Chiral Bis-chlorin: Enantiomer Resolution and Absolute Configuration Determination

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ABSTRACT
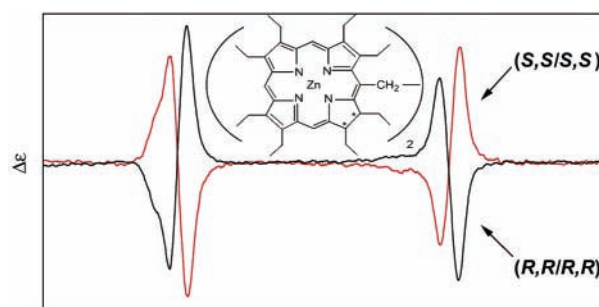
A racemic mixture of the ethane-bridged bis(zinc octaethylchlorin) was successfully resolved for the first time to yield two enantiomers that exhibit substantial CD signals in the regions of chlorin B and Q transitions. The absolute configuration of the corresponding enantiomers was assigned and the origin of its high optical activity was rationalized through a combined spectral, crystallographic, and theoretical analysis.

Bis- and multichlorin structures play an inevitable role in natural photosynthetic systems[1] and are widely employed as model compounds for mimicking a variety of biological processes.[2] Furthermore, recent advances in photodynamic therapy (PDT) of cancer revealed that they may also serve as potential candidates for second-generation sensitizers due to their unique spectral properties.[3] Thus, chlorins possess important structural features and spectroscopic advantages

over conventional porphyrins from the PDT point of view. This includes the reduced pyrrole ring, which causes a significant bathochromic shift and tremendous intensity enhancement of the lowest singlet-energy transition (Q) band. This allows more efficient use of the longer wavelength region of the chlorin absorption not only for PDT but also for various application purposes. For example, bis-chlorins are also good candidates for investigations of supramolecular chirogenesis phenomena and chirality sensors. Major prerequisites for this type of study are nonoverlapping host and guest absorptions and sufficient intensity of the monitoring transitions. Therefore, chlorins potentially offer greater advantages over porphyrin chromophores, which have recently been developed as chirality sensors.[4] In the course of


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(1) (a) Jordan, P.; Fromme, P.; Witt, H. T.; Klukas, O.; Saenger, W.; Krauss, N. *Nature* **2001**, *411*, 909. (b) McDermott, G.; Prince, S. M.; Freer, A. A.; Hawthornthwaite-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Isaacs, N. W. *Nature* **1995**, *374*, 517. (c) Kühlbrandt, W.; Wang, D. N.; Fujiyoshi, Y. *Nature* **1994**, *367*, 614.
(2) (a) Zheng, G.; Shibata, M.; Dougherty, T. J.; Pandey, R. K. *J. Org. Chem.* **2000**, *65*, 543. (b) Jaquinod, L.; Senge, M. O.; Pandey, R. K.; Forsyth, T. P.; Smith, K. M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1840.

(3) Pandey, R. K.; Zeng, G. In *The Porphyrin Handbook*; Kadish, K. M.; Smith, K. M., Guilard, R., Eds.; Academic Press: San Diego, 2000; pp 157−230 and references therein.

our studies on supramolecular chirogenesis, it was discovered earlier that ethane-bridged bis-porphyrins are achiral hosts suitable for the comprehensive investigation of various aspects of chirality induction processes upon noncovalent interactions with a variety of chiral guest compounds such as amines, alcohols, and others.[4a,5] Therefore, taking into account the above-mentioned spectral advantages of chlorin chromophores, it would be of particular interest to study chirogenic properties of chlorin analogue (**1**) (see Figure 1).[6]
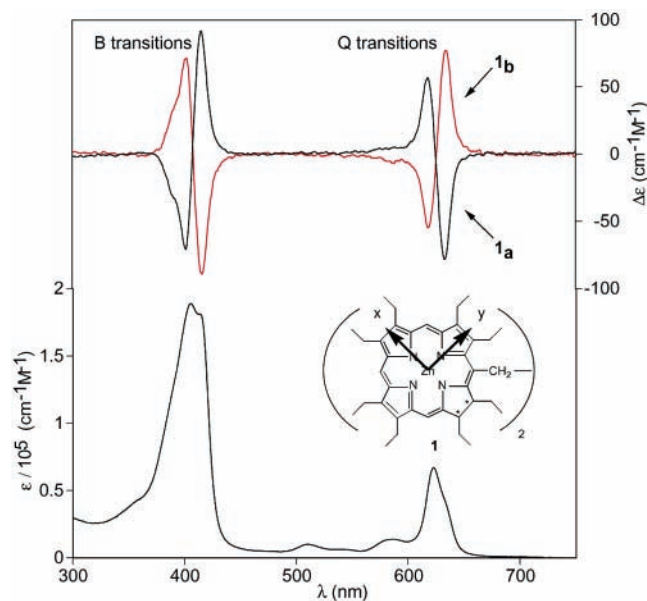
Figure 1. Experimental absorption (bottom) and CD (top) spectra of **1$_a$** and **1$_b$** in CH$_2$Cl$_2$ at room temperature.

