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Fluorescence-Detected Exciton-Coupled Circular Dichroism: Scope and Limitation in Structural Studies of Organic Molecules

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Abstract: The potential of fluorescence-detected circular dichroism (FDCD) to extend the scope of the exciton chirality method is rooted in the increased sensitivity and selectivity of fluorescence as compared with absorbance measurements. In this paper, several practical aspects of FDCD are addressed with model compounds possessing a (1*R*,2*R*)-*trans*-1,2-cyclohexanediol skeleton and two chromophores attached to the diol through ester linkages. We have probed the utility of fluorescent chromophores that have previously been shown to be excellent chromophores for the conventional absorbance-based circular dichroism (CD) exciton chirality method. Certain fluorophores, such as 2-naphthoate or 6-methoxy-2-naphthoate, provide FDCD spectra that are in good agreement with the conventional CD. In other cases, a bischromophoric derivative results in an FDCD spectrum that significantly deviates from the absorbance based data. Here it is shown that the extent of fluorescence polarization, an estimate of sample anisotropy, is directly correlated with the ability to extract a meaningful exciton-coupled FDCD spectrum. If fluorescence polarization is negligible, the solution is isotropic, and the FDCD and conventional CD are in good agreement. Fluorescence lifetime measurements are used to address the origin of solution anisotropy.

Introduction

The exciton chirality method, based on exciton-coupled circular dichroism,[1,2] provides intense bisignate circular dichroism (CD) spectra derived from the through-space coupling of two or more chromophores in chiral substrates and is a valuable tool for the determination of the absolute configuration or conformation of a natural product in solution. The sign of the split Cotton effect directly reflects the sense of chirality between the electric transition moments of the interacting chromophores. The exciton chirality method[1,2] requires no reference sample since it nonempirically establishes absolute configurations or conformations. This method is extremely versatile in that either preexisting chromophores[3,4] present in the molecule of interest can be used or chromophores can be introduced by derivatization of an appropriate functional group (e.g., alcohol or amine).[5] Theoretical calculations of exciton-split CD spectra[2] further expand the scope of the exciton chirality method and allow for rationalization of the origin of a bisignate curve. In addition, it has been found that the exciton-split CD spectra of molecules

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containing several interacting chromophores, either identical[6] or different,[5,7] can be reproduced by summation of the pairwise contributions of every possible bischromophoric interaction; this pairwise additivity principle, which has been confirmed by theoretical calculations,[8] is critical for simplifying the interpretation of complex CD spectra.

Fluorescence-detected circular dichroism (FDCD) spectra can be obtained with enhanced sensitivity and greater selectivity than that observed for conventional (absorbance) CD.[9,10] While CD measures the difference in absorption for left- and right-circularly polarized light, FDCD detects the difference in fluorescence intensity for left- and right-circularly polarized excitations. Under standard conditions, when the emission of a sample is directly proportional to absorbance, the same dichroic information can be obtained from both processes. Since fluorescence directly measures the amount of light emitted against zero background while absorbance is determined from an intensity difference of transmitted light, the fluorescence signal typically can be observed at much lower concentrations than absorbance. This enhanced sensitivity should allow for the measurement of fluorescence-detected exciton split CD spectra at sub-micromolar concentrations. Recently, we described FDCD measurements that can be applied successfully to compounds where the CD arises from exciton coupling of two or more identical fluorophores. That study showed that excellent agreement of FDCD with conventional CD and a remarkable 50−100 fold enhancement in sensitivity could be attained under favorable conditions, for example, when two strongly absorbing and fluorescent chromophores couple through space.[11] While increased sensitivity is a significant asset of FDCD, it is the selectivity of fluorescence detection that holds greater possibilities in terms of practical applications of FDCD. The ability to selectively analyze the chiral environment of fluorophores in systems that also contain nonfluorescent chromophores would clearly extend the overall scope of CD methods. This facet of FDCD has been explored in only a few cases, such as proteins that contain a single fluorescent tryptophan residue.[12] While our previous results confirm the CD/FDCD correlation for exciton chirality, the role of selectivity in exciton-coupled FDCD is an intriguing question; this can be examined directly by preparing an exciton-coupled system containing one fluorophore and one nonfluorescent chromophore. A more thorough analysis of exciton-coupled FDCD was undertaken to clarify the practical aspects and subtleties of this phenomenon.

Prior to our contribution on fluorescence-detected exciton-coupled systems,[11] several studies on FDCD,[12−17] extensions of FDCD methods, including multidimensional FDCD[18,19] and lifetime-resolved FDCD,[20−23] and several additional applications, such as FDCD-based detectors for capillary electrophoresis[24] and HPLC,[25] and the determination of enantiomeric excess,[26] have described the potential of this technique. In FDCD, the excitation alternates between left- and right-circularly polarized light, and the difference in the emission intensity is measured. The resulting emission can be characterized in one of two ways; either the emission is isotropic, or it is anisotropic, and photoselection becomes relevant.[27,28] In the case where photoselection exists, the FDCD can be significantly distorted so that the spectrum can no longer be directly related to conventional CD. A number of modified instrumental setups[29,30] have been used to attempt to eliminate the signal artifacts that appear in FDCD spectra due to photoselection. However, the development of FDCD as a general technique has been slowed considerably since these spectropolarimeters were substantially customized and were not commercially available. It is a significant advantage that the FDCD setup used in all of the studies described here and in our previous report[11] relies on a standard commercially available fluorescence attachment.

In this paper, the main impetus of the FDCD analysis is the identification of optimal fluorophores for fluorescence-detected exciton chirality studies. The criterion for what constitutes a reasonable fluorophore is the extent of agreement between the spectra obtained from FDCD and conventional CD. The fluorophores selected in this survey should have certain attributes, many of which are analogous to those required in exciton-coupled CD analysis. Namely, the fluorophore should have (1) a large extinction coefficient, ϵ, and a known direction of the electric transition moment, μ; (2) high fluorescence quantum yield; (3) chemical stability; (4) an appropriate substituent, e.g., carboxyl, for derivatizing common functional groups such as hydroxyls or amines; and (5) no or very limited effects due to photoselection. With these qualities in mind, four fluorophoric groups were selected for further analysis: 2-naphthoate **1**,[31,32] 6-methoxy-2-naphthoate **2**,[11] 2-anthroate **3**,[33] and *p*-phenylbenzoate **4**.[34] In addition, the effect on FDCD of four weakly fluorescent or nonfluorescent chromophores was examined: *p*-dimethylaminobenzoate **5**,[2] *p*-methoxycinnamate **6**,[35]

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p-bromobenzoate **7**,[36] and *p*-methoxybenzoate **8**.[37] All of these chromophores have been used extensively in conventional CD studies. The FDCD and fluorescence properties of a wide range of combinations of these fluorophores/chromophores conjugated to (1*R*,2*R*)-*trans*-1,2-cyclohexanediol were investigated.

To determine the conditions under which the CD and FDCD spectra were most closely correlated, the effects of fluorescence polarization and fluorescence lifetime were addressed. Fluorescence polarization (P_F),[28,38] the ratio of the difference between the intensity of the vertically and horizontally polarized emission components ($F_V - F_H$) to the sum of these two values ($F_V + F_H$) originating from a vertically plane-polarized excitation, can be used as a relative measure of sample anisotropy. The current studies have clarified that with systems in which the exciton-coupled FDCD spectrum deviated significantly from conventional CD, fluorescence polarization was relatively large; in cases where P_F was negligible, photoselection effects were not observed, and good agreement was observed between FDCD and CD.

