# Application of electronic circular dichroism in configurational and conformational analysis of organic compounds†

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This *tutorial review* is addressed to readers with a background in basic organic chemistry and spectroscopy, but without a specific knowledge of electronic circular dichroism. It describes the fundamental principles, instrumentation, data analysis, and different approaches for interpretation of ECD. The discussion focuses on the application of ECD, also in combination with other methods, in structural analysis of organic compounds, including host–guest complexes, and will emphasize the importance of the interplay between configurational and conformational factors. The tutorial also covers modern supramolecular aspects of ECD and recent developments in computational methods.

## 1. Introduction

Circular dichroism, or simply CD, is the difference between the absorption of left and right circularly polarized lights: it is strictly allied to chirality, because it is a manifestation of diastereomer discrimination, the two mirror image objects being the two light beams. CD may be regarded as one of the most powerful techniques for stereochemical analysis: it is sensitive to the absolute configuration as well as to conformational features, which are often completely obscured in the ordinary absorption spectrum. In the following we shall limit our discussion to some critical aspects regarding the interpretation of electronic CD spectra of organic solutes in isotropic media. The subject has been treated in many authoritative books,[1–4] and we hope that this review will stimulate the readers to familiarize themselves with this fascinating field by referring to the cited literature. We anticipate that the well-established and very popular application of circular dichroism to the investigation of secondary structure of biopolymers such as peptides and nucleic acids will be not surveyed here; exhaustive books and reviews are available on this topic.[1,5–9]

After brief notes on fundamental theoretical and practical aspects, we intertwine the introduction of the various methods for the interpretation of CD spectra with the discussion of practical applications. Often the same problem can be tackled at various levels of sophistication and we will try to show merits and limitations of each approach.

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† The HTML version of this article has been enhanced with additional colour images.

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## 2. Fundamentals

### 2.1 Phenomenological description

The interactions of any chiral non-racemic sample with left and right circularly polarized light beams, *i.e.*, two chiral physical entities one being the mirror image of the other, are of diastereomeric type. Accordingly, the circular dichroism is defined as the difference

$$CD = A^{\ell} - A^{\mathrm{r}} \tag{1}$$

where $A^{\ell}$and $A^{\mathrm{r}}$ are the absorptions of left and right circularly polarized light, respectively. For historical reasons, the output of CD instruments is usually measured as ellipticity $\theta$ (in mdeg), related to $CD$ through $\theta$ (mdeg) = 33000 $CD$. In analogy to the Lambert and Beer law, one can define a molar quantity

$$\Delta\varepsilon = \varepsilon^{\ell} - \varepsilon^{\mathrm{r}} = \frac{CD}{c \cdot b} \tag{2}$$

which is independent of the concentration $c$, expressed in mol·L$^{-1}$, and of the pathlength $b$, expressed in cm.

The definition of eqn (1) immediately tells us that CD can be measured *only in correspondence to absorption bands*; a dichroic peak is also called a Cotton effect, on account of the discoverer of the phenomenon. It is worth observing that CD is a signed quantity, because $\varepsilon^{\ell}$ may be smaller or larger than $\varepsilon^{\mathrm{r}}$ (and consequently $A^{\ell}$ and $A^{\mathrm{r}}$); it is easy to show that for each absorption band, the CD of two enantiomers are always exactly opposite.

A useful derived quantity is the $g$-factor, sometimes also called anisotropy or dissymmetry factor, defined as

$$g = \frac{\Delta\varepsilon}{\varepsilon} = \frac{A^{\ell} - A^{\mathrm{r}}}{A} \tag{3}$$

where $A$ represents the conventional absorbance of non-polarized light (or equivalently the average of $A^{\ell}$ and $A^{\mathrm{r}}$). It should be stressed that $g$ is independent of the concentration and of the pathlength (as long as the CD and absorbance measurements are performed on the same sample), which need not to be known or measured. It is defined only provided $\varepsilon \neq 0$, *i.e.* in correspondence with absorption bands.‡


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### 2.2 Theoretical bases

**2.2.1 Electronic transitions and rotational strength.** Although the description of light–molecule interactions goes far beyond our scope, it is useful to recall that for *each* electronic transition one can define an electric and a magnetic transition dipole. They are allied to the electron cloud redistribution taking place during the transition: if the initial and final states are labelled $i$ and $j$ respectively, a linear charge displacement brings about a non-vanishing electric transition dipole $\overrightarrow{\mu}_{ij} \neq 0$ whereas a rotation of electrons leads to a magnetic transition dipole $\overrightarrow{m}_{ij} \neq 0$. Both situations can lead to absorption of radiation: the intensity (or better the integral) of an absorption band is directly related to the oscillator strength $f$

$$\int \varepsilon \, \mathrm{d}\nu \propto f_{ij} \approx \left|\overrightarrow{\mu_{ij}}\right|^2 + \left|\overrightarrow{m_{ij}}\right|^2 \tag{4}$$

where the two vectors are expressed in suitable units. Very often, when it is not vanishing *e.g.* for symmetry reasons, the electric dipole term is very much larger than the magnetic dipole, which justifies the fact that often one makes a distinction between (electric-dipole) *allowed* and *forbidden* transitions, depending only on whether $\overrightarrow{\mu}_{ij} \neq 0$ or $\overrightarrow{\mu}_{ij} = 0$, respectively. On the contrary, in the context of chiroptical spectroscopy, both transition dipole moments play a critical role. The simplest chiral electronic displacement which can give rise to CD is along a helical path, which implies a simultaneous translation and rotation of charge, that is a transition with $\overrightarrow{\mu} \neq 0$ and $\overrightarrow{m} \neq 0$ and for which the two vectors are not orthogonal. By analogy with eqn (4), the integral of a CD band (in suitable units) is directly proportional to the scalar product

$$\int \Delta\varepsilon \, \mathrm{d}\nu \propto R_{ij} \approx \overrightarrow{\mu}_{ij} \cdot \overrightarrow{m}_{ij} \tag{5}$$

defined as *rotational strength*.§ It is apparent that $R_{ij}$ is a signed quantity and by vector algebra one can demonstrate that for mirror image arrangements $R_{ij}$ is of equal absolute value but of opposite sign.

Evaluation of $R_{ij}$ and its sign is the basis for non-empirical interpretation of CD spectra and configurational assignments, as will be discussed in the following sections.

Finally, it is useful to mention a general rule of CD spectroscopy: *the integral of CD over the whole electromagnetic spectrum is zero* or, in algebraic terms

$$\sum_{i,j}^{\text{all transitions}} R_{ij} = 0 \tag{6}$$

‡ A useful operational trick is to modify the definition of $g$, by adding a small constant value to the absorbance at the denominator (something between $1 \times 10^{-2}$ and $1 \times 10^{-3}$ may be a reasonable choice). This ensures that $g$ is defined over the whole spectrum and that it becomes numerically smoother also when the absorbance is very small. The error introduced through this stratagem is negligible as soon as the absorbance becomes sizeable.

§ In cgs units: $R_{ij} = 2.3 \times 10^{39} \int \Delta\varepsilon_{ij}(\nu)/\nu \mathrm{d}\nu$, with $\Delta\varepsilon$ in mol$^{-1}$ L cm$^{-1}$.

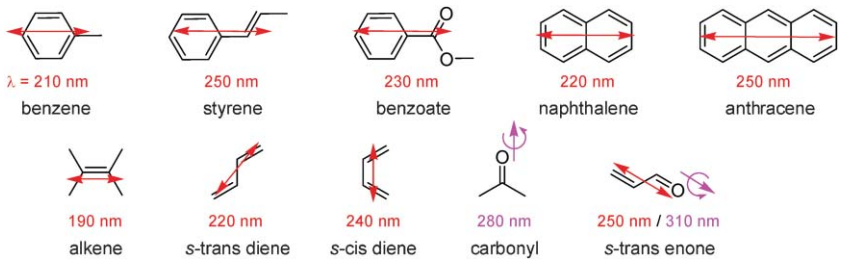



**Fig. 1** A few simple common organic chromophores with their main transitions; electric transition dipoles in red, magnetic in purple (last two structures). Notice that conjugated groups define one whole chromophore.

This is known as the sum rule and descends from very fundamental principles. According to this rule if we see a positive CD band in a spectral region, somewhere else in the spectrum we can expect one or more bands of negative sign. We limit our discussion only to electronic transitions of organic chromophores that are accessible with common instrumentation; "unseen counterparts" may be situated at higher energies, deeper into the far UV (<190 nm ), and therefore are difficult to measure and commonly overlooked.

**2.2.2 Chromophores.** A chromophore is a molecular moiety, responsible for one or more electronic transitions, allied to absorption bands in the UV or in the vis range. In the context of organic chemistry, it is usually a functional group or a combination of several groups with a more or less extended π electron system. A few common examples are depicted in Fig. 1.

The first and crucial step which must precede any attempt in analysing a CD spectrum requires a correct recognition of pertinent chromophoric unit(s). Let us consider the three dihydroisocoumarins depicted in Scheme 1.

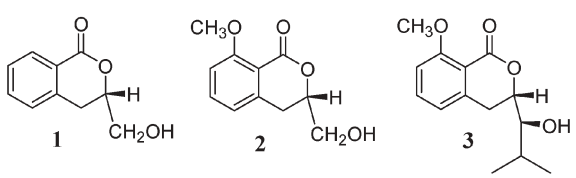
At first glance, **1** and **2** look similar, whereas **3** possesses an additional stereogenic centre. While the chromophores in **2** and **3** are the same, that in **1** is different due to the lack of $OCH_3$ group, which affects the delocalization of the π-system in **2** and **3**. Therefore, we are not allowed to directly compare the CD spectra of, *e.g.*, **1** and **2**. A careful estimation of similarity between two chromophores is an important issue not only when we compare experimental data of different compounds, but also when we apply computational methods. If we wish to calculate the spectrum of **3**, for the sake of saving computational time, we may decide to formally substitute the *i*-Pr group with a methyl, because this does not participate at all in the chromophoric systems. We may even consider exchanging the *i*-Pr for a hydrogen atom (thus running the calculation on the structure **2**), which means cutting off an entire stereogenic centre remote from the chromophore. But we are not allowed to neglect the methoxy group on the aromatic ring, because this implies the simulation of a different chromophore.

We can observe that many common chromophores have local symmetry planes, whereas certain groups, like for example the twisted *cis*-diene, are intrinsically chiral. This leads to a classification in terms of *chiral spheres*, which is useful in the context of chiroptical spectroscopy (Scheme 2).[10] According to this view, an intrinsically chiral chromophore, like a *cis*-diene, forms a first chiral sphere. Intrinsically achiral chromophores become locally dissymmetric due to higher-sphere chirotopic elements. If the chromophore is embedded in a chiral cyclic system, this is said to belong to the second chiral sphere, as in the dihydroisocoumarins **1–3**. Finally the third sphere refers to various situations where the chiral elements are more remote (like the second chiral centre in dihydroisocoumarin **3**).

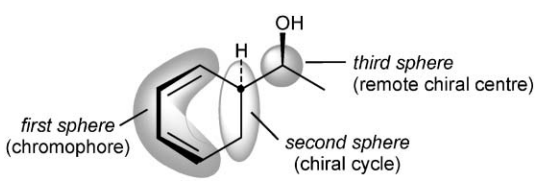
The twisted *cis*-diene is a paradigmatic example of an intrinsically chiral chromophore: a positive skew angle is predicted to be associated to a positive CD of the low lying π–π* transition at about 240 nm; of course for a negative angle a negative CD is expected. The transition between the highest occupied molecular orbital (HOMO) and the lowest unoccupied MO (LUMO) is associated with both non-vanishing electric and magnetic transition dipole moments, which are not orthogonal and give rise to a non-vanishing rotational strength according to eqn (5). The sign of *R* may be predicted in this simple case by multiplying MO lobes of the π-orbitals involved in the transition,[10] as depicted in Fig. 2; a similar reasoning applies to other intrinsically chiral chromophores, *e.g.*, helicenes (see the Graphical Contents entry).