However, contrary to the bis-porphyrin hosts, **1** is an intrinsically chiral compound with four stereogenic centers. Therefore, for detailed studies of the host−guest association and chirality induction processes, it is essential to obtain individual enantiomers, investigate their chiroptical proper-

ties, and assign the absolute configuration. Furthermore, these new optically active compounds themselves may open further attractive perspectives in the fields of chiral recognition, asymmetric synthesis and catalysis. Here, we report for the first time successful enantiomeric resolution of **1**, which consequently leads to the comprehensive understanding of the origin of optical activity at the molecular and electronic levels and to the assignment of their absolute configuration.

The enantiomeric separation of **1** was successfully achieved by preparative HPLC with a chiral column.[7] The enantiomeric excesses of both enantiomers were confirmed to be >88% by analytical HPLC,[7] and the CD spectra of the isolated two enantiomers (first eluting **1$_a$** and second eluting **1$_b$**) gave a pair of perfect CD mirror images that exhibit substantial optical activity in the regions of chlorin B and Q transitions (Figure 1).

As expected, UV−vis absorption profiles of **1$_a$** and **1$_b$** (Figure 1) are not distinguishable from each other and are the same as the spectrum of its racemic mixture,[6] showing considerable broadening and splitting of the corresponding B and Q bands due to strong excitonic interactions between the two chlorin moieties. Similar spectral features, particularly in the B transition region, have been observed also for anti conformations of the bis-porphyrin analogues, thus indicating some resemblance in their special arrangements.[4a,5] However, in the case of CD spectra, besides some similarities found in the B band region, there are drastic differences in the Q transition region between the bis-chlorin and bis-porphyrin systems due to their distinctive electronic structures. Thus, the B transition region of **1$_a$** and **1$_b$** exhibits a strong CD couplet (with an amplitude (A) of ca. ±163 cm^{-1} M^{-1}) consisting of two bisignate Cotton effects, the positions of which coincide closely with the maxima of the split B band in the UV−vis spectrum. However, in contrast to the bis-porphyrin systems, the Q band region also shows an intensive couplet ($A = \pm135$ cm^{-1} M^{-1}) with a remarkably high degree of the optical activity (the anisotropy factor of the first Cotton effect in the Q band region is 3.2 times larger than that in the B band region). Furthermore, importantly, the B and Q signals have opposite CD signs, which is a clear indication of distinct spatial orientation of the relevant coupling transitions, apparently due to its different polarization along the chlorin x- and y-axes (see Figure 1).

