行政院國家科學委員會補助專題研究計畫成果報告

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※ **生物催化劑之標示及其篩選開發之研究** ※

※ (Mechanism-Based Selection and Labeling of Biocatalysts) ※

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執行期間：89 **年** 08 **月** 01 **日至** 90 **年** 07 **月** 31 **日**

計畫主持人：羅禮強

執行單位：臺灣大學化學系

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行政院國家科學委員會專題研究計畫成果報告
NSC Project Reports

生物催化劑之標示及其篩選開發之研究
(Mechanism-Based Selection and Labeling of Biocatalysts)

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執行期限：89 **年** 08 **月** 01 **日至** 90 **年** 07 **月** 31 **日**
主持人：羅禮強 臺灣大學化學系 (lclo@ccms.ntu.edu.tw)
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中文摘要：

本研究乃是開發具有紅位移性質的螢光發色團之香荳素衍生物，配合 CD **方法來應用於**β-羥基-α-**氨基酸立體化學之鑑定。在色胺酸及絲胺酸甲基酯衍生物的模型化合物與其它胺基醇類衍生物的比較結果發現，**β-羥基-α-**氨基酸類化合物經香荳素螢光發色團做成衍生物後，會採取特定的構型，因此其正向的** CD **將可以用來判定是** L **型的**β-羥基-α-**氨基酸衍生物。**

關鍵詞：紅位移發色團、胺基醇、螢光、立體化學、香荳素衍生物

Abstract:

The absolute configuration of β-hydroxy-α-amino acids was studied by CD exciton chirality method using 7-diethylaminocoumarin-3-carboxylate as a red-shifted chromophore. The CD spectra of bischromophoric derivatives of (*S*)-serine and (2*S*,3*R*)-threonine methyl esters (**2** and **7**) were compared with those of acyclic *vic*-aminoalcohols and diols (**3**-**6** and **8**-**9**). This study indicates that the polar carboxylate group of β-hydroxy-α-amino acids makes them a unique subclass of *vic*-aminoalcohols. By combining the data of CD and NMR coupling constants, we are able to correlate their preferred conformer **B** and positive CD to the corresponding absolute configuration.

KEY WORDS: 7-diethylaminocoumarin-3-carboxylate, red-shifted chromophore, acyclic aminoalcohol, bischromophoric, fluorescent, stereochemistry

Introduction:

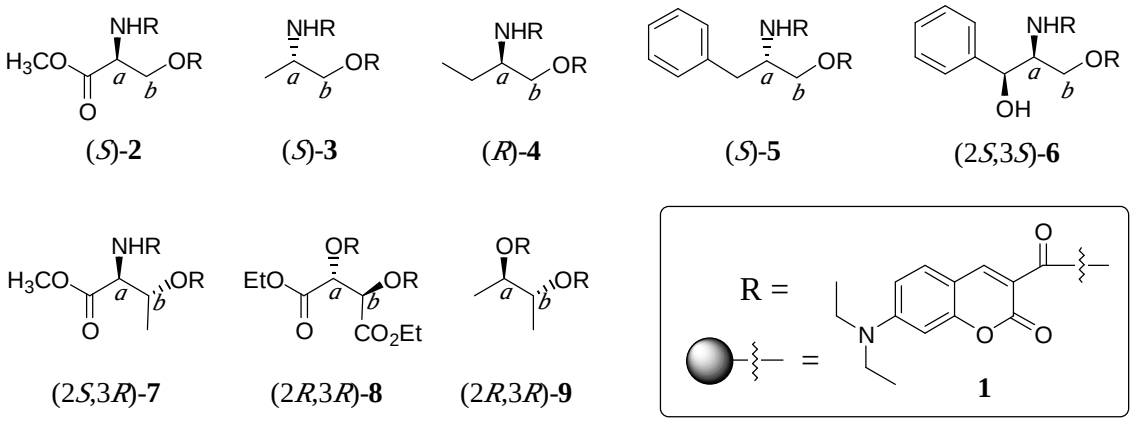
β-Hydroxy-α-amino acids belong to an important class of compounds. For example, L-serine and L-threonine, together with other L-amino acids, are the basic building blocks of proteins, which exhibit enormous diversity of functions in biological systems. β-Hydroxy-α-amino acids other than serine and threonine have also been found as constituents of cyclic peptides and other natural products possessing a wide range of biological properties, including antibiotics and immunosuppressants.[1] Therefore, they have become the targets of many synthetic efforts. A number of methods based on chemical and enzymatic approaches have been reported.[2-7] However, the establishment of the absolute configuration of these compounds still mostly relies on comparison of their optical rotations with those of authentic samples. Therefore, it is necessary to develop a spectroscopic method for the determination of the absolute configuration of this class of compounds.