Although this example clearly illustrates the effect of chirality on the sign of rotational strength, we have to point out that the contribution of intrinsic chirality of the diene chromophore to the observed CD can easily be overcompensated by other terms related to the second and/or third chiral sphere, which may have the same or opposite signs.[11]

There are many examples where a π-system with extended conjugation and/or aromaticity remains perfectly planar and




**Scheme 1**




**Scheme 2**

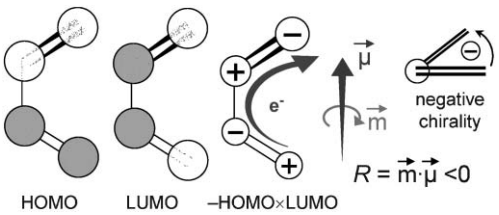



**Fig. 2** HOMO–LUMO transition in the twisted *s-cis* diene chromophore. The transition charge density is the direct (pointwise) product of initial and final orbitals multiplied by the electron charge (the sign inversion ensures agreement with the definition of CD as $\varepsilon^{\ell} - \varepsilon^{r}$). For a negative skew angle, the electron displacement obtained by formal multiplication of MOs defines a left-handed helical path, associated with magnetic and electric transition dipole vectors of opposite directions, resulting in a negative rotational strength.

locally achiral, thus raising an expectation that the symmetry of the associated transitions will prevent any CD. However, the experimental observations contradict such belief since chiral effects originating from second or third sphere easily lead to non-vanishing CD: all atoms and groups in the vicinity of the chromophore may exert symmetry-breaking perturbations of its electronic states. Some aspects concerning this point will be discussed in the following sections.

A special kind of perturbation arises when two chromophores, both endowed with non-vanishing electric dipole transition moments, are nearby in the same molecule. In this situation, each chromophore is at the same time active and passive toward the other (the perturber and the perturbed one), with the maximum effect observed when the two chromophores are identical. This case, known as exciton coupling, gives rise to a striking chiroptical response; it will be described in detail in § 4.2.

### 2.3 Instrumentation

The basic instrumentation for CD measurement is the conventional spectropolarimeter.[4,5] In the most common setup, there is a device between the monochromator and the sample compartment, called photoelastic modulator (PEM), which alternatively furnishes left and right CPL. It is based on a piezoelectric quartz crystal typically oscillating with a frequency in the 50 kHz range: during each cycle the light polarization changes, while the intensity remains constant. After passing through a chiral non-racemic sample, because the two CPL components become absorbed to a different extent, the light reaching the detector is time-modulated with the same frequency as the PEM. Phase-locked amplification of this signal can provide simultaneously absorbance (related to the DC component), and circular dichroism (AC component). Theoretically, a baseline correction should not be required for CD, since the baseline is expected to be 0. In real life, on the contrary, it is strongly recommended that any CD spectrum is corrected by baseline subtraction obtained from a measurement ideally of the racemic compound at the same concentration and in the same cell; as a common alternative, a blank of the same solvent provides acceptable results.

One should not forget that CD is essentially a spectrophotometric measurement and that the usual precautions taken into account for the latter apply to CD as well. Thus in the first place, one must make sure that the total sample absorbance is well below 1.5 a.u. (absorbance units): a value around 0.8 a.u. is recommended. This might imply that the same sample is either transferred into different pathlength cuvettes or is suitably diluted, considering the extinction coefficients of the pertinent absorption bands. Another relevant point is about the solvent choice: although it does not explicitly contribute to CD, a highly absorbing solvent may prevent a correct measurement in the high energy region with long path-lengths.

Optical spectroscopies in general—and thus even CD—are the "fastest" conventional techniques, which allows one to follow dynamic processes in the millisecond range (possibly by coupling it *e.g.* with a stopped-flow apparatus). In the case of an equilibrium mixture, each component contributes to the observed spectrum with a weight proportional to its molar fraction. This notably ensures that a conformational distribution can be treated by means of Boltzmann average over the individual species, which is done for example when comparing experimental and calculated spectra of flexible molecules (see § 4.6). Variable temperature (VT) CD spectra lend themselves as potent investigation tools.[2] In a classical example, axial-equatorial equilibrium of 3-methylcyclohexanone has been followed by means of VT CD of the 300 nm n–π* ketone band.

Selectivity and short response time also make CD an excellent detector for HPLC.[12]

It is not uncommon that organic chromophores are also good light emitters: in such a case, they can be defined as fluorophores and make possible an alternative experimental setup for recording CD spectra with increased selectivity and sensitivity. Fluorescence detected CD or FDCD is measured with the detector at right angle to the rest of the optics. Differential absorption (CD) leads to different excitation and consequently different intensity of the emitted light: the detector reveals a time-modulated signal, which is related to CD. In order to increase the intensity of detected light and to avoid some artefacts, the sample can be accommodated in one focus of an elliptical mirror, which conveys all emitted radiation into the second focus, where the detector is placed. This device is now commercially available as an optional extension to some common CD instruments. FDCD is a powerful alternative and complement to ordinary CD, because it benefits from the advantages of fluorescence, most notably its increased sensitivity; FDCD has been recently treated in a review article[13] and will not be further discussed here.

## 3. Structure–spectra relationships

Many experimental techniques aimed at structural determination, most notably NMR and X-ray diffraction methods, are based on the quantitative evaluation of peak amplitudes and positions, *i.e.*, on the extraction of numerical data, which are then used as input parameters in geometry optimization algorithms. It is rather rare that one can do the same with CD, since in this case the analysis relies largely on the visual comparison of spectra. The experimental CD of the molecule under investigation is commonly displayed together with either another experimental spectrum, or with the result of a more or

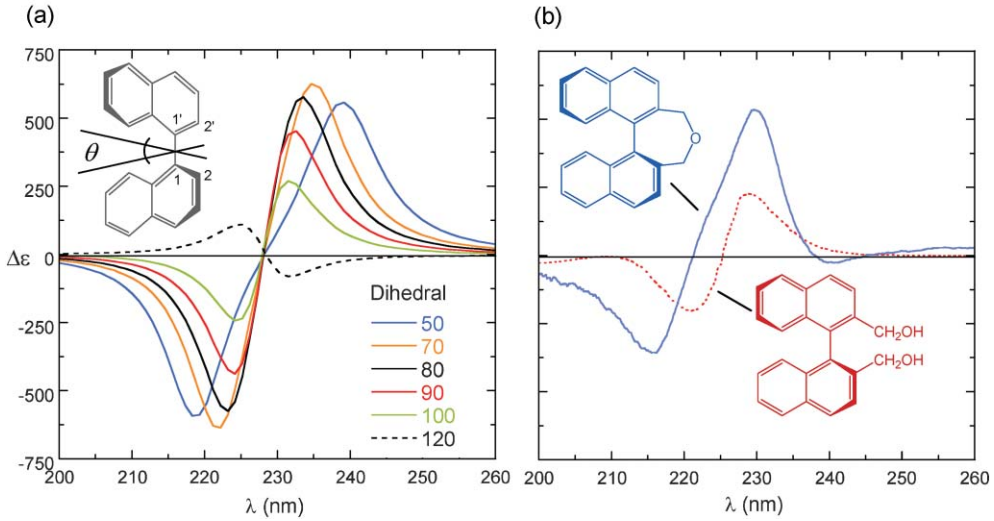



**Fig. 3** a) Calculated CD spectra as a function of the dihedral angle $\theta$ in (*S*)-1,1′-binaphthalene; b) experimental spectra of two compounds demonstrating the same effect (calculated dihedrals $\approx$ 55° for blue structure, solid line, and $\approx$90°; for red structure, dotted line).

less sophisticated calculated theoretical curve; in both cases it is very important to correctly choose the reference data. There may be another option, as well, consisting in the prediction of the sign of one or a few bands, by means of rules (§§ 4.1–4.2), which in turn stem from a generalization of an empirical or of a computational trend.

In this section we wish to clarify: 1) what kind of structural information can be derived from a CD analysis; 2) which are the key features of the test and reference molecules to be taken into account in order to ensure a reliable comparison.

Two enantiomers of a chiral molecule are expected to yield mirror image CD spectra. Thus in the first instance CD seems to provide a Boolean answer, of the type *R*/*S*. Such answer may be strictly obtained only if the test and the reference molecules are equal (or possibly enantiomers); in any other case, it should be borne in mind that CD senses the chromophore(s) embedded in its environment. Every conformational difference, every major alteration in the nature of the chromophore or of the perturbing groups between the test and reference can invalidate the comparison and determination of absolute configuration. The appearance of the CD spectrum, namely position, intensity (rotational strength) and sign of the bands, reflects the environment of each chromophore. A couple of examples will illustrate this crucial point.

1,1′-Binaphthalene provides an extremely useful chiral scaffold for preparing molecules endowed with the most diverse properties. This structure is so attractive mostly because of its pliancy: the rotation around the 1,1′-bond is practically unhindered for dihedral angles $\theta$ (2,1,1,2′) between $50° < \theta < 110°$. The CD spectrum of 1,1′-binaphthalenes is an extraordinary reporter of the dihedral angle $\theta$, as demonstrated in Fig. 3.[14] We can see that both the position (wavelength) and the intensity of the calculated CD bands change with $\theta$; there is a good agreement between the expected CD curves (Fig. 3a) and the experimental spectra (Fig. 3b) of molecules prepared *ad hoc* to provide models at predetermined conformations. In the extreme case, for a given absolute configuration and very large dihedral angles the CD can eventually change its sign; thus the CD appearance results from an interplay of configurational and conformational factors.

The two compounds shown on Fig. 4 provide another remarkable example.[15] They contain the same chromophores, but the 5- or 6-membered rings adopt different conformations which reflect into quasi-mirror image CD spectra for the same

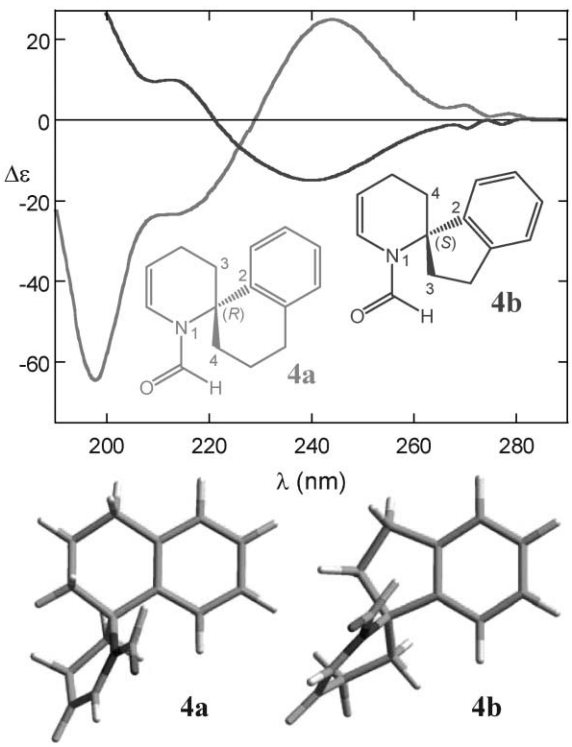



**Fig. 4** CD spectra of two apparently very similar compounds (adapted from ref. 15, with permission; copyright 1997 American Chemical Society): notice that actually the absolute stereochemistry is identical (even though, according to the sequence rules (see the numbering), **4a** is (1*R*), **4b** is (1*S*)). Bottom structures show the different conformation adopted by the two compounds.

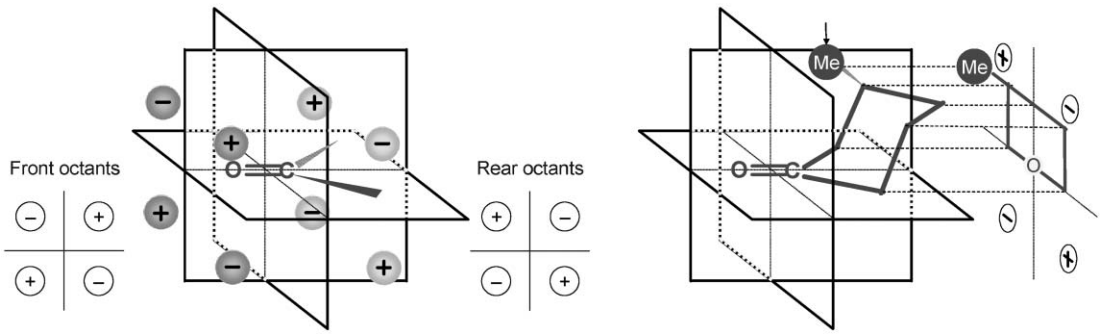



**Fig. 5** Saturated ketone octant rule. Left: the nodal planes of the chromophore define 8 octants; the signs of the contributions to the CD of the band around 300 nm are shown. Right: how the projection in the rear octant is built for (*R*)-3-methylcyclohexanone ($\Delta\varepsilon_{max}$ = +0.57 at 284 nm in EPA, ethyl ether/isopentane/ethanol 5 : 5 : 2).

absolute configuration.¶ This example is an extreme case and should finally convince one of the caution to be observed in considering spectra–structure relationships.