For assignment of the absolute configuration of **1$_a$** and **1$_b$**, the coupling direction of the chlorin's lowest-energy Q$_y$ electronic transitions is of the utmost importance because of their well-distinguished absorption characteristics.[8] In par-
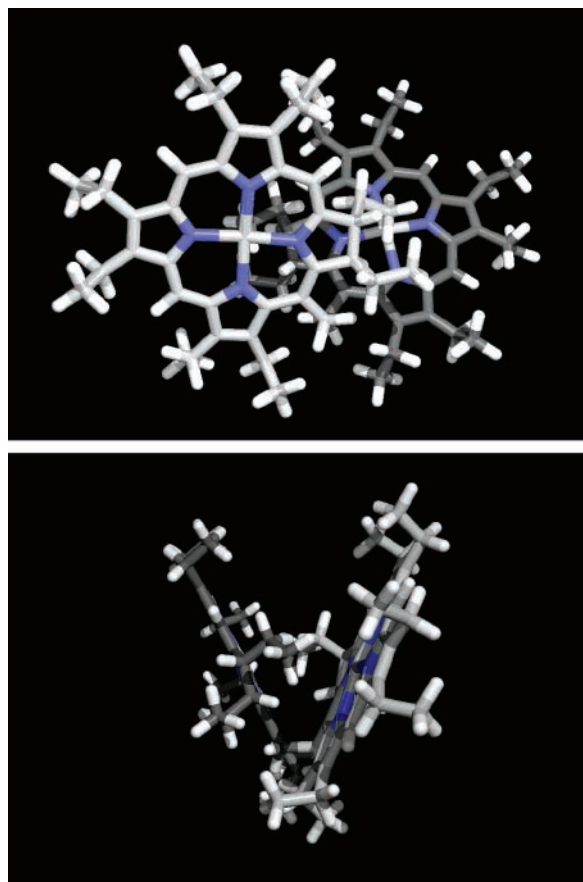
(4) (a) Borovkov, V. V.; Hembury, G. A.; Inoue, Y. *Acc. Chem. Res.* **2004**, *37*, 449. (b) Guo, Y.-M.; Oike, H.; Aida, T. *J. Am. Chem. Soc.* **2004**, *126*, 716. (c) Pescitelli, G.; Gabriel, S.; Wang, Y.; Fleischhauer, J.; Woody, R. W.; Berova, N. *J. Am. Chem. Soc.* **2003**, *125*, 7613. (d) Tsukube, H.; Shinoda, S. *Chem. Rev.* **2002**, *102*, 2389. (e) Ribó, M.; Crusats, J.; Sagués, F.; Claret, J.; Rubires, R. *Science* **2001**, *292*, 2063. (f) Jung, J. H.; Kobayashi, H.; Masuda, M.; Shimizu, T.; Shinkai, S. *J. Am. Chem. Soc.* **2001**, *123*, 8785. (g) Ogoshi, H.; Mizutani, T. *Acc. Chem. Res.* **1998**, *31*, 81.

(5) (a) Borovkov, V. V.; Fujii, I.; Muranaka, A.; Hembury, G. A.; Tanaka, T.; Ceulemans, A.; Kobayashi, N.; Inoue, Y. *Angew. Chem., Int. Ed.* **2004**, *43*, 5481. (b) Borovkov, V. V.; Hembury, G. A.; Inoue, Y. *Angew. Chem., Int. Ed.* **2003**, *42*, 5310. (c) Borovkov, V. V.; Lintuluoto, J. M.; Hembury, G. A.; Sugiura, M.; Arakawa, R.; Inoue, Y. *J. Org. Chem.* **2003**, *68*, 7176. (d) Borovkov, V. V.; Harada, T.; Inoue, Y.; Kuroda, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1378. (e) Borovkov, V. V.; Lintuluoto, J. M.; Sugeta, H.; Fujiki, M.; Arakawa, R.; Inoue, Y. *J. Am. Chem. Soc.* **2002**, *124*, 2993. (f) Borovkov, V. V.; Lintuluoto, J. M.; Inoue, Y. *J. Am. Chem. Soc.* **2001**, *123*, 2979.

(6) A racemic mixture of **1** has been synthesized according to the standard procedures (see: Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *Bioorg. Chem.* **1980**, *9*, 1 and Borovkov, V. V.; Lintuluoto, J. M.; Inoue, Y. *Helv. Chim. Acta* **1999**, *82*, 919) and compared with the reference spectral data (see: Chernook, A. V.; Shulga, A. M.; Zenkevich, E. I.; Rempel, U.; von Borczyskowski, C. *J. Phys. Chem.* **1996**, *100*, 1918).

(7) Enantiomeric separation was performed by preparative HPLC with a JAI LC-908 apparatus on a Chiralcel OJ-RH preparative column (Daicel Chemical Industries, Ltd., 20 × 150 mm, eluent MeOH, at 35 °C, flow rate 5.5 mL/min, UV detection at 380 nm) and a GL Sciences CO 705 column oven; the obtained fractions were analyzed by analytical HPLC on a JASCO GULLIVER apparatus with a Chiralcel OJ-RH analytical column (Daicel Chemical Industries, Ltd., 4.6 × 150 mm, eluent MeOH, at 35 °C, flow rate 0.3 mL/min, UV detection at 370 nm) and a GL Sciences CO 705 column oven.