Experimental Section

Abbreviations. The following abbreviations were used: CD, circular dichroism; FDCD, fluorescence-detected circular dichroism; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-(dimethylamino)pyridine; Φ_F, fluorescence quantum yield; P_F, fluorescence polarization; τ_F, fluorescence lifetime; ϕ, rotational correlation time; λ_{ex}, excitation wavelength; λ_{em}, emission wavelength; λ_{max}, UV maximum wavelength; λ_{ext}, CD extremum wavelength; ϵ, molar extinction coefficient; $\Delta\epsilon$, molar circular dichroism; F_L and F_R, fluorescence intensity for left- and right-circularly polarized excitation, respectively.

General. All UV/vis and CD spectra were recorded in acetonitrile (Aldrich, spectrophotometric grade), unless otherwise noted, on a Perkin-Elmer Lambda 40 UV/vis spectrometer and JASCO J-720 spectropolarimeter, respectively. In all cases, solution concentration was calculated based on sample weight. ^{1}H NMR spectra were recorded on a Bruker 300 or 400 MHz spectrometer. Chemical ionization (CI) was measured on NERMAG R1010 mass spectrometer with NH_3 as the ionization gas. Model compounds were prepared with appropriate materials purchased from Aldrich and TCI America (2-anthroic acid). Dichloromethane was distilled from calcium hydride under nitrogen and anhydrous acetonitrile used for syntheses was purchased from Aldrich. Analytical and preparative TLC were run on Analtech precoated silica gel plates (20 × 20 cm), 250 and 500 μm, respectively. Flash column chromatography was performed with Selecto Scientific silica gel, 32−63 mesh.

Fluorescence Properties. Fluorescence excitation and emission spectra were measured in acetonitrile on a SPEX FluoroMax-2 spectrometer. The fluorescence quantum yields for all model compounds in this report were determined by a relative method.[39] The reference solution for determination of quantum yield was 9,10-diphenylanthracene in acetonitrile, the quantum yield (0.90) of which was determined based on the previously reported quantum yield in ethanol (0.95) by employing the refractive index squared correction.[39] Fluorescence polarization experiments[38] were performed on the SPEX FluoroMax-2 spectrometer with Glan Thompson polarizers. The absorption maxima and other specific wavelengths of interest, if necessary, were used as excitation wavelengths, and vertical and horizontal components in emission were observed at emission maxima.

Fluorescence lifetimes[38,40] were determined by the phase and modulation method using a SPEX Fluorolog 2T2 double spectrometer and by single-photon counting (pulse method) using a FL900CDT spectrometer (Edinburgh Analytical Instruments). Since lifetime determinations required adequate emission for measurement accuracy, only compounds showing reasonable emission intensities were analyzed.

Rotational Correlation Time. Brownian movement in solution is approximated by the rotational correlation time (ϕ, in ns),[38] which can be calculated from the following equation: $\phi = (\eta V)/(RT)$, where η is the viscosity of the solvent (cP), V is the volume of the rotating unit (cm^3/mol) derived from the molecular weight and the density of the molecule (the density of each compound was assumed to be 1.3 g/cm^3), T is temperature (K), and $R = 8.314 \times 10^7$ erg·mol^{-1}·K^{-1}.

FDCD Measurements. To avoid intermolecular fluorescence quenching,[38] solutions for FDCD measurement had maximal absorbance below 0.2. In all cases, the same solution was used for both CD and FDCD measurements, ensuring that any observed difference between CD and FDCD spectra was not derived from sample preparation. The cell for CD, FDCD, and fluorescence measurements was a 1 cm square fluorescence quartz cell.

A JASCO-720 spectropolarimeter was fitted with a JASCO prototype FDCD attachment (containing a Hamamatsu phonics R376 photomultiplier tube), with the fluorescence detector placed at 90° to the excitation beam.[28] The transmitted light was trapped by a Rayleigh horn beam trap placed between the sample and the CD detector. The bandwidth was either 2.0 nm (highly fluorescent samples) or 5.0 nm (weakly fluorescent samples), and the voltage (HT) applied to the detector was always kept between 400 and 800 V, corresponding to a maximal DC output signal voltage from the detector of 1.0 V.

For FDCD measurements, the emission is observed over a range of irradiation wavelengths. The emitted light that is detected can be controlled by varying the cutoff of a long-pass filter placed between the sample and the fluorescence detector. Long-pass filters used were JASCO UV34 (340 nm), L38 (380 nm), and L42 (420 nm), CVI WG-320 (320 nm), and Coherent-Ealing WG-360 (360 nm). The appropriate filter was chosen in order to avoid scattered light from the excitation wavelengths which could contaminate the observed emission and to maximize the emitted light signal. In cases where scatter of the excitation wavelengths overlapped with the emission range, it was of primary concern to adjust the filter to a longer wavelength to eliminate the scattered excitation light. Analysis of the emission spectrum of each compound was used to select the filter cutoff prior to FDCD measurement. In experiments with a linear polarizer (plastic sheet polarizer), the polarizer was placed between the fluorescence detector and the long-pass filter. Rotation of the linear polarizer allowed for FDCD measurements detecting different orientations of light.[17,41]

FDCD raw data represent an excitation spectrum that corresponds to the difference in emission ($F_L - F_R$) and the total emission ($F_L + F_R$) resulting from differential absorption of left- and right-circularly polarized light. These data were converted into a conventional CD spectrum by the following equation: $\Delta\epsilon = \epsilon_L - \epsilon_R = \{2(1 - 10^{-A})S\}/\{cd10^{-A} \ln 10\}$, where A is the absorbance, c is the concentration (M), d is the cell length (cm), $S = k(F_L - F_R)/(F_L + F_R)$, and k is a derived instrumental constant (-3.49×10^{-5}).[9,11] The negative value of k inverts S so that FDCD and CD give Cotton effects with the same sign. (Note: Although the data derived from absorption and fluorescence measurements are the same, conventional CD is based on the detection of transmitted light, an indirect estimation of differential absorption. Since the spectropolarimeter takes into account the indirect nature of absorbance measurements, the fluorescence signal, which is measured directly by the detector, initially appears opposite in sign.)

Sample Preparation. Homochromophoric and Heterochromophoric Systems. All bis-ester model compounds were prepared from (1*R*,2*R*)-*trans*-1,2-cyclohexanediol and appropriate carboxylic acid(s) by either general procedure A or B, unless otherwise indicated. Relevant spectroscopic data for each compound is presented in Tables 1−5. Full characterization of compounds **1**−**4** and **9**−**30** is provided in the Supporting Information. UV−vis absorbance data for weakly or nonfluorescent chromophores **5**−**8** have been previously reported[2,35] and fluorescence measurements for weakly fluorescent chromophores

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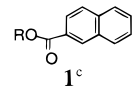
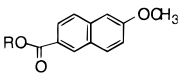
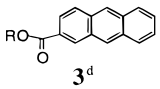
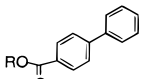
Table 1. UV and Fluorescence Properties of Fluorophores[a,b]

Compound	λ^{abs}_{max} / nm (ε)	λ^{em}_{max} / nm	Φ_F	P_F	τ_F / ns	ϕ / ns
 **1**[c]	234 (58,000)	359	0.32	0.0032	7.9	0.029
 **2**[c]	237 (48,000)	374	0.65	0.0026	5.0	0.032
 **3**[d]	258 (93,000)	434	0.62	0.0023	11.0	0.025
 **4**[c]	270 (21,000)	337	0.66	0.034	0.8	0.031

[a] Φ_F = fluorescence quantum yield, P_F = fluorescence polarization, τ_F = fluorescence lifetime, ϕ = rotational correlation time. [b] All data were obtained in acetonitrile. [c] (1*R*,2*R*)-*trans*-1,2-Cyclohexanediol monoester. [d] Methyl ester.