In the most common application of CD for absolute configurational assignment, the goal is much more easily achieved when the chiral compound possesses a rigid skeleton or is acyclic but of well predictable geometry. Flexible molecules pose a further issue to be taken care of, by means of a conformational analysis; sometimes it is preferable to carry out chemical manipulations of the analyte in order to provide a conformationally more homogeneous derivative. The necessity of conformational analysis becomes evident when dealing with virtual molecules, *in silico*. In this approach a computer simulation within an extended conformational space provides a manifold of more or less stable local minima for which the CD spectrum has to be calculated. Then the data of such strictly computational analysis, upon Boltzmann weighting of all the minima, can be compared with the experimental results. Unfortunately, often it is not easy to correctly account for the solvent or the intermolecular interactions: then, one can consider the possibility of obtaining other experimental evidence, *e.g.*, NMR data like NOE's and *J*-couplings. Such additional data allow one to introduce some constraints in the search for minimum energy structures or to provide evidence of the prevalence of one conformer in solution, possibly under the same conditions as those in which the CD data are collected. Often this hybrid method is very successful.

In conclusion, we can answer the questions raised at the beginning of this section: 1) CD provides information on the overall molecular stereochemistry (conformation as well as configuration); 2) before attempting any comparison, one must carefully check the identity of the chromophores, the nature of the perturbing groups, and if the aim is to determine the absolute configuration, also the preferred conformations.

## 4. Examples of applications

### 4.1 Semi-empirical treatments of isolated chromophores

An intrinsically achiral chromophore, characterized by a well-defined electronic transition at $\lambda_{ij}$, has by definition one or more symmetry planes, which may be thought to divide the space into sectors; each sector is associated with a positive or negative sign, so that enantiotopic sectors have opposite signs; any symmetry-breaking atom or group contributes to the CD band at $\lambda_{ij}$ with a term whose sign is determined by the sector, and whose intensity is related to the atom or group polarizability; the resulting CD($\lambda_{ij}$) is the algebraic sum of all contributions. This is the essence of sector rules, the most successful of which is the saturated ketone octant rule,[2] which will be very briefly discussed for its relevant historical role and because it clearly exemplifies symmetry-breaking interactions.

The carbonyl group is characterized by a weak, magnetic dipole allowed/electric dipole forbidden n $\rightarrow$ $\pi^*$ transition around 300 nm. The n and $\pi$-orbitals define three orthogonal nodal planes, as shown in Fig. 5, two of which are also symmetry planes. Overall, they divide the space into eight sectors, called octants, which are associated with the signs of contribution to the CD (300 nm) depicted in the figure.

It is easy to be convinced that the sign alternation within the front and the rear octants fulfils the expected trend for enantiotopic positions with respect to the chromophore; on the contrary, there are no symmetry arguments that can justify the inversion between the front and rear octants. The signs corresponding to the various sectors have been determined by extrapolating a large number of experimental data and are supported by theoretical considerations. That is why the octant rule is classified as semi-empirical: it is not based only on observations.

A classical example of application of the octant rule is (*R*)-3-methylcyclohexanone, shown in Fig. 5. It is apparent that the contribution of all atoms and groups cancel each other with the exception of the substituents at C-3 (a methyl group) and C-5 (a hydrogen). The larger polarizability of $CH_3$ with respect to H justifies the positive sign observed in the CD spectrum.

This example lends itself to a couple of very general considerations, which apply not only to sector rules, but to most stereochemical analyses through CD. 1) One must fully recognize not only the active chromophore but also the specific transition to be considered (in the present case the saturated ketone n–$\pi^*$); 2) atoms and groups can move around the chromophore, owing to conformational freedom, altering the nature (magnitude and sign) of the perturbations they exert on the electronic states and transitions of the chromophore. One

¶ Unfortunately the Cahn–Ingold–Prelog sequence rule brings about a formal inversion, owing to the presence of a longer chain in **4a** than in **4b**. This should not mislead our reader.

reason for the recent decline of sector rules is related to their inherent weakness. They focus on a single possible source of optical activity as arising from chiral elements belonging to a determinate sphere, while in principle any CD signal is due to the combination of several mechanisms. Moreover, as it is apparent from the example of methylcyclohexanone, one has to compare the contributions of two different substituents in enantiotopic positions: it might not be trivial to decide which one is dominant, in terms of making the larger perturbation.

### 4.2 Exciton coupled chromophores

**4.2.1 Theoretical basis.** Semi-empirical rules are often restricted to chiral molecules containing a single, isolated, chromophore; however, most of the "real" molecules are complex entities containing several light-absorbing groups in the UV-vis wavelength range. In such cases the interchromophoric interactions usually provide the most significant contributions to the CD spectra. When two (or more) chromophores are located near in space and have a proper (chiral) mutual orientation, the interactions between their transition dipoles is responsible for large rotational strengths, often surpassing those associated with the perturbations on each chromophore exerted by the chiral non-chromophoric skeleton. Among various possibilities of mixing between electric- and magnetic-dipole allowed transitions, the most significant case arises when two (or more) chromophores with strong electric-dipole allowed transitions couple to each other (exciton coupling).[3,16] As a consequence of the coupling between two equal chromophores, the two otherwise degenerate excited states split into two levels separated by a quantity $2V_{12}$, called Davydov splitting (Fig. 6). The potential $V_{12}$ for the interaction between electric transition dipoles can be approximated by a Coulomb dipole–dipole term

$$V_{12} = \frac{\mu_1 \mu_2}{r_{12}^3}\left[\vec{e_1}\cdot\vec{e_2} - 3\left(\vec{e_1}\cdot\overrightarrow{e_{12}}\right)\left(\vec{e_2}\cdot\overrightarrow{e_{12}}\right)\right] \quad (7)$$

where $\mu_1$, $\mu_2$ and $r_{12}$ are the intensities and mutual distance of the two transition dipoles, while $\vec{e_i}$ are the corresponding unit vectors. The splitting of excited states reflects in a split or broadened absorption band, centred around the wavelength transition $\lambda_0$ of the isolated chromophore. If the two transition moments are not coplanar, the magnetic moment generated by the oscillating dipole 1 at the end of the vector $\overrightarrow{r_{12}}$ will be non-orthogonal to dipole 2, and *vice-versa*.

As a result, a bisignate CD couplet is generated around $\lambda_0$ and allied with two opposite non-vanishing rotational strengths:

$$R_{1,2} \propto \pm \overrightarrow{r_{12}}\cdot\vec{\mu_1}\times\vec{\mu_2} \quad (8)$$

Taking into account the band-shapes and the mutual cancellation between the two oppositely signed bands, the resulting CD couplet is determined by the expression:

$$\Delta\varepsilon(\lambda) \propto \pm\Gamma(\lambda,\lambda_0)\, V_{12}\, \overrightarrow{r_{12}}\cdot\vec{\mu_1}\times\vec{\mu_2} \propto \pm\Gamma(\lambda,\lambda_0)\frac{\mu_1^2\mu_2^2}{r_{12}^2}\Omega(\alpha,\beta,\gamma) \quad (9)$$

Apart from the factor $\Gamma$ that accounts for the dispersive couplet shape, the CD depends only on the quadruple product $V_{12}\,\overrightarrow{r_{12}}\cdot\vec{\mu_1}\times\vec{\mu_2}$, which can be factorized into a module and a geometric term $\Omega$. This reveals that the CD couplet intensity is directly proportional to the fourth power of the dipole strength (and then to the square of the extinction coefficient $\varepsilon_{max}$, since this is $\propto \mu^2$), and inversely proportional to the square of the interchromophoric distance. For the non-degenerate coupling between two different chromophores, $R$ is also inversely proportional to the transition frequency separation. In practice, strongly absorbing chromophores located near in space and close in energy are expected to give rise to very intense CD couplets.[16,17] The intensity, and, more importantly, the sign of the couplet (defined by the sign of its longer wavelength component) are also related to the orientation expressed by $\Omega$, which depends on the molecular configuration and conformation. This term is a function of three

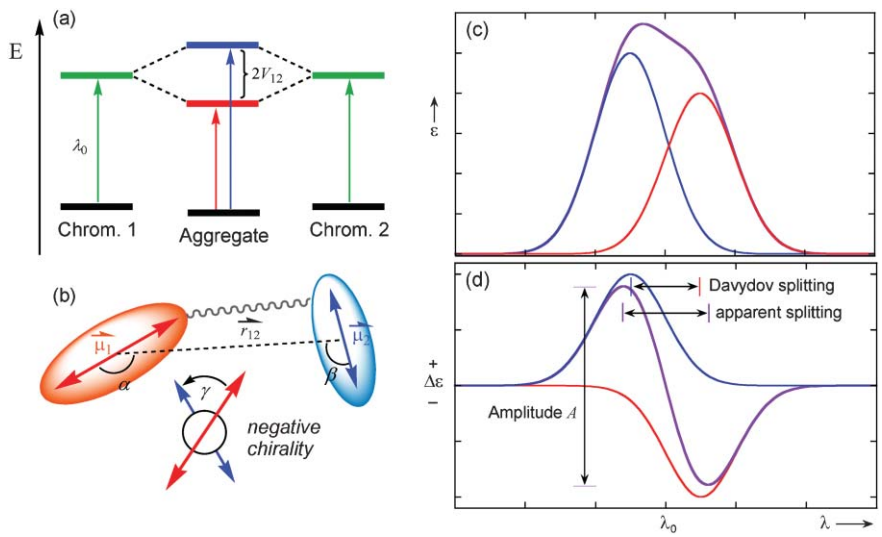



**Fig. 6** (a) Splitting of the excited states of two degenerate exciton-coupled chromophores linked by a chiral spacer. (b) Definition of geometrical parameters necessary for predicting CD sign and intensity through eqn (9). Expected absorption (c) and CD spectra (d) in case of exciton splitting as shown in (a): component spectra thin lines in blue/red, resultant spectra thick lines in violet. The distance between the peak and the trough of the split CD curve is called an amplitude or $A$.

angles $\alpha$, $\beta$, and $\gamma$, depicted in Fig. 6. The sign of exciton chirality, corresponding to that of $\Omega$, can be evaluated in the following way: upon looking through the centres of the two dipoles, a negative sign is defined when an anticlockwise rotation by an acute angle brings the dipole in the front onto that in the back.‖ The exciton chirality rule states that a positive chirality corresponds to a positive CD couplet and *vice-versa*; although such formulation may sound empirical, it relies on eqn (9) which derives from a solid (although approximate) theoretical treatment; therefore, the rule is entirely non-empirical. In addition to the qualitative prediction of couplet's sign and intensity, eqn (9) allows one to calculate a full exciton-coupled CD spectrum for the purpose of comparison with the experimental one (§ 4.3). In fact, the widely recognized potential of the exciton chirality approach stems from the straightforward relationship between geometrical and spectral properties, which allows fully feasible spectral predictions as well as reliable stereochemical assignments, including that of absolute configuration or conformation.[17,18]

**4.2.2 Application of the exciton chirality method. Absolute configurational analysis of cyclic, conformationally-defined systems.** Detailed accounts on the most important features regarding the determination of absolute configuration by means of the exciton chirality method, and practically pertinent aspects, can be found in refs. 17 and 19. Here we will present some examples of rigid and well-defined systems where application of the exciton chirality method for determining the absolute configuration is most straightforward, and uncomplicated by conformational ambiguities. Due to the presence of at least two "pre-existing" chromophores, some natural products have been successfully analyzed on the basis of their directly measured CD. Fig. 7 illustrates several combinations of pre-existing chromophoric pairs that have led to configurational analysis of structurally diverse natural products. As anticipated above, the interacting chromophores do not necessarily have to be identical, but their absorption bands should be close, so that they can easily and efficiently couple through space.[17]

Quite often the chiral sample subjected to configurational analysis lacks chromophores suitable for exciton coupling. In such cases, rather than resorting to more direct but less reliable approaches like sector rules (when applicable), or to accurate but less straightforward CD predictions (§ 4.6), one may still exploit the high sensitivity of the exciton chirality method by introducing extra chromophores with chemical derivatization procedures. The choice of the chromophores suitable for exciton coupling requires a careful selection; they have to be easily introduced at the reactive sites (usually free –OH and $–NH_2$ groups, but also –COOH and –C=C); they must have intense transitions with high $\varepsilon$ and well-known transition polarization. Some typical useful chromophores are represented by *para*-substituted benzoates and cinnamates, 2-naphthoates, 2- and 9-anthroates and so on; others are shown in the following examples; comprehensive lists may be

‖ The importance of "looking through the centres" is well demonstrated by the 1,1′-binaphthyl case: if one looks along the apparently most intuitive C1–C1′ chiral axis, the chirality defined at dihedral angles, say, of 90 and 120° would be the same, thus overlooking the couplet inversion at 110° (Fig. 3).