The CD exciton chirality method is a non-empirical means and has been widely used for determining the absolute configuration of many natural products.[8,9] The electric transition moments of two chromophores interact through space to give an exciton coupled CD spectrum. The sign and intensity of the couplets depend on the handedness of the two chromophores and the distance between them. The assignment of the stereochemistry is thus very straightforward for rigid systems. However, the acyclic systems, which are flexible and may exist in several conformers, present a more challenging task. Over the years, several methods have been developed for different occasions, including acyclic polyols,[10-14] aminopolyols[15,16] and sphingolipids.[17,18] In our continuing efforts to explore new red-shifted chromophores for CD exciton chirality application, we have previously introduced the use of 7-diethylaminocoumarin-3-carboxylate (**1**).[19] In this report, we further extend the use of this red-shifted chromophore to β-hydroxy-α-amino acids, an acyclic *vic*-aminoalcohol system, using serine and threonine derivatives (**2** and **7**) as model compounds.

Results and Discussion:

Red-shifted chromophores provide the advantage of high sensitivity (ε > 30,000)[8,9] and give CD couplets in the region where no intrinsic chromophores in the parent molecules will interfere.[20,21] This property also offers more freedom in choosing solvents for CD measurements. Red-shifted chromophore **1** is fluorescent (λ_{ex} 406 nm, λ_{em} 462 nm in acetonitrile) and gives extremely strong CD couplets on rigid systems such as (1*R*,2*R*)-1,2-diaminocyclohexane (*A* = −203) and (1*R*,2*R*)-1,2-cyclohexanediol (*A* = −161).[19] Since β-hydroxy-α-amino acids belong to a subclass of *vic*-aminoalcohols, we prepare two series of bischromophoric derivatives (**2**-**6**) and (**7**-**9**) from the corresponding chiral aminoalcohols and diols for this study (Fig. 1). (*S*)-Serine and (2*S*,3*R*)-threonine represent the simplest form of β-hydroxy-α-amino acids. The first line starts with the methyl ester of (*S*)-serine (**2**), a terminal *vic*-aminoalcohol. The second line starts with the methyl ester of (2*S*,3*R*)-threonine (**7**), which carries an extra methyl substituent on the carbinyl center (C_b) and is an internal *vic*-aminoalcohol. The bischromophoric derivatization of these aminoalcohols and diols could easily be achieved by a single coupling reaction using DCC/DMAP method in high yields.

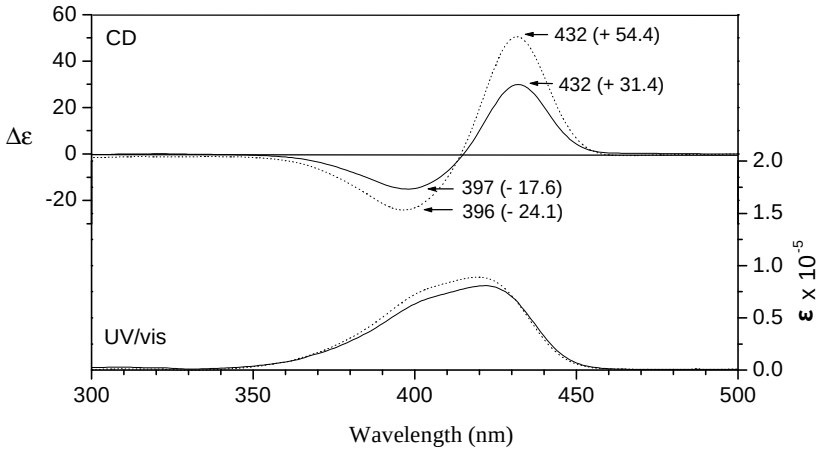
Fig. 1. Structures of the two series of bischromophoric derivatives **2**-**6** and **7**-**9**.