5 and **6** are presented in Table 2. The terms "homochromophoric" and "heterochromophoric" systems are used to describe bis-ester derivatives containing two identical and two different chromophores, respectively.

General Procedure A. Monoester. To a solution of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol (6.1 mg, 0.053 mmol) and *p*-phenylbenzoic acid (11.1 mg, 0.056 mmol) in dichloromethane (1.5 mL) were added DMAP (7.6 mg, 0.062 mmol) and EDC (16.7 mg, 0.0871 mmol) under nitrogen. The reaction mixture was stirred at room-temperature overnight, and the solvent was removed under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 5/1) to give the (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(*p*-phenylbenzoate) (6.5 mg, 42%)

Bis-ester. To a solution of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(*p*-phenylbenzoate) (6.5 mg, 0.022 mmol) and *p*-bromobenzoic acid (15.2 mg, 0.0756 mmol) in dichloromethane (1.5 mL) were added EDC (17.0 mg, 0.0887 mmol) and DMAP (13.2 mg, 0.108 mmol) under nitrogen. The reaction mixture was stirred overnight, and the solvent was removed under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 10/1) to afford (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(*p*-bromobenzoate)-2-(*p*-phenylbenzoate) (10.5 mg, 99%).

General Procedure B. **6-Methoxy-2-naphthoylimidazole**. A mixture of 6-methoxy-2-naphthoic acid (65.1 mg, 0.322 mmol) and 1,1′-carbonyldiimidazole (84.1 mg, 0.519 mmol) in acetonitrile (4.0 mL) was stirred overnight under a nitrogen atmosphere. After the solvent was removed under reduced pressure the resulting solid residue was purified on flash column chromatography (silica gel, hexane/EtOAc = 3/1) to give 6-methoxy-2-naphthoylimidazole (67.4 mg, 83%).

Monoester. To a solution of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol (27.9 mg, 0.240 mmol) and 6-methoxy-2-naphthoylimidazole (67.4 mg, 0.267 mmol) in acetonitrile (2.5 mL), was added DBU (eight drops) under a nitrogen atmosphere. The solution was stirred at room temperature for 1 h, and the solvent was removed under reduced pressure. The solid residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 5/1 and then 3/1) to give (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1,2-bis-(6-methoxy-2-naphthoate) (14.1 mg, 12%) and (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(6-methoxy-2-naphthoate) (47.8 mg, 66%).

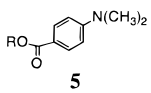
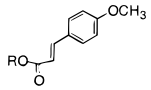
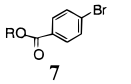
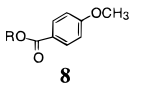
Bis-ester. To a solution of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(6-methoxy-2-naphthoate) (5.8 mg, 0.019 mmol) and 2-anthroic acid (8.8 mg, 0.039 mmol) in dichloromethane (1.0 mL), were added under a nitrogen atmosphere EDC (9.6 mg, 0.050 mmol) and DMAP (5.3 mg, 0.043 mmol), and the mixture was stirred at room-temperature overnight. To the reaction mixture, additional 2-anthroic acid (10.2 mg, 0.046 mmol), EDC (9.0 mg, 0.052 mmol), and DMAP (10.0 mg, 0.074 mmol) were added and stirred for 8 h. The solvent was removed under reduced pressure, and the solid residue was purified by flash column chromatography (silica gel, hexane/EtOAc = 8/1) to give (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(2-anthroate)-2-(6-methoxy-2-naphthoate) (9.7 mg, 99%).

Results and Discussion

Isolated Chromophores. The scope of exciton-coupled FDCD was probed with derivatives of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol, a simple and conformationally rigid skeleton with two hydroxyl groups suitable for fluorophoric derivatization. The strategy for determining favorable fluorophores for FDCD studies involved the systematic preparation and analysis of an extensive series of chromophoric derivatives. This report focuses on four chromophores that were expected to have advantageous fluorescence properties (Table 1) and four other chromophores previously reported as being suited for conventional exciton-coupled CD studies (Table 2).

The first four groups have been described as fluorophores with high fluorescence quantum yields and well-defined orientations of the strong electric transition moments that produce exciton couplets: 2-naphthoate **1**,[32] 6-methoxy-2-naphthoate **2**,[11] 2-anthroate **3**,[33] and *p*-phenylbenzoate **4**.[34] For the purpose of FDCD analysis, quantum yield (Φ_F), fluorescence polarization (P_F), and fluorescence lifetime (τ_F) were measured in acetonitrile (Table 1). All four fluorophores (**1**−**4**) have relatively high quantum yields, those of **2**−**4** exceeding 0.6, while that of 2-naphthoate **1** is ∼0.3. 2-Naphthoate, 6-methoxy-2-naphthoate, and 2-anthroate **1**−**3** exhibit relatively small fluorescence polarization (P_F < 0.0035) and long fluorescence lifetimes (τ_F ≥ 5.0 ns). Thus, the extent of fluorescence polarization can be correlated with the value of the fluorescence lifetime. It is well accepted that a high level of fluorescence polarization is observed when a molecule is unable to undergo sufficient Brownian motion to randomize molecular orientations before emission. In this case, when the probability of excitation depends on the sense of polarization of the incident light, the measured difference in fluorescence obtained by FDCD is no longer

Table 2. UV and Fluorescence Properties of Weakly or Nonfluorescent Chromophores[a,b]

Compound	λ^{abs}_{max} / nm (ε)	λ^{em}_{max} / nm	Φ_F	P_F
 **5**	307 (28,200)[c]	486	0.018	0.02
 **6**	306 (23,400)[d]	380	0.0005	0.4[e]
 **7**	244 (19,500)[c]	-[f]	-[f]	-[f]
 **8**	257 (20,400)[c]	-[f]	-[f]	-[f]

[a] Φ_F = fluorescence quantum yield, P_F = fluorescence polarization. [b] All data for methyl esters in acetonitrile except where indicated. [c] In ethanol, ref 2. [d] Reference 35. [e] Data for compound (1*R*,2*R*)-*trans*-1,2-cyclohexanediol monoester. [f] Not applicable due to weak fluorescence.

necessarily related to the difference in absorbed light measured by CD.[27] This type of anisotropy is generally observed either when a molecule is very large and/or rigid (e.g., biomacromolecules), or when the solvent is viscous and rotation is hindered. To understand the source of any fluorescence anisotropy observed in these experiments, rotational correlation times (ϕ) were compared with fluorescence lifetimes (Table 1).[38] All four fluorophores have calculated rotational correlation times (ϕ) ~0.03 ns, and, thus, are able to undergo >150 rotations since their emission lifetimes range between 5.0 and 11.0 ns. This randomization of the excited state leads to very small observed values of fluorescence polarization, indicating that samples containing only these fluorophores should be essentially isotropic. In contrast, the fluorescence lifetime (τ_F = 0.8 ns) of *p*-phenylbenzoate **4** is only 25 times longer than its rotational correlation time (ϕ = 0.031 ns), indicating that the emission of this fluorophore is more inclined to photoselection effects. The higher anisotropy of the emission of **4** is confirmed by the increased fluorescence polarization (P_F = 0.034).