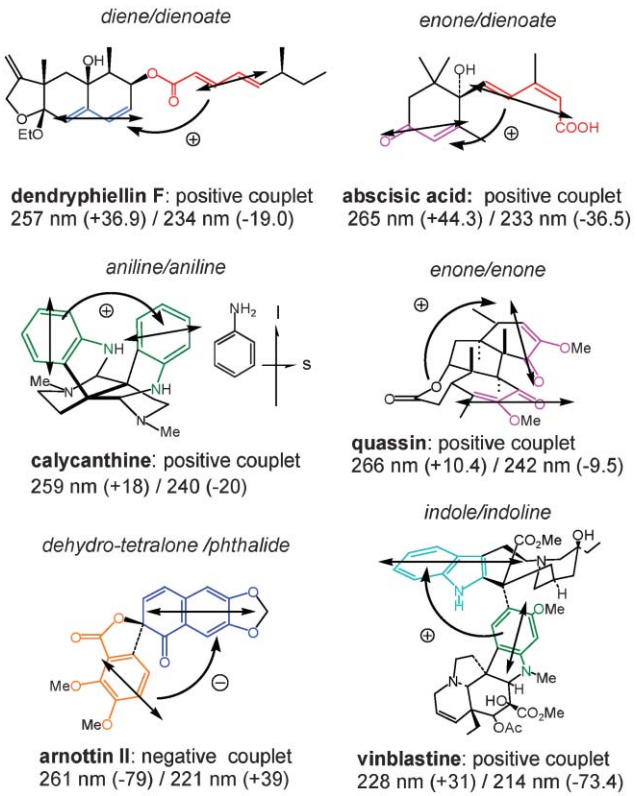



**Fig. 7** Configurational assignment of natural products on the basis of observed exciton coupling between two identical or different pre-existing chromophores (portions in colour).[17] CD extrema are given as $\lambda$ ($\Delta\varepsilon$).

found in refs. 16 and 17. While *monochromophoric* derivatization, or introduction of identical chromophores, is often preferred in order to achieve a stronger coupling in case of rigid substrates, many recent results have revealed that the most favourable choice for acyclic compounds is a *bichromophoric* approach. It consists of introducing two chromophores with very different $\lambda_{max}$, which, upon coupling, give a CD curve with unique, fingerprint shape, depending on the absolute twist between interacting chromophores and the conformational populations in the solvent employed. The comparison of such curves characteristic for each solvent with corresponding reference curves of known standards may lead to the configurational assignment, although in semi-empirical manner, of several stereogenic centres at the same time. The bichromophoric approach proved to be very fruitful in the structural analysis of 1,2- and mixed 1,2-/1,3-polyols and aminoalcohols, which are conformationally far more complex than, for example, 1,3-polyols with a rigid zigzag conformation.[17] Fig. 8 describes an example of submicroscale chemical protocol developed for the analysis of sphingosines and dihydrosphingosines isolated from new cell lines. By selective introduction of a naphthimido group at $NH_2$ group, followed by acylation of OH groups, D-*erythro*-sphingosine is converted into a bichromophoric derivative **5** which is sensitively detectable by HPLC, CD and fluorescence, thus leading to simultaneous identification of relative and absolute configurations. Such chromophores that show intense to moderate

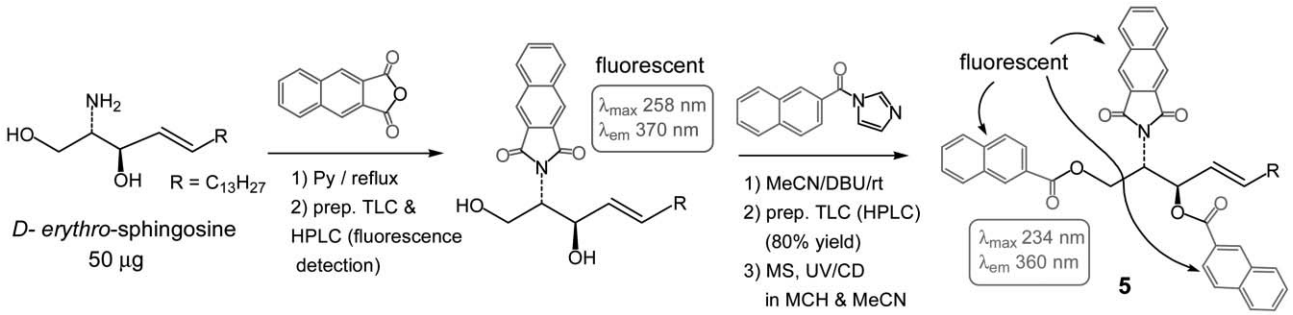



**Fig. 8** Bichromophoric selective chemical derivatization of D-*erythro*-sphingosine.

fluorescence are also attractive alternatives for application of the FDCD method.[13]

The notion that the interpretation of experimental exciton couplets of multichromophoric derivatives can be rationalized based on the pair-wise additivity principle allows significant simplification of the analysis when the sample contains more than just two derivatizable functional groups. When three or more chromophores are present in the same molecule, one should consider all possible coupling interactions, amounting to $n!/[2(n-2)!]$ combinations for $n$ transition dipoles. At first approximation, the couplings may be considered independent of each other, so that the overall spectrum results from the summation of all possible pair-wise terms.[16] For example, if three interacting chromophores are present in the molecule (with a rigid chiral framework), the observed CD of tris-derivative will be very close or even identical to the spectrum obtained by summation of the individual spectra of the corresponding three bis-derivatives. In terms of couplet amplitudes, for a molecule with three identical chromophores it holds that $A = A_{12} + A_{13} + A_{23}$; each term may be evaluated separately by means *e.g.* of eqn (9). However, it has to be emphasized that although the generality of pair-wise additivity rule is well established, the requirements for a preservation of similar mutual orientations of the chromophores and conformational equivalence between bischromophoric pairs and multichromophoric system should be cautiously examined; in general, a comprehensive treatment of multichromophoric systems would require calculations such those described in § 4.3.

Where one chromophore is already present in the substrate molecule, the introduction of second chromophore would be necessary, preferably with an absorption maximum close to that of the pre-existing chromophore. However, it frequently occurs that a natural substrate contains a chromophore of complex or not well understood electronic nature. In such cases a more attractive approach is to introduce two (or more) chromophores with absorbance shifted to the red so that they couple with each other, while an undesired overlap with some pre-existing bands is avoided. A typical example is taxinine, which belongs to the taxane group of diterpenes (Fig. 9). As its highly strained enone moiety shows a strong Cotton effect at 262 nm arising from a $\pi \rightarrow \pi^*$ transition, red-shifted chromophores, like chrom-4 shown on Fig. 9, are better suited for derivatization of 9- and 10-OH. In this way, on the basis of the CD spectrum of compound **6**, an unequivocal conclusion regarding the absolute configuration of taxinine can be made.[20] Red-shifted chromophores suitable for derivatization of OH groups are shown in Fig. 9; similarly, neutral and protonated Schiff bases have been introduced as red-shifted chromophores for the $NH_2$ group.[17]

The chromophores in Fig. 9 despite their useful absorbance, shifted to the red, possess moderate UV-vis absorptions. In

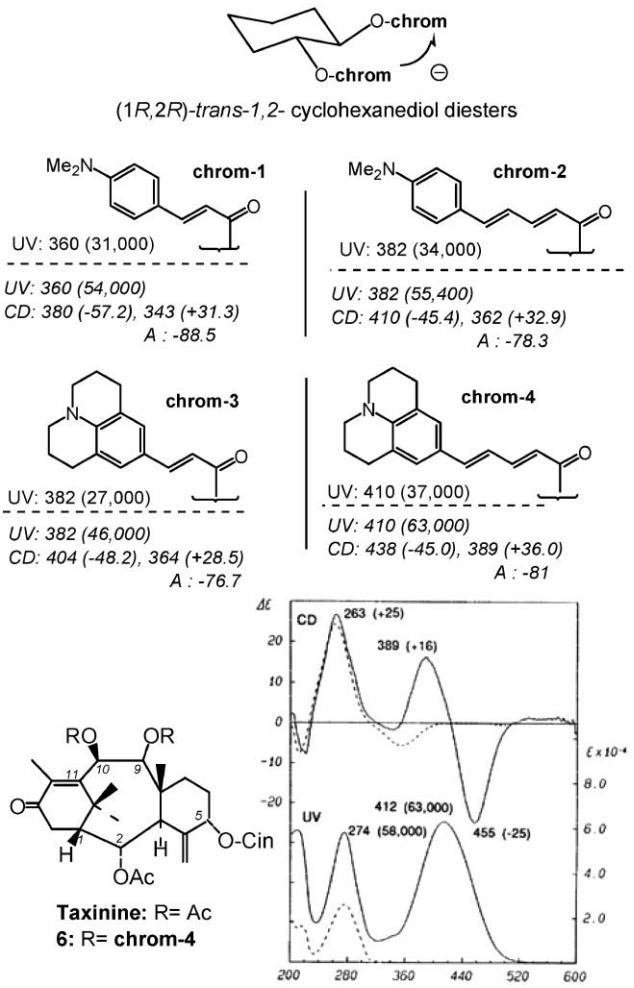



**Fig. 9** Top: UV ($\lambda$/nm, $\varepsilon$) and CD data ($\lambda$/nm, $\Delta\varepsilon$, $A$ value) in MeCN solution of bischromophoric derivative of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol. Also reported are the $\varepsilon$ values for the isolated chromophores. Bottom: CD/UV spectra of (9*R*,10*R*)-taxinine (dotted line) and taxinine bis chromophoric derivative **6** (solid line) in MeCN solution.

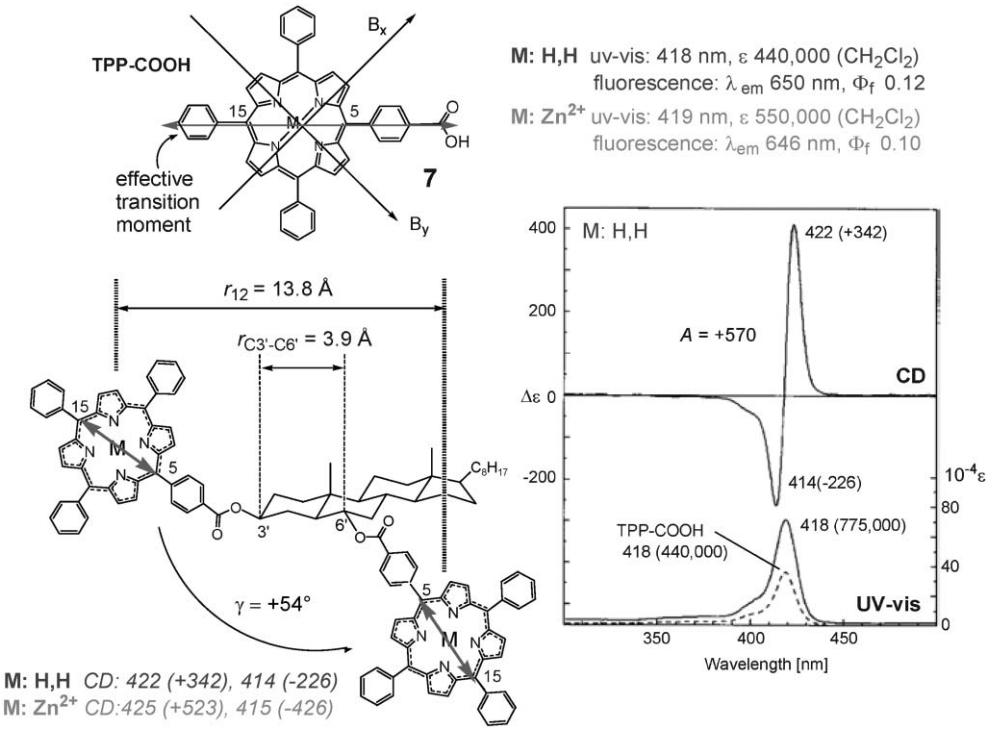



**Fig. 10** Top: Uv-vis and fluorescence data for TPP–COOH (**7**) and Zn–TPP–COOH. Bottom: UV-vis and CD spectra of bistetraarylporphyrin derivative of 5α-cholestane-3β,6α-diol in $CH_2Cl_2$. Angle $\gamma$ is the torsion between the two effective transition moments and $r_{12}$ the interchromophoric distance (as defined in Fig. 6). Justification of the effective transition moment is discussed in ref. 22.