(8) (a) Houssier, C.; Sauer, K. *J. Am. Chem. Soc.* **1970**, *92*, 779. (b) Gouterman, M. *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 3, Part A. (c) Keegan, J. D.; Stolzenberg, A. M.; Lu, Y.-C.; Linder, R. E.; Barth, G.; Moscowitz, A.; Bunnenberg, E.; Djerassi, C. *J. Am. Chem. Soc.* **1982**, *104*, 4305.
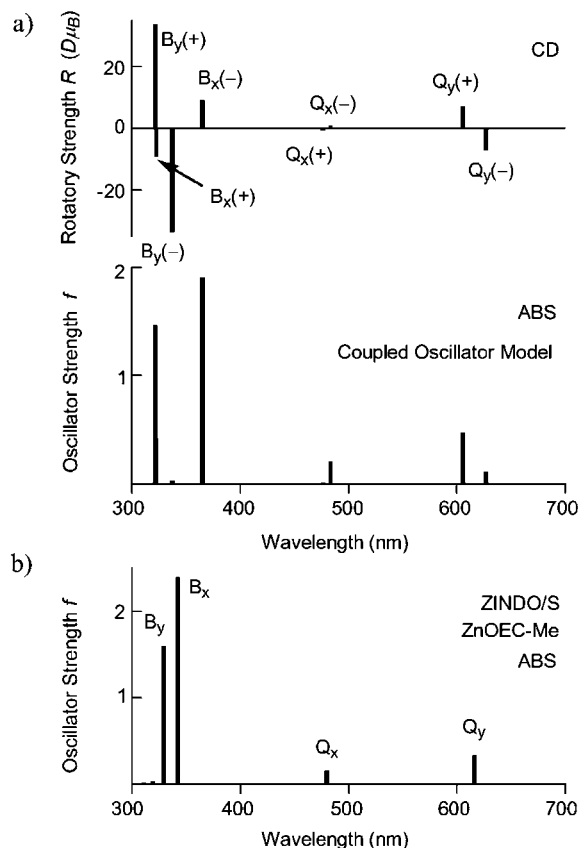
obtained at the semiempirical PM3 level using the corresponding crystallographic data of free base analogue[9] as a starting point for the optimization. In general, the resulting converged structure was found to be very similar to that of free base bis-chlorin with the interchromophoric distance being 8.16 Å (Figure 2). Under such conditions, a Coulombic, rather than electron exchange, interaction model is more appropriate to apply for the two chlorin chromophores. In the second step, spectral parameters (excitation energies and transition moments) of the chlorin monomer were obtained from CI calculations at the ZINDO/S level of zinc 5-methyloctaethylchlorin. The derived absorption spectrum of the monomer consists mainly of four well-distinguished (Q_y, Q_x, B_x, and B_y) transitions (Figure 3b) and is typical for

Figure 2. Top and side views of the optimized geometry of (R,R/R,R)-**1** at the semiempirical PM3 level (stick model).

ticular, the negative sign of the Q_y couplet of **1a** indicates anticlockwise orientation of the corresponding transitions, while in the case of **1b** the situation is exactly the opposite. Taking this into account, two crystallographic structures of the metal-free analogues of **1** obtained previously from the corresponding racemic mixture were analyzed.[9] One can clearly see that both forms are nearly mirror images despite the different solvent compositions used for crystallization, which is presumably a result of spontaneous antipodal resolution. Possessing C_2 symmetry, these bis-chlorins are V-shaped with the reduced pyrrole rings of two chlorin moieties located at the closest spatial position to each other. In this geometry, two Q_y transitions of the (S,S/S,S)-enantiomer form a clockwise turn that, in accordance with the exciton chirality method,[10] corresponds to positive chirality as determined for **1b**, while for the (R,R/R,R)-enantiomer the handedness of coupling and thus the corresponding chirality sign are opposite, as seen in the case of **1a**.

To theoretically confirm this assignment, the CD spectrum of (R,R/R,R)-**1** was calculated through the following approach. At first, the spatial geometry of this enantiomer was

Figure 3. (a) Calculated absorption and CD stick spectra of (R,R/R,R)-**1** based on the optimized structure. The notations (+) and (−) represent the in-phase and out-of-phase transitions, respectively. (b) Computed linear absorption spectrum of zinc 5-methyloctaethylchlorin.

chlorin-type chromophores.[8] Finally, on the basis of the geometrical and spectral parameters obtained by the point−dipole approximation and the Kuhn−Kirkwood coupled oscillator analysis,[11] the theoretical electronic absorption and CD spectra of (R,R/R,R)-**1** were calculated (see Figure 3a).

(9) Senge, M. O.; Hope, H.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 2* **1993**, 11.

