Porphyrins: Powerful Chromophores for Structural Studies by Exciton-Coupled Circular Dichroism

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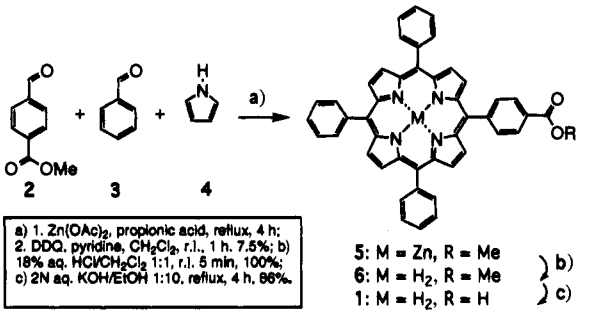
We introduce porphyrins as long-range interacting chromophores that extend the applicability of the exciton-coupled circular dichroic method to configurational studies of molecules with remote stereogenic centers and possibly to conformational studies of biopolymers. The interaction between excited states of chromophores in chiral environments give circular dichroism (CD) curves with split Cotton effects, i.e., exciton-coupled CD.[1] In this method for determining the absolute stereochemistry of organic molecules in solution, the signs and shapes of the characteristic CD curves are defined by the absolute skewness of interacting chromophores.[1] The extent of chromophoric coupling, i.e., the amplitudes of split Cotton effects, is inversely proportional to the square of interchromophoric distance[2] and proportional to the square of extinction coefficients[3] of the coupled chromophores. Therefore, the intensity of chromophoric absorptions is of prime importance in increasing the sensitivity over the large distance between interacting transition moments. Furthermore, when the original substrate contains a chromophore, new chromophores with bathochromic intense absorbances, ϵ = 31 000−58 000, may be used to deliberately avoid coupling with preexisting chromophores.[4] For the purpose of developing intense red-shifted chromophores, we had investigated cyanine dye chromophores; although unique from a spectroscopic viewpoint,[4b] they were unsuited for practical applications due to their instability, nontrivial microscale preparation, etc. This led to the introduction of other red-shifted chromophores which have wide and diverse applicabilities.[4c,d] In the following, we show that porphyrins further enhance the sensitivity of the exciton-coupled CD method by almost 10-fold and extend the applicability of the method to molecules that could not be studied so far. Thus, 5-substituted 10,15,20-triphenylporphyrins, e.g., **1**, with their intense red-shifted Soret band at 414 nm, ϵ = 350 000,[5] provide powerful exciton-coupled CD chromophores for absolute configurational studies of natural products with remote stereogenic centers 35−50 Å apart[6] as well as conformational studies of biopolymers, e.g., ligand−receptor interactions.

Scheme 1



CD of isolated porphyrin chromophores induced by protein complexes,[7] covalently bound oligopeptides,[8] or nucleic acid complexes[9] are well documented. Although porphyrin exciton coupling has been investigated in UV−vis[10] and CD,[11,12] it has not been studied as a tool for elucidating stereochemical problems.

We have selected 5-(*p*-carboxyphenyl)-10,15,20-triphenylporphin (**1**) as the chromophore to explore the usefulness of porphyrins in exciton-coupled CD structural analysis of large molecules (Scheme 1). The synthesis is based on published procedures.[15] One equivalent of *p*-methoxycarbonylbenzaldehyde (**2**), 3 equiv of benzaldehyde (**3**), and 4 equiv of pyrrole (**4**) were refluxed in propionic acid in the presence of zinc acetate to give zinc porphyrin **5**. Removal of zinc gave **6**, which was hydrolyzed to porphyrin **1** in 6.5% overall yield. Reactions of authentic glycols **7a**, **8a**, **9a**, and **10a** with porphyrin **1** using EDC/DMAP[16] afforded bisesters **7b**, **8b**, **9b**, **10b** in 60−80% yield[17] (Table 1); similarly, authentic 3-amino-17-hydroxy steroids **11a** and **12a**[18] were converted into the amide esters **11b** and **12b**. For comparison, glycols **7a**,[19] **8a**, and **10a** were also derivatized to the bis[*p*-(dimethylamino)benzoates] **7c**,[19] **8c**, and **10c** with triazole/DBU,[4d] while **10a** was converted to bis(benzoate) **10d** with EDC/DMAP. CD data are listed in Table 1.