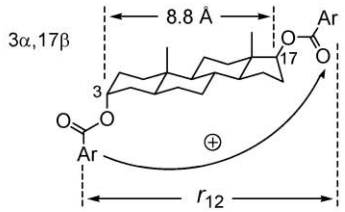
contrast, the tetraarylporphyrin shown on Fig. 10, in addition to red-shifted absorption, is endowed with many other unique structural and electronic properties. Due to the very intense sharp and narrow Soret band (ε 450 000–550 000 at *ca.* 420 nm), fluorescence, facile modification, variable solubility, approximately planar geometry, the porphyrins and their Zn or Mg derivatives belong to the most powerful and versatile chromophores. A detailed discussion on the application of porphyrins as CD reporter groups as well as an account of the theoretical analysis of porphyrin–porphyrin exciton interactions can be found in refs. 21 and 22.

It is worth demonstrating the remarkable ability of porphyrin chromophores to provide extremely intense CD couplets. At a distance of *ca.* 4 Å between the two points of attachment (usually coincident with stereogenic centres), many chromophores can be applied. Yet, even in case of moderate distances and proper interchromophoric twist (angle $\gamma \approx 55°$) as in the rigid 5α-cholestane-3β,6α-diol, the porphyrins provide an unsurpassed exciton couplet amplitude of $A$ = 570 (Fig. 10). Such large $A$ values of porphyrin–porphyrin couplet will be especially useful when the absolute configuration determination of a sample available in very limited amount is sought. The introduction of a porphyrin reporter group on secondary hydroxyl and amino groups, similar to that shown for cholestane-3,6-diol, can be performed in microscale using a sample of only *ca.* 50 μg. The CD can then be measured with a good S/N ratio down to concentrations around $10^{-6}$ M.

Superior properties of porphyrin reporter groups also become crucial when absolute configurational analysis of a very remote stereogenic centre is sought. Since the amplitude $A$ is inversely proportional to the square of the interchromophoric distance $r_{12}$ (see eqn (9)), in cases when the configurational analysis involves remote stereogenic centres with $r_{12}$ larger than 13–14 Å, the observed CD couplet becomes very weak or even undetectable with chromophores endowed with weak or moderate absorption bands. Such cases are presented in Fig. 11. The superb properties of tetraarylporphyrin (TPP) and its Zn derivative (Zn-TPP) are clearly seen at a distance $r_{12}$ of 24.0 Å. In this case the corresponding $A$-values are more than 10-fold larger than that of 4-dimethylaminobenzoate. Other examples for efficient porphyrin–porphyrin CD coupling over the large distance of 40–50 Å can be found in refs. 21 and 23.

### 4.3 Multichromophoric systems: configurational and conformational studies

The previous paragraph has demonstrated that for simple systems endowed with proper structural and spectroscopic properties the exciton chirality method does provide a straightforward and unambiguous approach for the absolute configurational assignment. However, this method allows going beyond a mere qualitative prediction of a couplet sign. A full calculation of CD spectrum within the exciton coupling framework is extremely useful in a number of situations. First, when a molecule contains three or more different excitonically-coupled chromophores with several transitions, the resulting CD spectrum can be rather complicated by the overlap of several exciton couplets. A complete comparison between experimental and computed spectra will be more reliable than




| Ar | | $Me_2N$ | | TPP | Zn-TPP |
|---|---|---|---|---|---|
| ε | 15,000 | 28,000 | 93,000 | 440,000 | 550,000 |
| $r_{12}$ (Å) | 13.6 | 14.5 | 18.9 | 24.0 | 24.0 |
| ***A*** | **nd** | **+21** | **+88** | **+193** | **+270** |

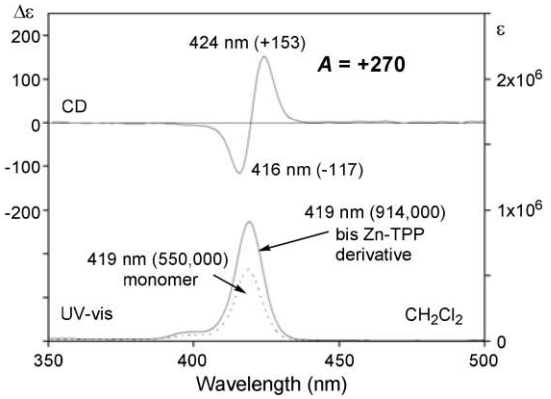



**Fig. 11** Exciton coupling over a large distance between chromophores at C-3,C-17 stereogenic centres of 5α-androstan-3α,17β-diol. The table reports ε values of isolated chromophores, interchromophoric distances and CD amplitudes *A* for the diesters. Right: UV-VIS and CD spectra of bis Zn-TPP (5-(4′-carboxyphenyl)-10,15,20-triphenylporphyrin) derivative.

just considering the CD signs arising from all possible pair-wise terms. Second, especially when the absolute configuration is known, one may want to explore the CD spectrum as a source of more complex information, namely, regarding preferred conformations. Although modern NMR techniques offer the most detailed answers to the majority of conformational problems, there are still cases where a straightforward NMR application is hampered for example by the molecular symmetry, causing unwanted isochrony between nuclei to observe (see 1,1′-binaphthalene derivatives in § 3), or because key structural information cannot be easily obtained (see tetra-1,1′-binaphthyl porphyrins below); in these situations, CD may represent a useful complement. Typically, one would need to generate a set of structures, evaluate the CD spectrum for each single geometry, and then compare experimental and computed CD to assess the most probable structure (or a restricted ensemble).

When some hypotheses on the source of optical activity are met, CD calculations may be affordable at a limited cpu expense. The common approach of dividing a molecule into subsets or spheres (chromophores and perturbers)[10] in practice allows one to describe the electronic transitions of each subunit independently, and then to consider their reciprocal interactions; this represents an independent system approximation (ISA), which is valid when strong interchromophoric communications are not operative (conjugation, resonance, charge-transfer). A general mathematical expression considering all possible sources of optical activity, within the IS approximation, was derived by Tinoco;[24] it is a first-order perturbational approach, in the sense that the overall rotational strength results from the sum of pair-wise couplings. While terms due to inherent chirality (*e.g.*, in twisted π-systems) need a quantum-mechanics description, the couplings between electric and magnetic dipoles allied to achiral chromophores may be profitably described by means of classical terms. In these cases, matrix-based treatments are available like Schellman's one.[25] It is based on an interaction matrix where the diagonal terms are the unperturbed ones (transition dipoles and energies of isolated chromophores) and off-diagonal terms are the perturbations (interaction potentials between the various transitions). The mixing of transition dipoles (either electric or magnetic) is then achieved through matrix algebra and corrected energies, dipole and rotational strengths are obtained.[26] For example, the CD spectra of compounds **4a** and **4b** (Fig. 4) were predicted by considering four aromatic transitions ($^1B_{a,b}$ and $^1L_{a,b}$), the enamide π–π* transition, and the n–π* carbonyl transition (overall, five electric and one magnetic moment, giving a $6\times6$ interaction matrix); the correct absolute configuration of the two compounds was thus assigned.[15] The input for such kind of computations[26] requires: 1) a complete description of each chromophore (transition dipoles energy, strength, direction and position); 2) a known molecular structure, *i.e.*, relative positions and orientations between chromophores. The first set of data can often be found in the literature or evaluated from experimental absorption spectra, and computed by suitable excited-state calculations *on the isolated chromophores*. The geometry can be assessed from spectroscopic (X-ray, NMR) or computational sources (geometry optimizations, conformational searches).

The full potentiality of ISA calculations becomes striking in conformational investigations where CD spectroscopy is employed as a complement to NMR and other techniques. Provided the ISA assumptions are met, matrix-based calculations offer a fast and reliable alternative where other methods are inapplicable. For example, for many complex systems, not only straightforward approaches such as the exciton chirality method become impossible, but also non-approximated calculations of CD spectra (§ 4.6) will be prevented due to large molecular size.

An interesting example is offered by the atropisomeric meso-tetra-1,1′-binaphthyl substituted porphyrins **8** (Fig. 12) whose iron complexes represent two enantioselective catalysts.[27] Given the rigidity of aromatic rings, the dihedral angles $\theta$ and $\psi$ (two for each substituent) represent the main conformational parameters to be investigated. Here, NMR offered no substantial help toward a more precise

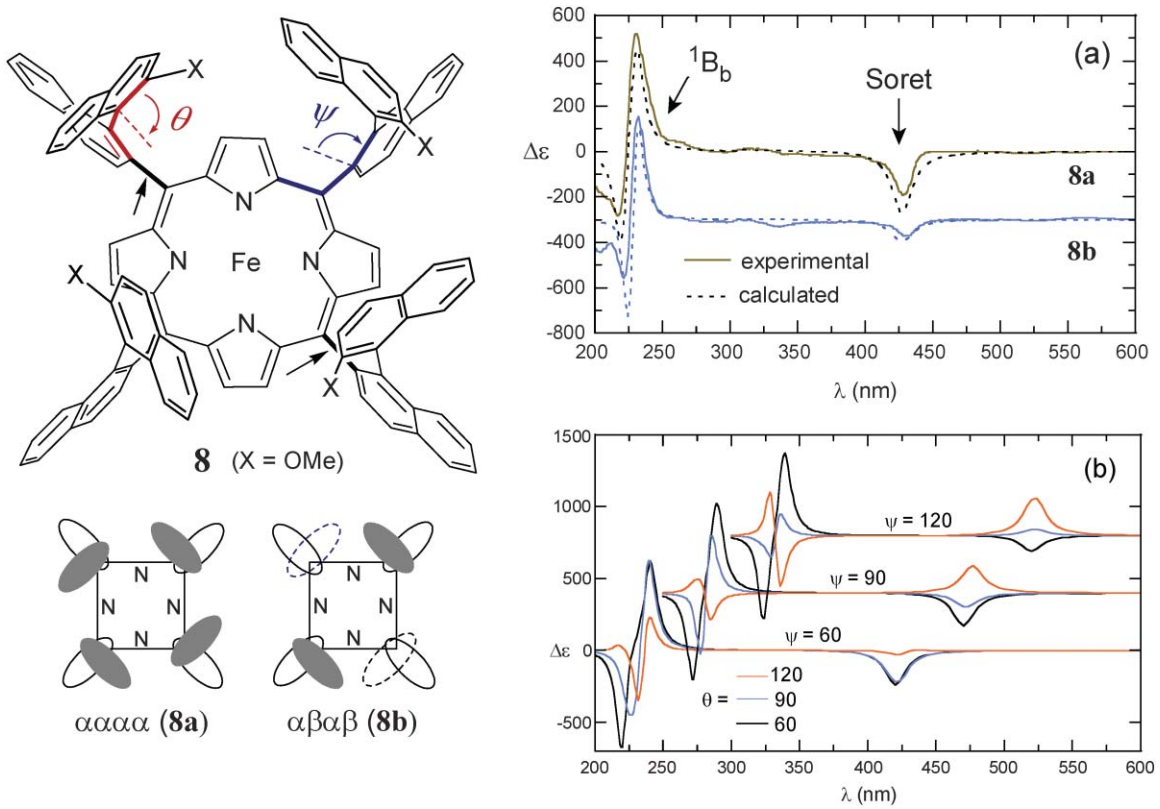



**Fig. 12** Left: Atropisomeric compounds **8a** and **8b** differ by the 180° rotation around the bonds indicated by arrows. Dihedral angles $\theta$ and $\psi$ are the conformational parameters varied in the CD calculations. Right: (a) CD spectra feature naphthalene transitions ($^1B_b$, $^1L_a$ and $^1L_b$) below 350 nm and porphyrin transitions (Soret and Q) above 400 nm. The dotted spectra are the best-fitting calculated ones with DeVoe method. (b) Calculated CD spectra for the set of geometries generated for compound **8a** upon varying $\theta$ and $\psi$. Only naphthalene $^1B_b$ and porphyrin Soret transitions were considered in DeVoe calculation. From bottom to top the sets of spectra are shifted by 50 nm.

quantification of the two angles, and crystals for X-ray analysis could not be obtained. A set of geometries was generated by a MM method upon systematic variation of $\theta$ and $\psi$ between 60–120° with 15° steps (25 overall structures), and absorption and CD spectra were calculated for each geometry. Focusing on the strongest transitions of the naphthalene and porphyrin chromophores ($^1B_b$ and Soret), 10 different electric dipoles giving rise to 44 distinct couplings had to be considered. In this case, a classical computational scheme known as coupled oscillator or DeVoe's approach was employed.[28] It is a matrix-based method useful for treating multi-chromophoric systems with several electric-dipole allowed transitions, broadly employed for a rapid quantitative calculation of exciton chirality.[29] The calculated absorption and CD spectra for the geometrical set of **8a** and **8b** (some are shown in Fig. 12b) feature a great variation as a function of $\theta$ and $\psi$, so that the spectra (dotted in Fig. 12a) best-fitting the experimental ones could be easily recognized with $\theta \approx 75$ and $\psi \approx 75°$ for **8a**, and with $\theta \approx 90$ and $\psi \approx 90°$ for **8b**.[27]

The above example demonstrates that matrix-type calculations are very useful and practical means for structural analysis of moderately complex systems, especially because they are not computationally demanding (the 25 different spectra for **8a** were generated in a few seconds). However, they require as a prerequisite an independent knowledge of spectroscopic properties of chromophores involved.