The cyclohexanediol scaffold was derivatized not only with strong fluorophores, but also with moderately fluorescent or nonfluorescent chromophores. Chromophores previously described as being suited for conventional exciton-coupled CD studies, namely *p*-dimethylaminobenzoate **5**,[2] *p*-methoxycinnamate **6**,[35] *p*-bromobenzoate **7**,[36] and *p*-methoxybenzoate **8**,[37] were employed for these studies (Table 2). The *p*-dimethylaminobenzoate chromophore, **5**, has a weak fluorescence signal (Φ_F = 0.018) and a relatively large fluorescence polarization (P_F = 0.02). A second weak-fluorophore, *p*-methoxycinnamate **6**, has even lower quantum yield (Φ_F = 0.0005) and, moreover, larger fluorescence polarization (P_F = 0.4) than **5**. Two chromophores, *p*-bromobenzoate **7** and *p*-methoxybenzoate **8**, did not exhibit significant emission and were considered nonfluorescent.

Bischromophoric derivatives. All model compounds described here possess a (1*R*,2*R*)-*trans*-1,2-cyclohexanediol skeleton and two chromophores attached to the diol through ester linkages. Our initial report of fluorescence-detected exciton-coupled CD described the excellent agreement between FDCD and CD seen in bis-2-naphthoate **9** and bis-6-methoxy-2-naphthoate **10**,[11] and clearly demonstrated the enhanced sensitivity of FDCD. In the present study, **9** and **10** were used as standards to compare the extent of agreement between FDCD and CD for all new derivatives. In addition, a more detailed analysis of the fluorescence polarization and lifetime of **9** and **10** was performed in order to determine the extent of solution anisotropy for these systems (Table 3).

It was expected that the fluorescence properties of bis-2-naphthoate **9** would be similar to those of the isolated chromophore in **1**. Despite the fact that the two fluorophores in **9** are identical to that in **1**, the bis-ester showed a slight broadening of its emission spectrum from 400 to 500 nm as compared to the monochromophoric ester (Figure 1). The fluorescence lifetime and polarization of **1** and **9** were essentially identical (Tables 1 and 3).

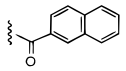
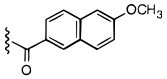
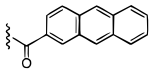
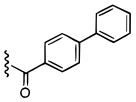
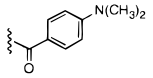
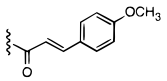
Based on the emission spectrum of **9** (Figure 1B), a 320 nm long-pass filter was chosen for FDCD measurement since most emission occurs above 340 nm and excitation wavelengths between 200 and 300 nm were sufficient for observation of the exciton couplet. The FDCD raw data ($F_L - F_R$; $F_L + F_R$) for **9** in acetonitrile are shown in Figure 2A. Using the conversion equation (see Experimental Section), these data were translated into an FDCD spectrum ($\Delta\epsilon/\lambda$). A comparison of the converted FDCD and the spectrum obtained from conventional CD clearly showed excellent agreement (Figure 2B). In this case, the ratio of the lifetime (τ_F = 8.0 ns) to the rotational correlation time (ϕ = 0.045 ns) indicated negligible photoselection effects. Indeed, this was confirmed by the low fluorescence polarization value (P_F = 0.004). In addition, a very close agreement between FDCD and CD was seen in the case of bis-6-methoxy-2-naphthoate **10** (Figure 3A). As with **9**, the fluorescence polarization, fluorescence lifetime and the rotational correlation time of **10** (Table 3) indicated that this solution could also be considered isotropic. The FDCD analyses of **9** and **10** thus demonstrate the utility of 2-naphthoate and 6-methoxy-2-naphthoate chromophores for fluorescence-based CD measurements.

While 2-anthroate **3** was previously described as a fluorophore for FDCD,[11] attempts to prepare bis-2-anthroate **11** were complicated by the light sensitivity of this compound. Although small amounts of **11** could be purified, light exposure led to significant decomposition as monitored by silica thin-layer chromatography; therefore, further studies with **11** were not pursued. However, in certain cases, compounds containing only a single 2-anthroate fluorophore were stable under conditions employed for routine FDCD measurements (see below); the factors affecting the stability of compounds containing 2-anthroate have not been established.

Although the fluorescence quantum yield of *p*-phenylbenzoate **4** is 0.66, its relatively large P_F (Table 1) is a significant drawback for FDCD. As expected, bis-ester **12** showed a relatively large fluorescence polarization (Table 3), and the agreement between FDCD and CD (Figure 3B) was less satisfactory than that observed for **9** and **10**. The FDCD spectrum, when compared with the CD spectrum, exhibited a difference in intensity and position of corresponding Cotton effects. Thus, this is a case where photoselection effects cannot be neglected.

Bis-derivatives containing one of the two weak-fluorophores, *p*-dimethylaminobenzoate **5** and *p*-methoxycinnamate **6**, were also prepared. The CD and FDCD of bis-*p*-dimethylaminobenzoate **13** were very similar in both position and intensity (Table 3), but the low quantum yield (0.021) and moderate fluorescence polarization (P_F = 0.01) make *p*-dimethylaminobenzoates

Table 3. Bis-homochromophoric Esters of (1*R*,2*R*)-*trans*-1,2-Cyclohexanediol[a,b]

	R	Φ_F	P_F	τ_F / ns	ϕ / ns	λ_{ext} / nm ($\Delta\varepsilon$)
9		0.29	0.004	8.0	0.045	*CD* 229 (+223) / 242 (-321) *FDCD* 229 (+210) / 242 (-269)
10		0.64	0.003	6.9	0.051	*CD* 234 (+124) / 255 (-144) *FDCD* 232 (+116) / 255 (-114)
11[c]		-	-	-	-	-
12		0.65	0.03	1.8	0.050	*CD* 257 (+23) / 286 (-41) *FDCD* 259 (+31), 286 (-28)
13		0.021	0.01	n.d.[d]	n.d.[d]	*CD* 293 (+39) / 320 (-79) *FDCD* 292 (+41) / 320 (-63)
14		0.0014	0.4	n.d.[d]	n.d.[d]	*CD* 282 (+33) / 320 (-55) *FDCD* 307 (+246)

[a] Φ_F = fluorescence quantum yield, P_F = fluorescence polarization, τ_F = fluorescence lifetime, ϕ = rotational correlation time, λ_{ext} = extremum wavelength. [b] All data were obtained with acetonitrile. [c] Due to photochemical instability, the data for **11** were inconsistent. [d] Not determined.

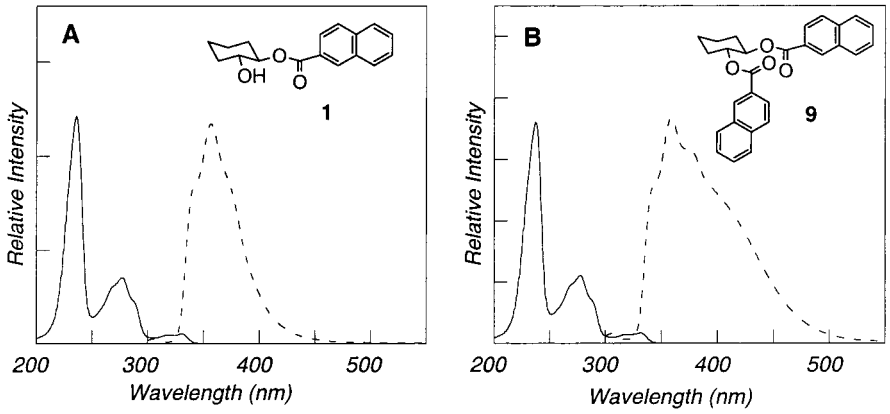


Figure 1. Excitation and emission spectra of the naphthoate chromophore. (A) Excitation (solid line) and emission (dashed line) spectra of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol mono-naphthoate (**1**). (B) Excitation (solid line) and emission (dashed line) spectra of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol bis-naphthoate (**9**).

generally unsuitable for FDCD. In addition, this chromophore is believed to give rise to a twisted intramolecular charge-transfer (TICT) excited state,[42] which may lead to environment-sensitive fluorescence that distorts FDCD data. In certain cases, it may be that the TICT state leads to high levels of emission anisotropy. Therefore, this chromophore cannot be used for exciton-coupled FDCD studies.