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(14) Osuka and Maruyama[10a] assumed a 5−15 direction of the Soret transition of a C(5)-substituted porphyrin and estimated its magnitude (ref 10).

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(17) All new compounds exhibited satisfactory spectral data. A representative procedure is exemplified by the preparation of **7b**: To a solution of **1** (24.4 mg, 37.1 μmol), EDC (7.1 mg, 37.1 μmol), and DMAP (4.0 mg, 37.1 μmol) in absolute CH_2Cl_2 (1 mL) was added a solution of **7a** (5 mg, 12.4 μmol) in CH_2Cl_2 (1 mL) at room temperature. The mixture was stirred at room temperature for 12 h, diluted with CH_2Cl_2 (20 mL), extracted with a solnution of saturated aqueous NH_4Cl (3 × 20 mL), washed with brine (2 × 20 mL), dried (Na_2SO_4), and evaporated (15 Torr) to give 29 mg (139%) of product, which was purified (CH_2Cl_2/hexane 3:1, R_f = 0.36) to yield pure **7b** (16.3 mg, 78%), deep purple powder. HRMS (FAB): *m/z* 1685.8280 ($C_{117}H_{105}N_8O_4$ requires 1685.8260).

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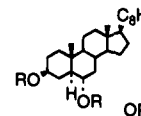
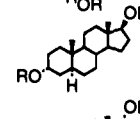
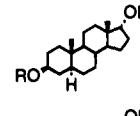
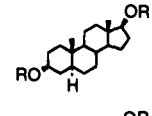
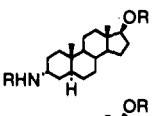
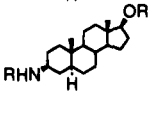
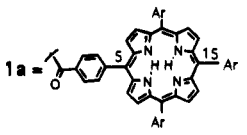
Table 1. CD Data of Bisporphyrins, Bis[*p*-(dimethylamino)-benzoates], and Bisbenzoates of Various Glycols and Amino Alcohols[a]

	Compound	1st λ(Δε)	2nd λ(Δε)	A
	7a: R = H			
	7b: R = 1a	423(+412)	414(-263)	+675
	7c: R = 1b	319(-30)	294(-30)	+89[b]
	8a: R = H			
	8b: R = 1a	423(+111)	414(-77)	+188
	8c: R = 1b	316(+17)	289(-4)	+21
	9a: R = H			
	9b: R = 1a	423(-61)	414(+48)	-109
	10a: R = H			
	10b: R = 1a	423(-12)	415(+21)	-32
	10c: R = 1b	318(-3)	293(+5)	-8
	10d: R = 1c	226(+3)		[c]
	11a: R = H			
	11b: R = 1a	422(+116)	415(-66)	+182
	12a: R = H			
	12b: R = 1a	424(-6)	414(+11)	-17



[a] Unless otherwise mentioned, all CD spectra were measured in CH_2Cl_2 on a Jasco 720 spectropolarimeter, $c = 1$ μM, λ_{max} in nm, $\Delta\epsilon$ and A in L mol^{-1} cm^{-1}. [b] Data from ref 19; measured in 20% dioxane–ethanol. [c] Measured in MeCN.

The directions of electric transition moments of the chromophores have to be known for the moments to be used in exciton-coupled CD. In the case of porphyrins, this still remains unsettled, due to the highly complex electronic nature of the chromophore. However, the CD data (Table 1) have allowed us to qualitatively estimate the direction, which suffices for practical applications of exciton-coupled CD. The Soret or B transition of symmetrically substituted porphyrins consists of two perpendiculary oriented transitions B_x and B_y; the more intense electric transition moment, B_x, is in the NH–NH direction at ~420 nm, while a weaker transition moment, B_y, is in the N–N direction at ~400 nm (see **1a** and Figure 1).[7h,13] Since the NH–NH and N–N groups interchange, and the influence of monosubstitution in tetraarylporphyrins is unknown, the direction of the B transitions is unsettled.[14] The interacting transition moments of *p*-substituted benzoates in exciton-coupled CD lie in the longitudinal C-4/C-1 axis, or in the C–O/C–N bond directions, because of the the known *syn* orientation of the ester and the amide carbonyl with respect to the methine hydrogens.[1] The exciton-coupled CD signs of steroidal bis-[(dimethylamino)benzoates] and bisporphyrin esters are identical (Table 1); this suggests that the interacting electric transition moments of the substituted porphyrin **1** are also in the direction of the C–O/C–N bond or C-5/C-15. Indeed, in view of the C_2 symmetry of the porphyrin chromophore,[14] in the present case **1a**, we can conclude that the electric transition moment in **1** (**1a**) runs in the C-5/C-15 direction. Thus, the exciton-coupled CD of bisporphyrin esters and amides correctly represent the chiral sense of twist between the C–O and/or C–N bonds at the corresponding stereogenic centers.