### 4.4 Supramolecular systems

The enormous attention and advance in supramolecular chemistry in the past few decades has not surprisingly stimulated the interest toward the observation of circular dichroism arising from different types of *intermolecular* interactions. Four typical situations are encountered. 1) A chiral (non-racemic) "guest" and an achiral chromophoric compound as "host", for example, crown ethers, calixarenes, atropisomeric biaryls and bis-porphyrin systems, can form a chiral host–guest complex which exhibits an induced CD (ICD) within the absorption bands of the host.[30] 2) Inversely, a small guest molecule which is achiral and hence its chromophores are optically inactive, upon binding to a biopolymer host, such as proteins,[31] polypeptides, oligonucleotides,[32] oligosaccharides (notably including cyclodextrins)[30] may produce an induced CD due the chiral perturbation by the biopolymer host. 3) The coupling between several guest-molecules bound to different sites of a macromolecular host may result in a significant and diagnostic CD spectrum.[33] 4) A chiral, non-chromophoric ligand binds to a metal ion with observable *d* or *f*-type transitions in the UV-vis spectrum, making them CD-active. In several cases, CD lends itself not only to a detection of host–guest interactions, but also to the analysis of binding modes, association–dissociation kinetics and thermodynamics (see § 4.5). A significant example from each type 1)–4) above is presented in the following.

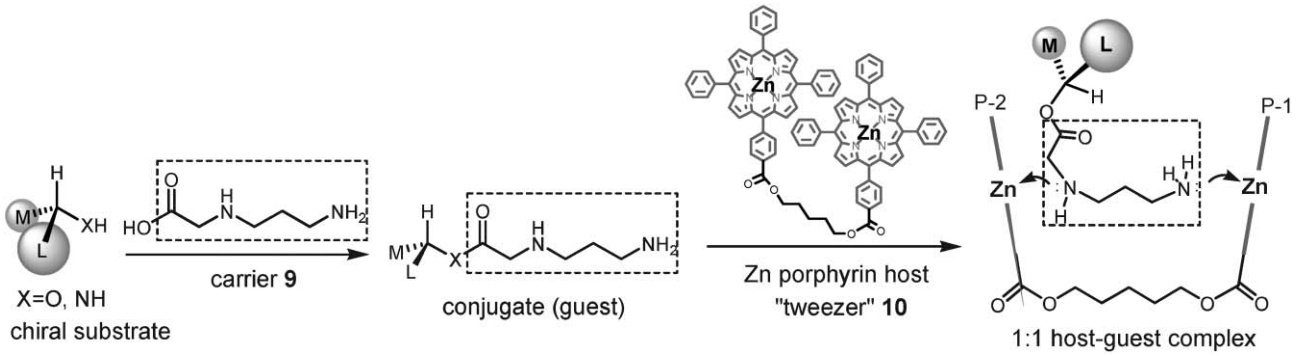



**Fig. 13** Formation of 1 : 1 host guest complex between Zn-porphyrin tweezer **10** as host and a conjugate as guest, prepared from starting chiral substrate (primary monoamine or secondary alcohol).

**4.4.1 Bis-porphyrin tweezers in the determination of absolute configurations.** As a first relevant and recent CD application in the field of supramolecular chemistry, we return to the tetraarylporphyrins, this time regarding their use as reporter groups in absolute configurational studies of chiral compounds that contain a single stereogenic centre and only one site for chromophoric derivatization, a common situation where the conventional bischromophoric exciton chirality approach is inapplicable. To this type belong chiral monoamines, secondary monoalcohols, α-substituted carboxylic acids, and various natural products carrying only a single functionality: they cannot fulfil the requirement of having at least two intramolecularly interacting chromophores. To overcome this difficulty, a totally new supramolecular concept was recently developed. A new dimeric zinc porphyrin reagent, now available under the name "Zn-tweezer" (**10**, Fig. 13) was first prepared by linking the zinc derivative of TPP–COOH (**7** in Fig. 10) to 1,5-pentanediol. It was found that the tweezer is capable of forming 1 : 1 host–guest complexes upon adding to a solution of a *N*,*N*-bidentate ligand, such as a 1,3-diamine. Based on this observation a supramolecular approach was developed where the tweezer is used as a sensitive chiroptical probe for absolute configurational analysis of single stereogenic centres of primary and secondary amines, secondary alcohols, as well as α-substituted carboxylic acids.[34] According to this method the chiral substrate, for example a primary monoamine, reacts with an achiral trifunctional bidentate carrier, such as **9**, to form a bidentate conjugate capable of undergoing facile N/Zn coordination to Zn-porphyrin tweezer (achiral host **10**) to form a 1 : 1 sandwiched chiral host–guest complex (Fig. 13).

Such complex exhibits a very intense bisignate CD spectrum in the Soret region due to porphyrin–porphyrin exciton coupling, which follows from the stereodifferentiation in the complexation resulting in a preferred porphyrin–porphyrin chiral orientation. Since the absolute sense of twist between the two porphyrins in the complex is dictated by the stereogenic centre of the substrate, the sign of the couplet determines the absolute configuration at this centre (Fig. 14).

Over the past few years the method has undergone several important developments. While in the beginning the preferred interporphyrin helicity of the host–guest complex was rationalized on the basis of relative steric sizes of the groups flanking the stereogenic centre, such that the bulkier group (L) protrudes from the complex sandwich, other approaches have been developed in subsequent years. One of them uses the porphyrin ring-current induced $^1$H chemical shifts of the complex for assignment of relative steric size of the L (large)/M (medium) substituents attached to the stereogenic centre.[35] More recently it was found that a prediction of preferred

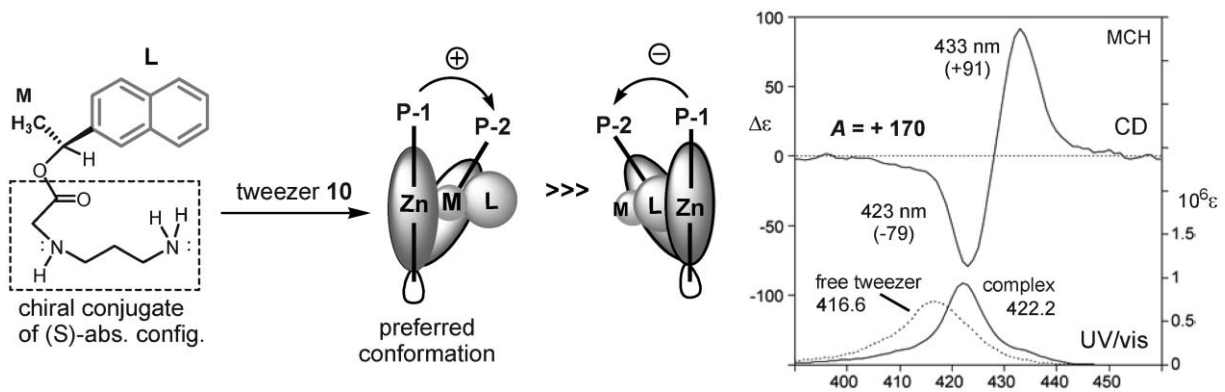



**Fig. 14** Complex formation between conjugate of (*S*)-α-(2-naphthyl)ethanol and Zn-porphyrin tweezer leads to two conceivable conformations with opposite sense of twist. The predominant conformation is of positive twist, with the L (large) group protruding away from the P-1/P-2 sandwich. It gives rise to a typical CD couplet with amplitude $A = +170$, which is related to the sense of twist between the two porphyrins and hence the absolute configuration at the stereogenic centre. Spectra measured in methylcyclohexane.

porphyrin helicity of the complex could be made by molecular mechanics calculations using the Merck Molecular Force Field (MMFFs) within a Monte Carlo-based conformational search.[36] The porphyrin tweezers method is now well established and its successful application to new types of chiral compounds, where the conventional exciton chirality approach does not work, has been reported in several recent publications.[37,38] At the same time, many other applications following a similar host–guest approach have appeared in the literature. Yang *et al.* have applied the same porphyrin tweezers host (**10**) for analysis of chiral carboxylic acids (guests) by introducing aromatic diamines as carrier molecules for preparation of the necessary bidentate ligand. In this case also, an exciton split CD band serves for determining the guest absolute configuration.[39] Borovkov *et al.* introduced a different achiral metalloporphyrin host, namely, a *syn*-folded ethane-bridged bis(zinc) porphyrin, which upon binding of chiral amines and alcohols (monodentate guests) also forms a host–guest complex. The latter adopts in solution an extended *anti*-conformation with a preferred porphyrins twist which leads to exciton split CD controlled by the absolute configuration of the guest amine.[40] It has been also recently shown that the absolute configuration of chiral primary amines can be determined as well upon derivatization with 2-bromomethyl quinolines and subsequent formation of tripodal ligand–metal complexes. The binding fixes the spatial arrangements of aromatic groups in a stable propeller-like *anti* conformation which exhibits an intense and diagnostic bisignate CD curve (220–260 nm) with sign governed by chirality of primary amine. The reliability of this model, applicable to structurally diverse primary amines, was proved by independent methods.[41]

**4.4.2 Cyclodextrin inclusion compounds.** Cyclodextrins are well known for their capability of forming inclusion compounds: their cavity provides a chiral nest for a variety of apolar guests. Since natural cyclodextrins (or their non-chromophoric derivatives) are CD-transparent above 200 nm, when an achiral but chromophoric guest is bound, an ICD may be easily detected arising from the chromophore embedded into a chiral environment. One can distinguish two main situations (Fig. 15a): the chromophore on the guest is surrounded by the macrocycle or it lies above or below the host rim (while possibly a different portion of the guest is actually included). It has been demonstrated that the ICD of transitions, whose electric transition dipole is aligned along the cyclodextrin symmetry axis, is positive if the chromophore is inside, negative if it is outside the host. The opposite applies for electric transition dipoles lying perpendicular to the axis.[30]

**4.4.3 Serum albumin as a carotenoid carrier.** Serum albumin is a carrier protein, responsible for the transport of apolar, poorly water-soluble molecules in blood. There are several hydrophobic pockets in the protein structure, where guest molecules can be accommodated. Interestingly, occupancy of one site may change the binding parameters of another one, in a typical scheme of allosteric interactions. Albumin has strong absorptions, associated to CD only below 300 nm, therefore complexation of achiral guests endowed with red-shifted

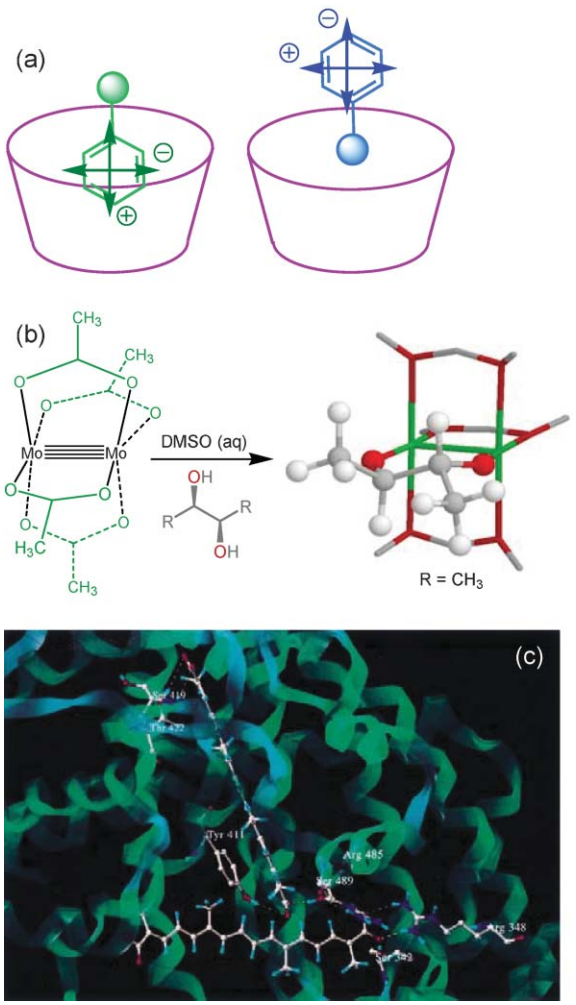



**Fig. 15** Examples of supramolecular species exhibiting ICD. (a) An achiral chromophore bound to cyclodextrins shows a CD with sign (shown) dependent on binding mode and transition polarization. (b) A chiral diol–dimolybdenum complex with observable CD above 300 nm. (c) Two crocetin molecules bound to HSA give exciton-coupled CD (from ref. 33, with permission; copyright 2003 John Wiley & Sons).