Bis-*p*-methoxycinnamate **14** (Table 3) has an extremely large fluorescence polarization of 0.4 and the FDCD was greatly perturbed by photoselection effects.[27,30,43] Comparison of its CD spectrum with that obtained by conventional CD revealed no similarity. Although *p*-methoxycinnamate is a valued red-shifted chromophore for absorbance-based exciton chirality studies, similar to *p*-dimethylaminobenzoate, it is not useful for FDCD studies.

The above studies on bis-homochromophoric cyclohexanediol derivatives indicated that fluorophores with high quantum yield and small fluorescence polarization gave rise to similar FDCD and CD spectra. When fluorescence polarization was large, regardless of quantum yield, FDCD spectra were no longer correlated with conventional CD.

While it was demonstrated that certain pairs of identical fluorophores lead to well-defined exciton-coupled FDCD, the effect of having nonidentical fluorophores in the fluorescence-detected exciton-coupled system has not been addressed. It was of interest to determine the type of FDCD that would result from the coupling of a single fluorophore with a nonfluorescent chromophore. The selectivity of fluorescence detection may lead to a CD spectrum where only the Cotton effect of the fluorophoric group is observed. Alternatively, it was possible that the dipole−dipole exciton coupling interaction would allow for the manifestation of the entire bisignate curve. Similar to the previous cases, heterochromophoric systems were analyzed

(42) Grabowski, Z. R.; Dobkowski, J. *Pure Appl. Chem.* **1983**, *55*, 245−252.

(43) Tinoco, I.; Ehrenberg, B.; Steinberg, I. Z. *J. Am. Chem. Soc.* **1977**, *66*, 916−920.

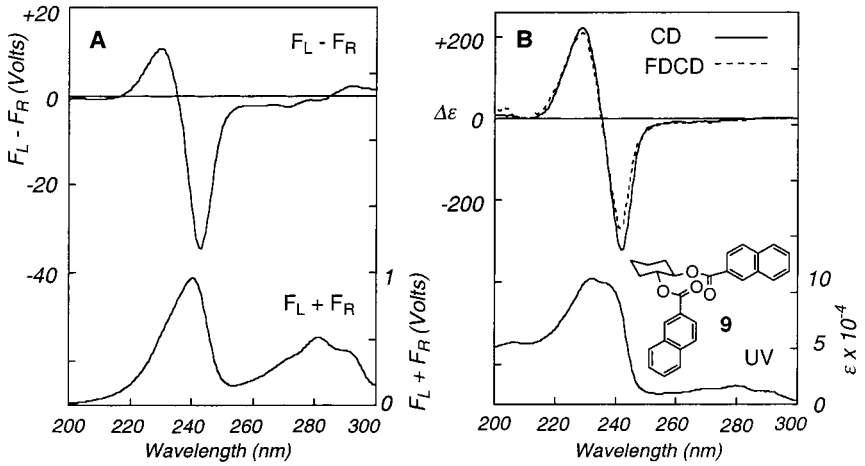


Figure 2. FDCD spectrum of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1,2-bis-(2-naphthoate) (**9**). (A) Raw data obtained from spectropolarimeter. (B) Processed FDCD spectrum (dashed line), as described in the Experimental Section. The FDCD spectrum is compared with the conventional CD spectrum (solid line). All spectra were obtained in acetonitrile (1.93 μM).

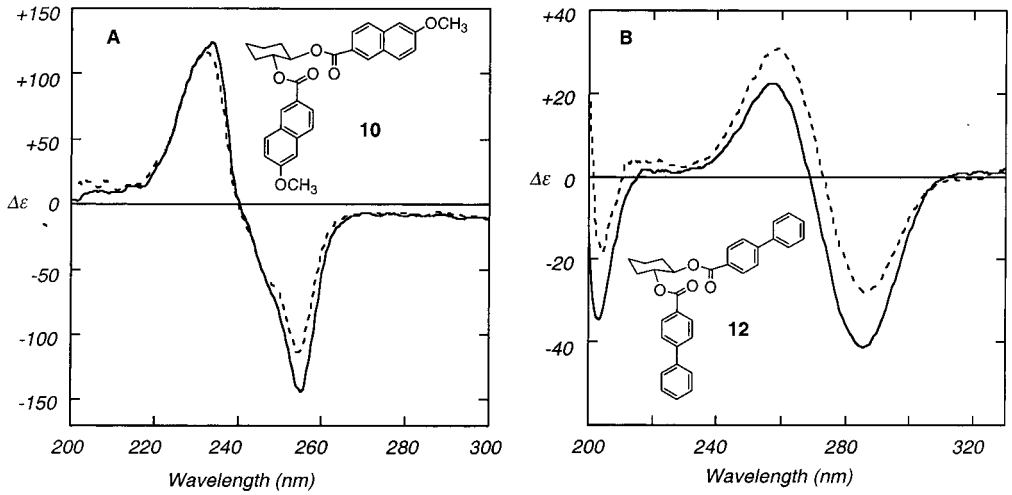


Figure 3. FDCD spectra of homochromophoric-bis-esters of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol. FDCD (dashed lines), CD (solid lines). (A) Bis-6-methoxy-2-naphthoate (**10**) in acetonitrile (2.24 μM), (B) bis-*p*-phenylbenzoate (**12**) in acetonitrile (5.48 μM).

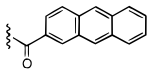
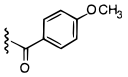
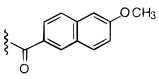
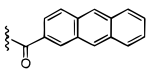
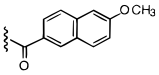
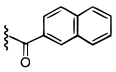
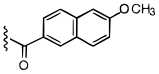
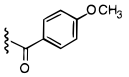
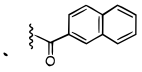
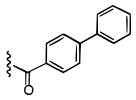
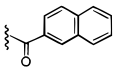
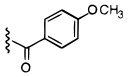
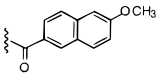
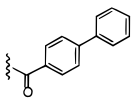
by comparing the converted FDCD spectra with conventional CD and searching for further correlation between fluorescence polarization and lifetime measurements.

In certain cases, bis-derivatives exhibited high Φ_F, small P_F, and good agreement between FDCD and CD (Table 4). The heterochromophoric system **15**, containing the fluorescent 2-anthroate **3** and nonfluorescent *p*-methoxybenzoate **8**, provided FDCD spectra that were in excellent agreement with CD. Thus, when these two chromophores couple, although only one is a fluorophore (2-anthroate, $\Phi_F = 0.62$), the resulting exciton couplet can be surprisingly well represented by FDCD**.** Excitation and emission spectra of 2-anthroate **3** as a single fluorophore (Figure 4A) and bis-ester **15** (Figure 4B) were very similar, confirming that the nonfluorescent *p*-methoxybenzoate is silent in terms of fluorescence. The emission spectrum of the hetero-bis-ester **15** (Figure 4B) indicated that most of the emission is at wavelengths greater than 400 nm. The exciton couplet is expected around the absorbance maxima of the two chromophores, between 250 and 260 nm. With these data in mind, circularly polarized excitation wavelengths from 200 to 320 nm were scanned and a cutoff filter at 380 nm was employed. The resulting raw data (Figure 5A) were converted into an FDCD spectrum which closely resembled the absorbance-based CD (Figure 5B). It is clear that there is good agreement between the two curves, and the analysis of the fluorescence properties of **15** indicated that it has very small levels of fluorescence polarization (Table 4, $P_F < 0.001$).