(10) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy. Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

The calculated spectral features are in good agreement with the experimental spectra of 1_a.[12] It is clearly seen that the intensity of the out-of-phase $Q_y(-)$ transition is smaller than that of the in-phase $Q_y(+)$ transition due to the partially forbidden nature as a result of the V-shaped orientation of these dipoles (see Supporting Information). A negligibly small CD signal arising from the Q_x-Q_x coupling is also predicted properly, because its intensity is proportional to the square of the magnitude of the Q_x transition moment. Furthermore, the lower energies of the Q_y and B_x transitions, in comparison to the corresponding Q_x and B_y transitions, allow us to attribute the origin of their opposite chirality signs to the different handedness of the coupled transitions: i.e., clockwise and anticlockwise for the x and y polarizations, respectively (see Supporting Information). Thus, this theoretical model nicely reproduces the relative energy levels and intensities of the coupling transitions, particularly the three degenerate Q_y-Q_y, Q_x-Q_x, and B_x-B_x couplings. Although there is some overestimation of the CD intensities arising from the B_y-B_y coupling, the actual CD signals for the B_y bands are expected to be less pronounced due to the smaller excitonic splitting than those for the B_x bands. More importantly, this analysis predicts the negative chirality of the most red-shifted and energetically isolated Q_y transitions of 1_a, thus supporting unambiguously the assigned absolute configuration.

Additional support in favor of the optimized structure of $(R,R/R,R)$-**1** in nonpolar solvents comes from a simple state dipole model analysis.[13] Since chlorin chromophores have a relatively large dipole moment because of their less-symmetrical skeleton due to the reduced pyrrole ring, the most stable conformation of this bis-chlorin in nonpolar media in the absence of any external or internal interactions (such as hydrogen binding, coordination, or solvent−solute interactions) is achieved by minimizing the total dipole moment as a result of the dipole−dipole interaction between two chlorin moieties. Hence, the calculated magnitude of the permanent moment of this conformer is found to be the smallest (1.51 D) among the other low-energy conformers. Furthermore, the calculated absorption spectra of other conformers are significantly different from the observed one, particularly in the Q_y band region (see Supporting Information).

In summary, we succeeded in the enantiomeric resolution of bis-chlorin **1** for the first time, unambiguously assigned its absolute configuration, and further rationalized the origin of its high optical activity through a combined CD spectral, crystallographic, and theoretical analysis. These results should have important implications for the complete understanding of the mechanisms and driving forces operating in a variety of artificial and natural chiral bis- and multichromophoric systems and contribute to the sophisticated design and synthesis of advanced supramolecular systems and chiroptical devices, particularly utilizing porphyrin and chlorin chromophores.

Supporting Information Available: Experimental and calculated spectral data and orientation of electronic transitions. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) (a) Kirkwood, J. G. *J. Chem. Phys.* **1937**, *5*, 479. (b) Tinoco, I. *Adv. Chem. Phys.* **1962**, *4*, 113. (c) Kuhn, W. *Trans. Faraday Soc.* **1930**, *26*, 293. The validity of this approach was further confirmed by comparison of the theoretical absorption spectra obtained from the coupled oscillator analysis and ZINDO/S level calculations (see Supporting Information): both spectra are almost identical, thus suggesting purely electrostatic interactions between the chlorin moieties in **1**.

(12) Assignment of the corresponding transitions was further confirmed by MCD measurements (see Supporting Information). The MCD signal patterns corresponding to the intense CD couplets were a positive absorption shape for the Q region (622 nm) and negative absorption shape for the B region (400 and 414 nm); therefore, these MCD signals were assigned to the Faraday *B* terms. Since the exciton contribution to the Faraday *B* term MCD of chlorophyll-like dimers is extremely small (see: Hughs, J. L.; Pace, R. J.; Krausz, E. *Chem. Phys. Lett.* **2004**, *385*, 116), the MCD signals should simply reflect the electronic structure of the chlorin chromophore, i.e., the polarization and the molecular orbital. The correspondence of the same MCD signs to the two oppositely signed CD signals observed in the B and Q regions implies that the CD signals arise from the degenerate coupling mechanism. As a result, it is confirmed that the intense CD signals seen at 618 and 633 nm arise from the Q_y-Q_y coupling, while those at 402 and 415 nm arise from the B_x-B_x coupling. The CD signal seen at 395 nm may be due to the B_y band, judging from the positive MCD signal.

(13) Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry*; John Wiley & Sons: Chichester, 2000.