chromophores can be very efficiently analysed by studying their ICD.[31,42] Carotenoids can be hosted in the manifold of binding sites usually occupied by fatty acids. The observed ICD for crocetin–human serum albumin complex shows a typical negative couplet with crossover around 420 nm, *i.e.* in correspondence to the $\lambda_{max}$ of crocetin; this demonstrates the simultaneous presence of two guest molecules, arranged in well defined chiral orientation, which gives rise to the exciton interaction (Fig. 15c). Interestingly, by adding palmitic acid one crocetin molecule is displaced from its site, leading to the ternary complex albumin–crocetin–palmitic acid. Since in this suprastructure there is only one chromophore with a transition above 400 nm, a monosignated CD is observed, centered on the absorption maximum of crocetin.[33]

**4.4.4 Metal complexes as reporter of chirality of bidentate ligands.** In this review, we have not said much on the CD allied to metal-centred transitions, which are usually characterized by weak absorption spectra in the visible range, but may be

associated with moderately strong CD, on account of their essentially magnetic-dipole character.[43] A complex between a metal core and a chiral ligand may provide a Cottonogenic species, *i.e.*, a species endowed with observable CD bands which can be used, for example, for the determination of absolute configuration of the ligand. Since reducing the conformational freedom of the ligand is often beneficial, many empirical methods of this kind have been developed for bidentate ligands such as diols, diamines, amino alcohols and carboxylic acids; metals employed include transition metals such as Cu, Os, Ni, Rh and Mo, and lanthanides such as Pr, Eu, and Yb. Such methods, which have been quite popular in the past, are well exemplified by one of the most versatile ways for assigning the stereochemistry of 1,2-diols and similar compounds, also known as Snatzke's method.[44] Upon simple mixing of dimolybdenum tetracetate $Mo_2(AcO)_4$ with a chiral, non-racemic, but CD-transparent substrate (Fig. 15b), one observes a CD around 300–400 nm allied to the transitions of the $Mo_2$ core; the sign of the most intense CD band correlates with the absolute configuration of the substrate. In spite of the widely acknowledged success of this and similar methods, most of them remained essentially empirical, mainly because of the complete lack of information on the Cottonogenic species (with a few exceptions, see § 4.5).

### 4.5 Quantitative analysis of intermolecular interactions by ICD

CD spectroscopy has been very successfully used for investigating the thermodynamic parameters of binding interactions in many host–guest systems. An ideal situation for this kind of investigation is when one partner is chiral and non-chromophoric (or with blue-shifted transitions), while the other is achiral and endowed with strong electric dipole-allowed transitions, so that the only observable CD is that allied to the complex. This induced CD signal then represents a very convenient quantity for detecting and following complex formation. More or less conventional analyses of the magnitude of Cotton effects during the course of a titration, *e.g.* by means of Scatchard's, Job's or Hildebrand–Benesi's methods, may allow one to determine binding parameters, such as stoichiometry and affinity constants. When the above mentioned (or equivalent) conditions are met, CD may be superior to other spectrophotometric techniques because it is sensitive only to the adduct and does not require care in subtracting the contributions from the free species.

Data analyses of this kind have been reported for very different cases including those discussed above (§ 4.4): from complexes between bis-porphyrin tweezers and chiral hosts[35,36] to cyclodextrin inclusion compounds,[30] from serum proteins acting as carriers for drugs, toxins and nutrients,[31,33,42] to interactions involving nucleic acids.[45] In the context of metal complexes used for the determination of absolute configurations (§ 4.4.4), quantitative studies carried out by CD have helped elucidating the nature of the dimolybdenum–diol Cottonogenic species.[46]

### 4.6 Full CD calculations

Most applications of CD spectroscopy considered so far consist in the prediction of CD signs or spectra for a given compound, after a certain structure is assumed (*e.g.*, conformation), and then in the comparison with the experimental spectrum to obtain a second piece of structural information (*e.g.*, the absolute configuration). Whatever approach is used, one needs a basic knowledge of chromophore transitions and some hypotheses about the way they interact with each other and/or with the skeleton. Would it not be possible to use a computational package where, using a certain molecular structure at input, a predicted full CD spectrum is obtained at the output, without making troublesome underlying assumptions or approximations? This idea for direct CD or ORD calculation has fascinated chemists for a long time, but it is only in recent years that technological progress has made it workable with a sufficient reliability, and for moderately complex chemical compounds. In principle, any quantum mechanical method for excited state calculations[47] can provide rotational strengths; however, high accuracy is required, since the sign inversion in $R$ may be a matter of only a few degrees in the angle between electric and magnetic dipoles. We will discuss the currently most popular methods for calculating electronic CD, namely ZINDO-S/CI and TDDFT, and refer the interested reader to more specialized literature for exhaustive discussion.[48,49]

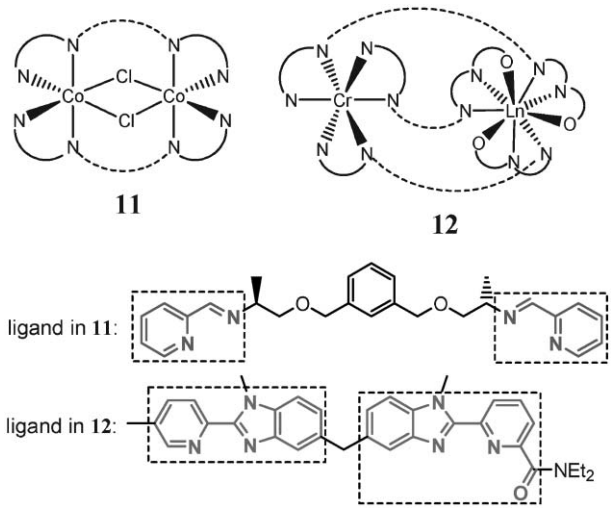
ZINDO-S/CI is a version of the semi-empirical method known as "intermediate neglect of differential overlap", modified by Zerner for spectroscopic purposes, and includes configuration interaction (CI) correction of singly excited states. In general, semi-empirical quantum mechanical methods (not to be confused with the semi-empirical CD approaches like the octant rule!) depend on the robustness and quality of parameters, and may not be universally applicable; for example, the old but still used Pariser–Parr–Pople (PPP) model is restricted to π conjugated systems. On the other hand, these methods are quite fast (cpu time of minutes for a medium molecule) and applicable to large molecular systems. The Time Dependent Density Functional Theory (TDDFT) is an example of *ab initio* methods: they are in principle applicable to any molecule, but tend to be very computationally demanding, which in practice limits their application to small to medium-sized systems; DFT approaches, however, can lead to predictions of high accuracy at a reasonable cost. For such calculations, one needs to choose a functional and a basis set. The best performing functionals are the so-called hybrid ones, such as B3LYP, BH&HLYP, MPW1PW91, PBE0. Commonly employed basis sets are of the split-valence type and include polarization and possibly diffuse functions, for example, in order of size, 6–31G+(d,p), TZVP, 6–311G++(2df,2pd), aug-cc-pVDZ and aug-cc-pVTZ (the larger the basis set, the more accurate are the results, but longer the calculation time).

Any quantum mechanical calculation requires an input geometry, which needs to be determined independently. TDDFT is very time consuming (several hours or days for a medium size molecule), thus extensive conformational analyses (like those one may perform with matrix-type calculations, see § 4.3) are almost impossible, and even configurational assignments of very flexible molecules may represent a formidable task. For molecules with a limited number of minimum-energy conformers, one would usually go through several steps: 1)

estimate geometries *and* relative energies computationally (various levels are available, from molecular mechanics to DFT); 2) compare the calculated geometries with experimental structural data (NMR or others), if available; 3) calculate the CD spectrum for each conformer (this will appear as a series of rotational strengths at discrete wavelengths); 4) apply a band-shape form to translate rotational strengths into a simulated spectrum; 5) evaluate conformer populations with Boltzmann distribution (here the approximation of free energies with computed energies – enthalpies – is implicit); 6) calculate the average spectrum to be compared with the experimental one. Although lengthy and computationally demanding, this procedure is *exact* and *direct*, in the sense that no approximations are used. Unfortunately, this does not imply that it is also accurate. Imperfect estimation of intensities and, above all, transition energies is quite common, especially for transitions involving high-lying states.[50] A careful and critical comparison between the *entire* computed and experimental spectra is therefore essential. In addition to structural studies, the quantum mechanical CD calculations are fundamental for theoretical investigations of the origin of observed chiroptical properties. We will illustrate the application of ZINDO and TDDFT methods with two examples.

A series of binuclear metal complexes with dimeric ligands (Scheme 3) based on 2-pyridinealdimine (**11**) and 2-(2-pyridinyl)benzoimidazole (**12**) have been recently investigated.[51] It was found that the CD spectra in the UV region, although clearly dominated by the exciton couplings between the aromatic chromophores, were difficult to analyze due to several expected different coupling types, both of intra-nuclear (between ligand portions on the same metal centre) and inter-nuclear nature (between portions on different metal centres). ZINDO-S/CI calculations on the X-ray structures revealed that observed CD spectra do result from a complicated combination of all couplings, and that internuclear long-range couplings play an unexpectedly decisive role. It is interesting to discuss the choice of the calculation technique in this case. In fact, only the planar conjugated chromophores were included in the calculations, with the skeletons and metal ions removed (see framed portions in Scheme 3); therefore, actually, only exciton-like terms were evaluated by ZINDO, which could also have been treated by a coupled oscillator method. Nonetheless, since a quantum mechanical method such as ZINDO would be required to assess transition dipoles positions and polarizations, the overall computational cost of a full CD calculation will be just slightly larger.




**Scheme 3**

The absolute configuration of some enantioselective aza-Diels Alder products **13** (Fig. 16) has been recently assigned by means of TDDFT CD computations.[52] Any other simplified approach for interpreting CD spectra was impractical due to the presence of many overlapping transitions of aromatic and β-aminoenone chromophores. In particular, an earlier established semi-empirical rule for cyclic enones provided the wrong answer, because it overlooks enone distortion from planarity. An exciton-coupling based theoretical analysis was unsuitable

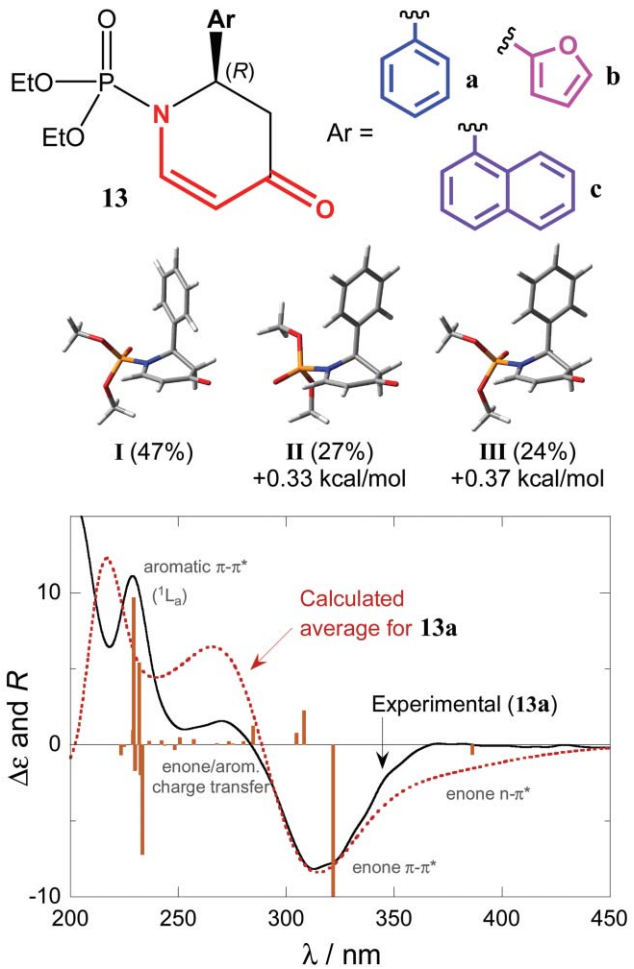



**Fig. 16** (a) Structure of compounds **13a–c**, with chromophoric portions in bold and colour. (b) Computed structures for the three miminum energy conformers **I–III** of **13a** (with population at 298 K and relative energies). (c) Experimental and Boltzmann-average calculated CD spectrum of **13a** with band assignment based on computed MOs. Vertical bars represent rotational strengths *R* computed for **I** (in $10^{-39}$ cgs units). Geometry optimizations run with DFT B3LYP/6–31G(d) method, CD calculated with TDDFT B3LYP/TZVP; calculated spectrum is shifted by 50 nm to the right for better comparison with the experiment.

for the lack of a clear-cut CD couplet. Moreover, the calculations have shown substantial orbital overlap between the two chromophores, and revealed the occurrence of charge-transfer transitions.