Compounds **16**, **17**, and **18**, containing different combinations of the fluorescent 2-naphthoate **1**, 6-methoxy-2-naphthoate **2** and 2-anthroate **3**, and the nonfluorescent *p*-methoxybenzoate **8**, also showed satisfactory agreements between their FDCD and CD curves (Figure 6). In all three cases, their polarizations were relatively small (Table 4, $P_F < 0.009$) and their fluorescence lifetimes were relatively long (Table 4, $\tau_F > 5.0$ ns). The relationship between the fluorescence lifetime, the rotational correlation time of a molecule, and fluorescence polarization is also evident by **16**−**18**; e.g., in the case of hetero-bis-ester **16**, where $\tau_F = 11$ ns and $\phi = 0.053$ ns, more than 200 rotations can occur during its emission lifetime. This situation led to low levels of fluorescence polarization ($P_F = 0.002$), which, in turn, gave rise to the reasonable agreement between FDCD and CD. The long τ_F and small P_F relation was also seen in cases **17** and **18**, which showed satisfactory agreement between CD and FDCD.

It is remarkable that the presence of only one fluorophore in esters **15** and **18** still led to exciton coupled FDCD. Since in this case the fluorescent and nonfluorescent chromophores interact through space as a coupled oscillator forming two new split energy levels, the light absorbed by either chromophore can be emitted by the fluorophore. The interaction between the

Table 4. Bis-heterochromophoric Esters of (1*R*,2*R*)-*trans*-1,2-Cyclohexanediol Showing Good Agreements between FDCD and CD[a,b]

	R_1	R_2	Φ_F	P_F	τ_F / ns	ϕ / ns		λ_{ext} / nm ($\Delta\varepsilon$)
15			0.50	<0.001	12.5[d]	0.049	*CD*	249 (+66) / 271 (-95)
							FDCD	250 (+52) / 270 (-81)
16			0.65	0.002	11.0[c], 11.5[d]	0.053	*CD*	237 (+124) / 259 (-171)
							FDCD	237 (+127) / 259 (-157)
17			0.36	0.006	9.9[c]	0.048	*CD*	231 (+159) / 244 (-161)
							FDCD	231 (+142) / 244 (-120)
18			0.79	0.009	5.0[c]	0.046	*CD*	237 (+24) / 258 (-44)
							FDCD	237 (+28) / 258 (-35)
19			0.29	0.02	7.7[d]	0.048	*CD*	237 (+24) / 272 (-30)
							FDCD	237 (+28) / 273 (-25)
20			0.26	0.02	7.7[d]	0.043	*CD*	236 (+54) / 248 (-43)
							FDCD	236 (+51) / 251 (-34)
21			0.38	0.016	7.4[c]	0.053	*CD*	244 (+35) / 261 (-55)
							FDCD	243 (+33) / 262 (-40)

[a] Φ_F = fluorescence quantum yield, P_F = fluorescence polarization, τ_F = fluorescence lifetime, ϕ = rotational correlation time, λ_{ext} = extremum wavelength. [b] All data were obtained in acetonitrile. [c] Determined by phase and modulation method. [d] Determined by pulse method.

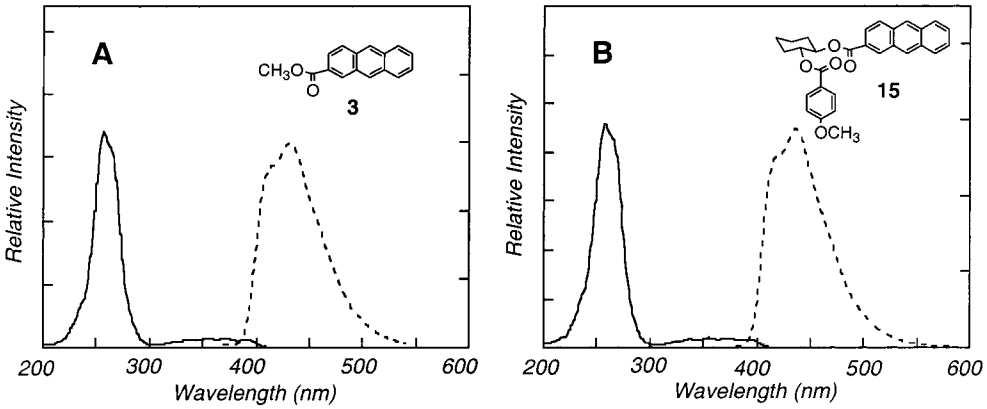


Figure 4. Excitation and emission spectra of 2-anthroate and its derivative. (A) Excitation (solid line) and emission (dashed line) spectra of 2-anthroate (**3**). (B) Excitation (solid line) and emission (dashed line) spectra of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 1-(2-anthroate)-2-(*p*-methoxybenzoate) (**15**).

fluorophore and non- (or weak-) fluorophore in exciton-coupled systems leads to bisignate curves in FDCD where the Cotton effect derived from the nonfluorophore is apparent and both CD and FDCD couplets are almost identical. Therefore, the selectivity of FDCD does not extend to systems in which two chromophores are involved in through-space dipole−dipole interactions. Presumably, an additional nonfluorescent chromophore that is isolated from the exciton-coupled system would be silent in FDCD measurements. This case will be submitted for further studies to probe the selectivity of FDCD analysis. Additional FDCD studies of nonfluorescent chromophores as parts of exciton-coupled systems may allow for a better understanding of the fundamental nature of the exciton coupling phenomenon.

Exciton-coupled CD[2] and fluorescence energy transfer[44] are both derived from dipole−dipole chromophoric interactions. The heterochromophoric bis-ester **16**, containing 2-anthroate and 6-methoxy-2-naphthoate, was used to investigate the effect of energy transfer on FDCD.[45,46] As shown in Figure 7A, there is an overlap between the emission of 6-methoxy-2-naphthoate **2** (donor) and the absorption of 2-anthroate **3** (acceptor).[45] In fact, the emission spectrum of **16** (solid line, Figure 7B) did not show the emission corresponding to that of the donor 6-methoxy-2-naphthoate; this peak was observed in the mixture of the two

(44) Lakowicz, J. R. In *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1984; pp 303−339.
(45) Schnepp, O.; Levy, M. *J. Am. Chem. Soc.* **1962**, *84*, 172−177.
(46) Latt, S. A.; Cheung, H. T.; Blout, E. R. *J. Am. Chem. Soc.* **1965**, *87*, 995−1003.

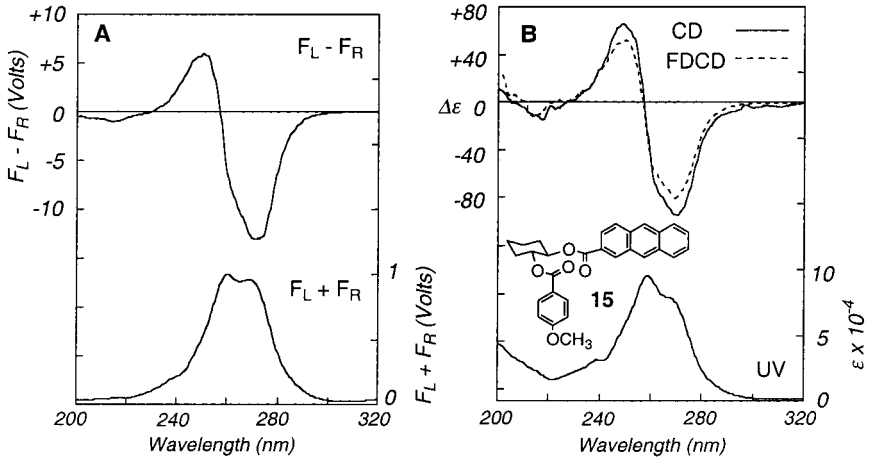


Figure 5. FDCD spectrum of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 2-anthroate, *p*-methoxybenzoate (**15**). (A) Raw data obtained from spectropolarimeter. (B) Processed FDCD spectrum (dashed line), as described in the Experimental Section. The FDCD spectrum is compared with the conventional CD spectrum (solid line). All spectra were obtained in acetonitrile (1.26 μM).

