be interpreted as arising from exciton coupling between nondegenerate chromophore systems, i.e., a cation chromophore with 522-nm absorption and a neutral chromophore with 405-nm absorption.

Concluding Remarks

The origin and nature of the large separation of the two intense bands observed in UV–vis and CD spectra of chiral biscyanine dye **4b** has been clarified by theoretical calculation of UV–vis and CD spectra using the π-electron SCF-CI-DV MO method. The big exciton separation of the two bands as large as 70 nm is due to the strong exciton interaction (2890 cm^{-1}) between two intense cyanine $\pi \rightarrow \pi^*$ transitions and their narrow $1/e$ bandwidth, which is as small as 1524 cm^{-1}. The fact that in both the biscation and the neutral species, the signs of the exciton split Cotton effects are opposite those expected from the data of dibenzamide and bis(*p*-methoxycinnamide) derivatives indicates that cyanine side chains adopt a unique conformation in which the exciton chirality between the two cyanine transition moments is negative. On the other hand, in the case of dibenzamide and the bis(*p*-methoxycinnamide) **2** the exciton chirality is positive as predicted in a straightforward manner from molecular models. This example thus demonstrates the importance in knowing the spatial disposition of interacting electric transition moments in the CD exciton chirality method. The chiral biscyanine **4** appears to be the first example for which both the visible absorption and CD spectra in solution display two distinct, largely separated exciton split bands.

The cyanine dyes were initially explored to check the feasibility for use in application of the exciton chirality method. Although they exhibited properties of spectroscopic and theoretical interest,[9] it has become clear that they are not suited for practical purposes because of difficulty in preparation hence unsuited for microscale derivatizations. However, the present studies have led to the development of several new chromphores which readily yield stable acylates and Schiff bases with red-shifted intense UV–vis maxima. These chromophores and their microscale applications in CD will be reported shortly.

Experimental Section

General Procedures. IR spectra were obtained as $CHCl_3$/MeOH solutions using a NaCl cell on a Perkin-Elmer 1600 FT-IR spectrophotometer. UV–vis and CD spectra were recorded as CH_2Cl_2 solutions on a Perkin-Elmer Lambda 4B UV/vis spectrophotometer and Jasco 500A spectropolarimeter driven by a Jasco DP500N data processor, respectively. Smoothing and other manipulations of spectra were carried out by use of software developed in house: DFT (Discrete Fourier Transform) procedure for smoothing. The concentration of biscyanine neutral dye 3 in solutions used for the measurements of UV–vis and CD spectra was determined from the experimental ϵ value: λ_{max} 383 nm (ϵ 80 000). In the case of monocyanine compound 7, the ϵ value reported by Griffiths[8] for protonated asymmetrical cyanine in CH_2Cl_2 was used to determine the concentration: λ_{max} 519 nm (ϵ 207 000). FAB-MS spectra were run on a VG-7070 EQ spectrometer, glycerol matrix, Xe ionizing gas. Trifluoroacetic acid (TFA), cyclohexylamine, and (1*S*,2*S*)-(+)-*trans*-1,2-cyclohexanediamine were purchased from Aldrich and used without further purification.

Synthesis of Bis-Schiff Base (1*S*,2*S*)-3. A solution of (1*S*,2*S*)-(+)-*trans*-1,2-cyclohexanediamine **1** (1.12 mg, 0.00981 mmol) in methanol (0.5 mL) was added to a solution of 7-piperidinohepta-2,4,6-trienal (**6**) (merocyanine,[17] 3.92 mg, 0.0205 mmol) in dry methanol (1.5 mL) under argon. The mixture was stirred at refluxing temperature for 4 h in the dark. Progress of the reaction was monitored by measurements of UV–vis spectra: disappearance of the merocyanine band with λ_{max} at 422 nm. Upon completion of the reaction, the UV–vis profile showed a band with λ_{max} at 383 nm. The resulting solution containing biscyanine neutral compound 3 was subjected to flash chromatography on a large excess of silica gel (MeOH/$CHCl_3$/1 M aqueous HCl, 20:80:0.4) to yield biscyanine hydrochloride salt **4a**: bright red color in a CH_2Cl_2 solution; UV–vis, λ_{max} 480 and 550 nm. The NMR spectrum of this compound could not be measured because of the instability of this compound. FT-IR: ν_{max} 2400, 1529, 1389, 1211. FAB-MS *m/z* 461 ($C_{30}H_{45}N_4^+$, i.e., (M − 2HCl + H)$^+$).

Biscyanine neutral compound 3 was prepared from **4a** by addition of a few microliters of 0.7 M NaOH in $CHCl_3$/MeOH until complete neutralization of **4a** was detected by UV–vis measurements. Compound 3 was yellow in CH_2Cl_2 solution. FT-IR: ν_{max} 1477, 1371, 1195.

Synthesis of Mono-Schiff Base 8. Cyclohexylamine (1.21 mg, 0.0122 mmol) was added to a stirring solution of 7-piperidinohepta-2,4,6-trienal (**6**) (merocyanine, 1.93 mg, 0.0102 mmol) in dry methanol (1.0 mL) under argon. The resulting solution was stirred at refluxing temperature for 3 h. Progress of the reaction was monitored by measurements of UV–vis spectra: disappearance of the merocyanine band with λ_{max} at 422 nm and appearance of a band with λ_{max} at 392 nm. The resulting solution containing **8** was subjected to flash chromatography on a large excess of silica gel (MeOH/$CHCl_3$/1 M aqueous HCl, 14:86:0.3) to yield monocyanine hydrochloride salt **7a**, which showed a purple color in CH_2Cl_2. UV–vis: λ_{max} 515 nm. The NMR spectrum of this compound could not be measured due to its instability. FAB-MS: *m/z* 273 ($C_{18}H_{29}N_2^+$, i.e., (M − Cl)$^+$). Mono-Schiff base **8** was prepared from **7a** by addition of a few microliters of 0.7 M NaOH in $CHCl_3$/MeOH until complete neutralization of **7a** was detected by UV–vis measurements. Compound **8** showed a pale yellow color in CH_2Cl_2.

Titration of Bis-Schiff Base (1*S*,2*S*)-4 and Mono-Schiff Base 8 with Trifluoroacetic Acid. Deprotonation of **4a** and **7a** to the corresponding bis-Schiff base 3 and mono-Schiff base **8** was achieved in a UV cell by addition of a few microliters of 0.7 M NaOH in H_2O/MeOH. UV–vis and CD spectra were measured after addition of 3 μL of a solution of TFA in CH_2Cl_2 (1.56×10^{-3} μmol of TFA) until no change was observed in the recorded spectra.

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