Conformational molecular-mechanics search, refined by DFT optimizations and checked against NMR data, led to identify three minimum energy conformers **I–III** (Fig. 16). TDDFT calculations on the three single geometries followed by Boltzmann weighting resulted in an average CD spectrum in good agreement with the experimental; a safe configurational assignment of **13a** was then possible.[52] It is important to notice that the TDDFT calculations were performed on the full molecular structures, while for using *e.g.* ZINDO it would be necessary to remove the phosphoric group because parameters for phosphorous were not available. Also noteworthy is that large differences were observed in the computed spectra of the three single conformers,[52] despite their apparent structural similarity. This finding demonstrates how sensitive may CD be to subtle differences in the molecular structure, a point that perhaps would be difficult to prove without employing computational tools. In fact, any experimental CD spectrum is a weighted average of all contributions over an entire molecular ensemble, and it is usually impossible to extract a single component spectrum, for example that corresponding to a certain conformer. In contrast, calculations are performed *only* on single conformers of defined geometry, and eventually they are averaged.

In summary, quantum mechanical methods, implemented in commercially available software, have become in the past decade powerful theoretical tools affordable to many who are interested to perform direct CD calculations; these methods are applicable to any system, independent of its electronic properties; they do not require any *a priori* hypotheses or approximation. However, at present their usage still remains significantly limited (especially TDDFT) by the molecular size and/or conformational flexibility.

## Conclusions and perspectives

Electronic circular dichroism, one of the best established chiroptical methods, has undergone in the past few decades a tremendous technological development towards improved data acquisition and analysis. Modern ECD instrumentation allows broad (from 163 nm to 1100 nm) range measurements of differential UV-vis to near IR absorption of left and right circularly polarized light by conventional detection in transmission, or more sensitively in emission. Efforts are underway to extend the far UV region into the vacuum UV. The strength and advantages of electronic CD over other chiroptical methods include its high sensitivity ($\Delta A/A \sim 10^{-2}$, while for vibrational CD $\Delta A/A \sim 10^{-4}$ to $10^{-6}$), and requirement for much smaller sample amounts (less than 1 mg, or even few micrograms). These attractive characteristics of ECD have recently been further improved by development of new CD reporter groups, among them porphyrins and metallo-porphyrins with very favourable attributes, such as intense UV-vis absorption, fluorescence and capability to form host–guest complexes. Nevertheless, electronic CD has some inherent weaknesses and limitations stemming from the low signal resolution and difficulties in assigning the signals to specific chromophoric sites without resorting to excited-state quantum-mechanical calculations.

ECD undoubtedly remains one of the most broadly used chiroptical techniques for solving various stereochemical and analytical problems. Its unsurpassed power is best seen in the determination of absolute configurations, as the first alternative (along with its vibrational counterpart, VCD) to X-ray techniques when these are not applicable; and in the elucidation of conformational aspects, especially in the field of biopolymers. The past decade has seen a dramatic progress in theoretical methodologies for prediction of CD properties. In particular, *ab initio* methods have significantly improved the interpretation and application of experimental CD spectra. While the role of empirical, semi-empirical sector and helicity rules shows some decline, methodologies based on coupled-oscillator formalism and on *ab initio* treatments will continue to gain increasing significance in structural analyses through CD.

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## References

1 *Circular Dichroism: Principles and Applications*, ed. K. Nakanishi, N. Berova and R. W. Woody, Wiley-VCH, New York, 2nd ed., 2000.
2 D. A. Lightner and J. E. Gurst, *Organic Conformational Analysis and Stereochemistry from Circular Dichroism Spectroscopy*, Wiley-VCH, New York, 2000.
3 S. F. Mason, *Molecular Optical Activity and the Chiral Discrimination*, Cambridge University Press, Cambridge, 1982.
4 A. Rodger and B. Nordén, *Circular Dichroism & Linear Dichroism*, Oxford University Press, Oxford, 1997.
5 *Circular Dichroism and the Conformational Analysis of Biomolecules*, ed. G. D. Fasman, Plenum Press, New York, 1996.
6 S. M. Kelly and N. C. Price, *Curr. Protein Pept. Sci.*, 2000, **1**, 349–384.
7 N. J. Greenfield, *Methods Enzymol.*, 2004, **383**, 282–317.
8 D. M. Gray, R. L. Ratliff and M. R. Vaughan, *Methods Enzymol.*, 1992, **211**, 389–406.
9 K. D. McReynolds and J. Gervay-Hague, *Tetrahedron: Asymmetry*, 2000, **11**, 337–362.
10 G. Snatzke, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 363–377.
11 P. Salvadori, C. Rosini and L. Di Bari, Conformation and chiroptical properties of dienes and polyenes, in *The Chemistry of Dienes and Polyenes*, ed. Z. Rappoport, Wiley, Chichester, 1997, vol. 1, ch. 4, pp. 111–147.
12 P. Salvadori, L. Di Bari and G. Pescitelli, HPLC-CD: Stereochemical Analysis at Work, in *Circular Dichroism: Principles and Applications*, ed. K. Nakanishi, N. Berova and R. W. Woody, Wiley-VCH, New York, 2nd ed., 2000, ch. 28, pp. 797–817.
13 K. Tanaka, G. Pescitelli, K. Nakanishi and N. Berova, *Monatsh. Chem.*, 2005, **136**, 367–395.
14 L. Di Bari, G. Pescitelli and P. Salvadori, *J. Am. Chem. Soc.*, 1999, **121**, 7998–8004.
15 L. Ripa, A. Hallberg and J. Sandström, *J. Am. Chem. Soc.*, 1997, **119**, 5701–5705.
16 N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy - Exciton Coupling in Organic Stereochemistry*, Oxford University Press, Oxford, 1983.

 

17 N. Berova and K. Nakanishi, Exciton Chirality Method: Principles and Application, in *Circular Dichroism: Principles and Applications*, ed. K. Nakanishi, N. Berova and R. W. Woody, Wiley-VCH, New York, 2nd ed., 2000, ch. 12, pp. 337–382.
18 See the monograph issue: Exciton Chirality: Fundamentals and Frontiers, *Monatsh. Chem.*, 2005, **136**(3).
19 N. Berova, N. Harada and K. Nakanishi, Electronic Spectroscopy: Exciton Coupling, Theory and Applications, in *Encyclopedia of Spectroscopy and Spectrometry*, ed. J. Lindon, G. Tranter and J. Holmes, Academic Press, London, 2000, pp. 470–488.
20 G. Cai, N. Bozhkova, J. Odingo, N. Berova and K. Nakanishi, *J. Am. Chem. Soc.*, 1993, **115**, 7192–7198.
21 X. Huang, K. Nakanishi and N. Berova, *Chirality*, 2000, **12**, 237–255.
22 G. Pescitelli, S. Gabriel, Y. Wang, J. Fleischhauer, R. W. Woody and N. Berova, *J. Am. Chem. Soc.*, 2003, **125**, 7613–7628.
23 K. Tanaka, Y. Itagaki, M. Satake, H. Naoki, T. Yasumoto, K. Nakanishi and N. Berova, *J. Am. Chem. Soc.*, 2005, **127**, 9561–9570.
24 I. Tinoco, Jr., *Adv. Chem. Phys.*, 1962, **4**, 113–160.
25 P. M. Bayley, E. B. Nielsen and J. A. Schellman, *J. Phys. Chem.*, 1969, **73**, 228–243.
26 J. Sandström, Determination of Conformations and Absolute Configurations by Semiempirical Calculations of CD Spectra, in *Circular Dichroism: Principles and Applications*, ed. K. Nakanishi, N. Berova and R. W. Woody, Wiley-VCH, New York, 2nd ed., 2000, ch. 16, pp. 459–490.
27 L. Di Bari, G. Pescitelli, G. Reginato and P. Salvadori, *Chirality*, 2001, **13**, 548–555.
28 H. DeVoe, *J. Chem. Phys.*, 1964, **41**, 393–400; H. DeVoe, *J. Chem. Phys.*, 1965, **43**, 3199–3208.
29 S. Superchi, E. Giorgio and C. Rosini, *Chirality*, 2005, **16**, 422–451.
30 S. Allenmark, *Chirality*, 2003, **15**, 409–422.
31 G. A. Ascoli, E. Domenici and C. Bertucci, *Chirality*, 2006, **18**, 667–690.
32 B. Nordén and T. Kurucsev, *J. Mol. Recognit.*, 1994, **7**, 141–156.
33 M. Simonyi, Z. Bikadi, F. Zsila and J. Deli, *Chirality*, 2003, **15**, 680–698.
34 T. Kurtán, N. Nesnas, Y.-Q. Li, X. Huang, K. Nakanishi and N. Berova, *J. Am. Chem. Soc.*, 2001, **123**, 5962–5973.
35 T. Kurtán, M. Nesnas, F. E. Koehn, Y.-Q. Li, K. Nakanishi and N. Berova, *J. Am. Chem.*, 2001, **123**, 5974–5982.
36 X. Huang, N. Fujioka, G. Pescitelli, F. E. Koehn, T. R. Williamson, K. Nakanishi and N. Berova, *J. Am. Chem. Soc.*, 2002, **124**, 10320–10335.
37 A. Solladie-Cavallo, M. Roje, M. Giraud-Roux, Y. Chen, N. Berova and V. Sunjic, *Chirality*, 2004, **16**, 196–203.
38 J. W. Van Klink, S.-H. Baek, A. J. Barlow, H. Ishii, K. Nakanishi, N. Berova, N. B. Perry and R. T. Weavers, *Chirality*, 2004, **16**, 549–558.
39 Q. Yang, C. Olmsted and B. Borhan, *Org. Lett.*, 2002, **4**, 3423–4326.
40 V. V. Borovkov, J. M. Lintuluoto and Y. Inoue, *J. Am. Chem. Soc.*, 2001, **123**, 2979–2989.
41 J. M. Castanetto, X. Xu, N. D. Berova and J. W. Canary, *Chirality*, 1997, **9**, 616–622; J. Zhang, A. E. Holmes, A. Sharma, N. R. Brooks, R. S. Rarig, J. Zubieta and J. W. Canary, *Chirality*, 2003, **15**, 180–189.
42 L. Di Bari, S. Ripoli and P. Salvadori, Serum albumin and natural products, in *Progress in Biological Chirality*, ed. G. Palyi, C. Zucchi and L. Caglioti, Elsevier, Oxford, 2004, pp. 271–295.
43 R. Kuroda and Y. Saito, Circular Dichroism of Inorganic Complexes: Interpretation and Applications, in *Circular Dichroism: Principles and Applications*, ed. K. Nakanishi, N. Berova and R. W. Woody, Wiley-VCH, New York, 2nd ed., 2000, ch. 20, pp. 563–599.
44 J. Frelek, M. Geiger and W. Voelter, *Curr. Org. Chem.*, 1999, **3**, 117–146.
45 F. Zsila, Z. Bikadi and M. Simonyi, *Org. Biomol. Chem.*, 2004, **2**, 2902–2910.
46 L. Di Bari, G. Pescitelli and P. Salvadori, *Chem.–Eur. J.*, 2004, **10**, 1205–1214.
47 J. C. Cramer, *Essentials of Computational Chemistry*, 2nd ed., Wiley, New York, 2004; A. Dreuw and M. Head-Gordon, *Chem. Rev.*, 2005, **105**, 4009–4037.
48 A. Koslowski, N. Sreerama and R. W. Woody, Theoretical Approaches to Electronic Optical Activity, in *Circular Dichroism: Principles and Applications*, ed. K. Nakanishi, N. Berova and R. W. Woody, Wiley-VCH, New York, 2nd ed., 2000, ch. 3, pp. 55–95; T. D. Crawford, *Theor. Chem. Acc.*, 2006, **115**, 227–245.
49 C. Diedrich and S. Grimme, *J. Phys. Chem.*, 2003, **107**, 2524–2539.
50 D. M. McCann and P. J. Stephens, *J. Org. Chem.*, 2006, **71**, 6074–6098.
51 S. G. Telfer, N. Tajima, R. Kuroda, M. Cantuel and C. Piguet, *Inorg. Chem.*, 2004, **43**, 5302–5310; S. G. Telfer, N. Tajima and R. Kuroda, *J. Am. Chem. Soc.*, 2004, **126**, 1408–1418.
52 L. Di Bari, S. Guillarme, S. Hermitage, D. A. Jay, G. Pescitelli and A. Whiting, *Chirality*, 2005, **17**, 323–331.