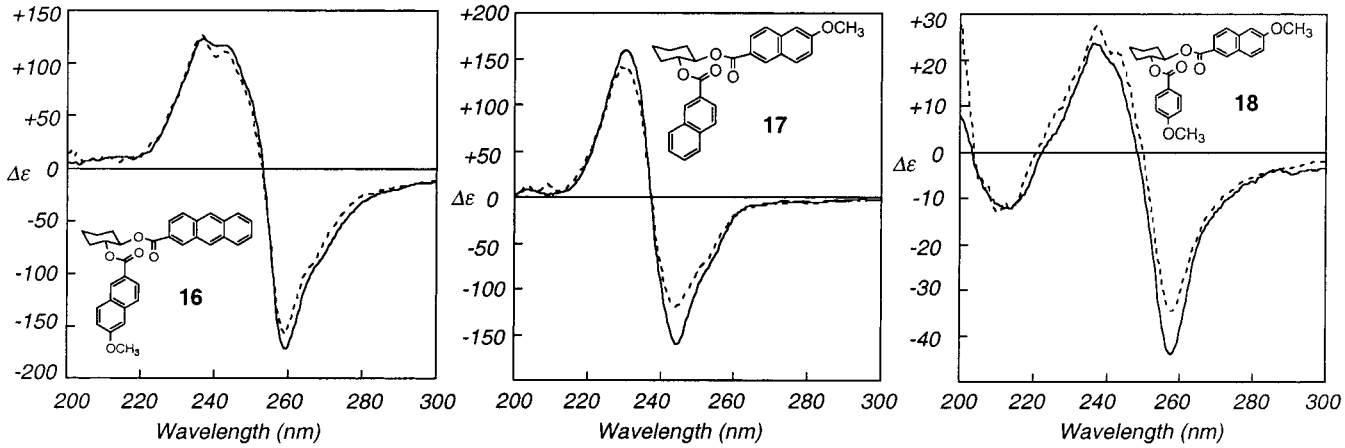


Figure 6. FDCD spectra of hetero-bis-esters of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol. FDCD (dashed lines), CD (solid lines) (A) 6-Methoxy-2-naphthoate, 2-anthroate (**16**) in acetonitrile (1.49 μM), (B) 6-Methoxy-2-naphthoate, 2-naphthoate (**17**) in acetonitrile (3.60 μM), (C) 6-Methoxy-2-naphthoate, *p*-methoxybenzoate (**18**) in acetonitrile (4.41 μM).

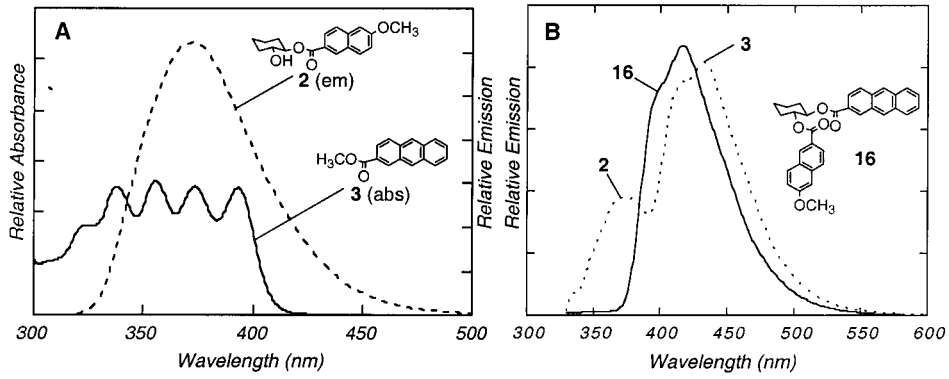
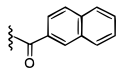
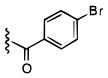
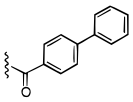
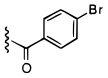
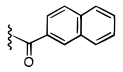
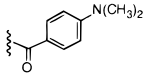
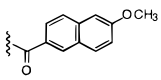
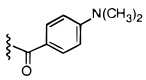
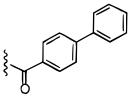
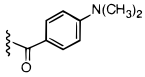
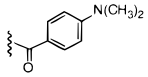
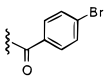
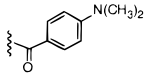
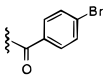
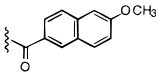
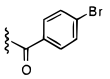
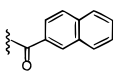
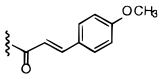
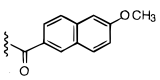
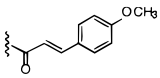


Figure 7. Fluorescence properties of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol 2-anthroate, 6-methoxy-2-naphthoate (**16**). (A) Overlap of 6-methoxy-2-naphthoate emission (**2**) (dashed line) and 2-anthroate absorbance (**3**) (solid line). (B) Emission spectra of **16** (solid line) and an equimolar mixture of **3** and **2** (dashed line).

monochromophoric compounds **2** and **3** (Figure 7B). Despite this evidence of energy transfer, **16** showed good agreement between FDCD and CD.

In the cases of compounds **19**−**21** (Table 4), good agreements between CD and FDCD were observed. Although there was a slight increase in the fluorescence polarization ($P_F \approx 0.02$) as compared with derivatives **9** and **10**, the ability of FDCD to provide the same dichroic information that is obtained from conventional CD was not compromised. In exciton-coupled systems where higher values of P_F are observed, an independent method for confirming the presence of fluorescence anisotropy may be required. One simple method for checking this, which has previously been described and does not require instrumental modification,[17,41] is the placement of a linear polarizer between the sample and the fluorescence detector. The linear polarizer will allow only certain orientations of light to reach the detector. The FDCD and CD spectra obtained at different orientations of the polarizer should be compared. The angle of the linear

Table 5. Bis-heterochromophoric Esters of (1*R*,2*R*)-*trans*-1,2-Cyclohexanediol leading to poor agreement between FDCD and CD[a,b]

	R_1	R_2	Φ_F	P_F	τ_F / ns	ϕ / ns		λ_{ext} / nm ($\Delta\varepsilon$)
22			0.18	0.02	2.5[c]	0.048	*CD*	232 (+49) / 244 (-91)
							FDCD	235 (+34) / 251 (-28)
23			0.53	0.04	0.6[c], 0.66[d]	0.051	*CD*	243 (+13) / 262 (-22)
							FDCD	240 (+16) / 256 (-7) / 282 (+5)
24			0.0093	0.02	n.d.[e]	n.d.[e]	*CD*	240 (+29) / 315 (-20)
							FDCD	238 (+22) / 291 (-8)
25			0.015	0.08	n.d.[e]	n.d.[e]	*CD*	252 (+14) / 313 (-25)
							FDCD	243 (+20) / 313 (-17)
26			0.011	0.02	n.d.[e]	n.d.[e]	*CD*	274 (+22) / 306 (-36)
							FDCD	272 (+20) / 304 (-21)
27			0.0054	0.09	n.d.[e]	n.d.[e]	*CD*	249 (+7) / 309 (-11)
							FDCD	-[g]
27[f]			0.051	0.1	n.d.[e]	n.d.[e]	*CD*	249 (+9) / 302 (-11)
							FDCD	235 (-14) / 304 (+44)
28			0.053	0.02	n.d.[e]	n.d.[e]	*CD*	234 (+40) / 254 (-76)
							FDCD	236 (+23) / 253 (-22)
29			0.001	0.1	n.d.[e]	n.d.[e]	*CD*	236 (+28) / 308 (-15)
							FDCD	236 (+48) / 307 (+69)
30			0.004	0.1	n.d.[e]	n.d.[e]	*CD*	250 (+22) / 312 (-23)
							FDCD	309 (+72)