The UV/vis and CD spectra of (*S*)-**2** and (2*S*,3*R*)-**7** are shown in Fig. 2. The UV/vis spectra of these two compounds measured in CH_2Cl_2 show λ_{max} around 419-421 nm, which is typical for the derivatives of red-shifted chromophore **1**. Since all the bischromophoric derivatives are freely soluble in CH_2Cl_2, all the following UV/vis and CD measurements were carried out in this solvent. The CD of (*S*)-**2** exhibits an exciton couplet with a positive first

Cotton effect (CE) at 432 nm (Δε +31.4) and a negative second CE at 397 nm (Δε -17.6), leading to an *A* value of +49. This positive handedness of the two chromophores could be explained from their conformational analysis. There are three staggered conformers for (*S*)-**2**, as shown in Fig. 3 (R^1 = CO_2Me, R^2 = H, X = NH). The sign of the CD exciton couplets depends on the relative positions of the two chromophores. The two chromophores are in *gauche* positions for conformers **A** and **B**, and in *anti* positions for conformer **C**. Since the dihedral angle between the two chromophores in conformer **C** is close to 180°, it is not expected to have a significant CD. Conformer **A** will give a negative CD and conformer **B** will lead to a positive CD. ^{1}H-NMR study provides useful information regarding which conformer will be predominant. The coupling constants between one H_a and two H_b protons were measured for (*S*)-**2**. The corresponding coupling constants were 3.7 and 4.0 Hz in $CDCl_3$ (Table 1). These data reveal that the H_a proton is *gauche* to both H_b protons, as shown in the conformer **B**, which is predominant and responsible for the observed positive CD.

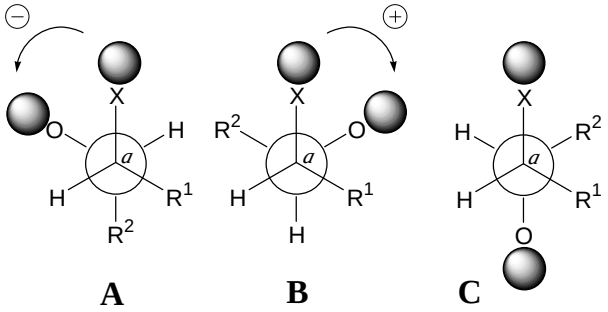
Fig. 2. CD and UV/vis spectra of bischromophoric derivatives of serine **2** (——) and threonine **7** (·····) in CH_2Cl_2.



In order to compare (*S*)-**2** with other terminal *vic*-aminoalcohols, we also prepared bischromophoric derivatives **3**-**6**, each respectively carrying a Me, Et, Bn and hydroxybenzyl group in place of the carboxylate. The UV/vis, CD and ^{1}H-NMR coupling constants of these derivatives are shown in Table 1, and their conformational structures in Fig. 3. Their UV/vis spectra have λ_{max} around 419-424 nm in CH_2Cl_2, which are the same as that of (*S*)-**2**. This λ_{max} is slightly red-shifted as compared with the spectra taken in polar solvents such as acetonitrile and acetone (λ_{max} 413-418 nm). The *vicinal* coupling constants of H_a and H_b protons for compounds **3**-**6** fall in the range of 3.6-4.9 Hz (Table 1), which are similar to those of (*S*)-**2**. This evidence indicates that compounds **2**-**6** all favor conformer **B**. The only difference is to what extent the conformer **B** is predominant over the other two conformers. The relative abundance of the conformers could be deduced from Karplus equation;[22] the smaller the coupling constants are, the more abundant the conformer **B** is populated. The conformer **B** is more abundant in compounds **2** (R^1 = CO_2Me) and **6** (R^1 = CH(OH)Ph) than in compounds **3** (R^1 = Me), **4** (R^1 = Et) and **5** (R^1 = Bn). It implies that the polarity of the substituent plays a dominating role in the distribution of conformers. Here, a polar substituent, such as carboxylate and hydroxybenzyl group, at C_a favors the conformer **B**. This observation of conformational preference is different from what Harada *et al.* have reported on terminal *vic*-diols using *p*-substituted benzoates as the chromophore, where the conformer **A** is the most populated conformation regardless of the polarity of the

substituents.[13] The intensity of the *A* values (Table 1) for compounds **2-6** obtained from CD measurements are also parallel to this NMR conformational analysis, (**2**, **6**) > (**3**, **4** and **5**). Therefore, in the first series of the β-hydroxy-α-amino acids, a positive CD is linked to the (*S*)-configuration of serine derivative.