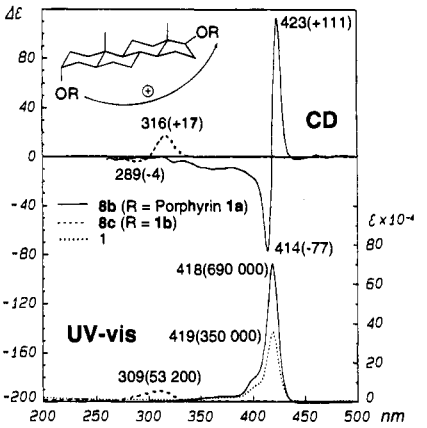


Figure 1. UV–vis and CD spectra of **1**, **8b**, and **8c** in CH_2Cl_2.

Of the known steroidal bis[*p*-(dimethylamino)benzoates], **7c** exhibited the strongest coupling, with amplitude $A = +89$.[19] Compared to **7c**, the CD of bisporphyrin ester **7b** exhibited bisignate CD with the same sign but with a >7-fold stronger A value of +675. Exciton coupling between C-3/C-17 chromophores of steroids is a typical example of long-distance interaction.[1,20] In the case of 3α,17β-bis[*p*-(dimethylamino)-benzoate] **8c**, $\epsilon_{309} = 53\,200$, with an interchromophoric distance R of 14.5 Å, A is +21, whereas for the corresponding bisporphyrin ester **8b**, ϵ_{418} 350 000, with $R = 24.4$ Å, the A value is +188, or enhanced 9-fold, despite the greater distance (Figure 1). The shape of the UV–vis spectrum of **8b** (Figure 1) is typical for all bisporphyrin esters and is similar to that of monomer **1**. The decreasing absolute A values encountered in the series 3α,17β- (**8b**, $R = 24.4$ Å, +188), 3β,17α- (**9b**, $R = 23.4$ Å, −109), and 3β,17β-bisporphyrins (**10b**, $R = 22.7$ Å, −32) reflect the decreasing chiral twist between the two chromophores. The 3β,17β-bis-[*p*-(dimethylamino)benzoate] **10c**, $R = 13.6$ Å, exhibits a bisignate CD splitting with $A = -8$, but in 3β,17β-bis(benzoate) **10d**, $R = 13.6$ Å, $\epsilon_{230} = 30\,000$, the small ϵ results in a complete breakdown of coupling. Thus, the distinct bisignate CD in bisporphyrin ester **10b**, $A = -32$, demonstrates the intensity of porphyrin coupling. Comparisons of amplitudes of bisporphyrin esters **8b** (+188) and **9b** (−32) with the corresponding 3-amide 17-ester series **11b** (+182) and **12b** (−17) show that although the amplitude is smaller in the latter two, porphyrin **1** is an excellent chromophore for amino groups as well.

The above data demonstrate that porphyrin **1**, with its λ_{max} in the visible range of 420 nm and the very intense ϵ of 350 000, provides a new promising chromophore to extend exciton-coupled CD to unexplored areas of conformational analysis of biopolymers, e.g., drug (ligand)/receptor interaction, proteins, nucleic acids, lipids, etc.; the superb sensitivity of the chromophore allows exciton-coupled CD to be scaled down to the ~0.1–1 nM range. The use of porphyrin derivatives with stronger absorption coefficients,[21] greater water solubility, and "push–pull" substituents[22] is in progress, together with conformational studies of biopolymers involving long-distance coupling.

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Supporting Information Available: Experimental procedures and spectroscopic data of all synthesised compounds (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthhead page for ordering information and Internet access instructions.

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