[a] Φ_F = fluorescence quantum yield, P_F = fluorescence polarization, τ_F = fluorescence lifetime, ϕ = rotational correlation time, λ_{ext} = extremum wavelength. [b] All data were obtained in acetonitrile. [c] Determined by phase and modulation method. [d] Determined by pulse method. [e] Not determined. [f] In cyclohexane. [g] Very weak FDCD signal.

polarizer will have no effect on FDCD when the solution is isotropic;[17] however, in the case where photoselection is relevant, rotation of the linear polarizer should result in nonidentical FDCD spectra. In the case of **21**, the use of a linear polarizer had no effect on the measured FDCD (data not shown).

Compounds that displayed larger fluorescence polarization, regardless of quantum yield, gave less satisfactory agreement between FDCD and CD (Table 5). The fluorescence polarization of **22**, containing 2-naphthoate **1** and *p*-bromobenzoate **7**, varied significantly depending on the excitation wavelength ($P_F^{237\ nm} = 0.02$; $P_F^{278\ nm} = 0.006$). Its fluorescence lifetime was relatively short ($\tau_F = 2.5$ ns) compared to systems that showed good CD/FDCD agreement. The CD and FDCD were similar in the area where P_F is small (>260 nm), but were different where P_F is larger (230−250 nm) (Figure 8A). Although the overall bisignate shape of the FDCD curve was maintained, the position and intensity of the peaks differed significantly from those of the CD spectrum.

Compound **23**, with *p*-phenylbenzoate **4** and *p*-bromobenzoate **7**, has a short fluorescence lifetime of 0.6 ns and rotational correlation time of 0.051 ns (Table 5). The fluorescence polarization of 0.04 is larger than that for **22** ($P_F = 0.02$), while the fluorescence lifetime is shorter than any other compound in this study. No agreement is seen between FDCD and CD for **23** (Figure 8B). This compound can only rotate approximately 10 times during its emission lifetime and clearly photoselection effects play an important role in the FDCD spectrum of this compound. Since it is unlikely that this situation will result in a randomized excited state, fluorescence polarization is high ($P_F = 0.04$). In contrast to the result observed with **21**, the presence of photoselection artifacts for the analysis of **23** was further confirmed since the use of a linear polarizer lead to altered FDCD spectra (data not shown). The use of modified spectropolarimeters[29,30] may alleviate these effects, but this type of analysis requires substantial instrumental modification which was beyond the scope of the current studies.

Compounds containing *p*-dimethylaminobenzoate (**24**−**27**) and *p*-bromobenzoate chromophores (**27** and **28**) were weakly fluorescent and were sensitive to photoselection artifacts. Compound **28** gave results similar to **22**. The FDCD of **28**, possessing weak fluorescence ($\Phi_F = 0.053$) and significant fluorescence polarization ($P_F = 0.02$), showed a distorted and weak spectrum as compared with conventional CD analysis. Compounds containing *p*-dimethylaminobenzoate and *p*-bromobenzoate, in general, showed significant photoselection effects and the agreement between FDCD and CD was not satisfactory. Heterochromophoric bis-esters **29** and **30**, contain-

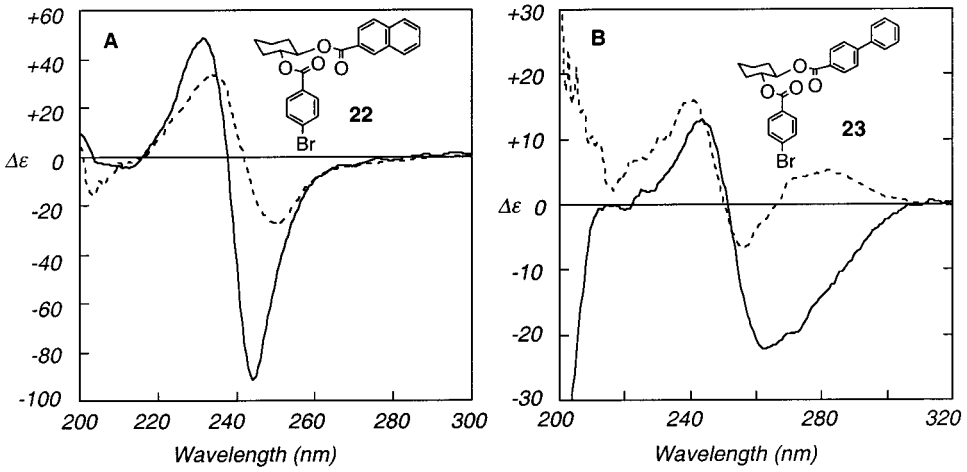


Figure 8. FDCD spectra of hetero-bis-esters of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol. FDCD (dashed lines), CD (solid lines) (A) 2-Naphthoate, *p*-bromobenzoate (**22**) in acetonitrile (4.81 μM), (B) *p*-Phenylbenzoate, *p*-bromobenzoate (**23**) in acetonitrile (6.34 μM).

ing *p*-methoxycinnamate, displayed the largest photoselection artifacts in FDCD. Despite the utility of this chromophore in absorbance-based CD studies, the *p*-methoxycinnamate chromophore is thus unsuited for FDCD studies.

In summary, the value of fluorescence polarization (P_F) is crucial for predicting the agreement between circular dichroic spectra obtained by absorbance and fluorescence. Although additional studies are necessary, the present results suggest that when the fluorescence polarization is smaller than 0.01, a good agreement between the shape of the exciton-coupled CD and FDCD curves can be expected. To our knowledge, this represents the first extensive quantitative analysis of the relationship between FDCD and fluorescence polarization. This trend is valid among all model compounds described in this report. Namely, measurements of fluorescence polarization can be used to check whether the sample is suitable for exciton-coupled FDCD studies; in addition, the fluorescence quantum yield Φ_F of the compound should be larger than 0.2 in order to achieve a significant sensitivity enhancement. Further investigations are ongoing to clarify the factors involved in general FDCD/CD correlations as well as in exciton-coupled FDCD.

Conclusion

FDCD, which in general allows measurements with much higher sensitivity than conventional CD, can be used for the determination of absolute configuration of small molecules by the exciton chirality method. Optimal fluorophores for the fluorescence-detected exciton chirality method have strong absorbance, high fluorescence quantum yield, and negligible polarization of fluorescence. Only isotropic samples, i.e., samples with fluorescence polarization below 0.01 yield exciton coupled FDCD curves corresponding closely to those of absorbance-based CD. A short fluorescence lifetime leads to an increase in polarization since the molecule does not rotate sufficiently to fully randomize molecular orientation before light emission. Clear exciton-coupled FDCD spectra may be observed even in the case where one of the chromophores is nonfluorescent.

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Supporting Information Available: Detailed characterization of compounds **1**−**4** and **9**−**30**, including ^{1}H NMR, mass spectrometry, UV−vis absorbance, CD, fluorescence emission and excitation, fluorescence quantum yield and polarization, and FDCD data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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