Fig. 3. Three staggered conformers (**A-C**) of the bischromophoric derivatives **2-9**.



Compound	R^1	R^2	X
(*S*)-**2**	CO_2Me	H	NH
(*R*)-**3**	Me	H	NH
(*R*)-**4**	Et	H	NH
(*R*)-**5**	Bn	H	NH
(2*S*,3*S*)-**6**	CH(OH)Ph	H	NH
(2*S*,3*R*)-**7**	CO_2Me	Me	NH
(2*S*,3*S*)-**8**	CO_2Et	CO_2Et	O
(2*R*,3*R*)-**9**	Me	Me	O

Table 1. UV, CD and NMR coupling constants of bischromophoric derivatives **2-9**.

compd	UV/Vis (CH_2Cl_2): λ_{max} (ε)	CD (CH_2Cl_2): λ_{ext} (Δε)	***A*** value	Coupling Constant[a] $J_{Ha,Hb}$ (Hz)
(*S*)-**2**	422 (82,000)	432 (+31.4), 397 (–17.6)	+49	3.7, 4.0
(*S*)-**3**	420 (80,000)	428 (–5.9), 396 (+4.0)	–10	4.6, 4.9
(*R*)-**4**	419 (75,000)	428 (+8.6), 395(–5.3)	+14	4.0, 4.2
(*S*)-**5**	421 (71,000)	428 (–21.7), 395 (+8.7)	–30	3.9, 4.6
(2*S*,3*S*)-**6**	424 (84,000)	431 (+44.4), 398 (–15.5)	+60	3.6, 4.7
(2*S*,3*R*)-**7**	419 (88,000)	432 (+54.4), 396 (–24.1)	+79	2.0
(2*R*,3*R*)-**8**	422 (88,000)	435 (–70.9), 401 (+33.5)	–104	3.2[b]
(2*R*,3*R*)-**9**	420 (82,000)	432 (–31.9), 395 (+21.1)	–53	–

[a]Spectra were taken in $CDCl_3$.
[b]Obtained from the ^{13}C satellite bands.

Threonine derivative (**7**) is an extension to serine (**2**). It has two stereocenters with a *threo* relative stereochemistry, as shown in Fig. 3 (R^1 = CO_2Me, R^2 = Me, X = NH). The *vicinal* coupling constant of the H_a and H_b protons is 2.0 Hz in (2*S*,3*R*)-**7** (Table 1). The CD of (2*S*,3*R*)-**7** gives a positive first CE at 432 nm (Δε +54.4) and a negative second CE at 396 nm (Δε -24.1), an *A* value of +79. Therefore, (2*S*,3*R*)-**7** preferably adopts conformer **B**.

Besides, the population of conformer **B** is higher in (2*S*,3*R*)-**7** than in (*S*)-**2**, which could be deduced from their *A* values (+79 *vs.* +49) (Fig. 2). This could be attributed to the steric effect of the extra methyl group at C_b of (2*S*,3*R*)-**7**. The crystal structure of (2*S*,3*R*)-**7** is shown in Fig. 4. It is consistent with the conformer **B** in the crystalline state and the two chromophores have a dihedral angle of +75.0°.

The second series of β-hydroxy-α-amino acids can be compared with internal *vic*-diols (2*S*,3*S*)-**8** and (2*R*,3*R*)-**9** (Fig. 3). Compound **8** has two polar carboxylate substituents (R^1 = R^2 = CO_2Et, X = O), whereas compound **9** has two non-polar methyl substituents (R^1 = R^2 = Me, X = O). The *vicinal* coupling constant of the H_a and H_b protons in (2*R*,3*R*)-**8** is 3.2 Hz, which is obtained from the ^{13}C satellite bands.[23] It therefore excludes the conformer **A**. Since conformer **C** gives no significant CD, the intense CD of (2*R*,3*R*)-**8** (*A* = -104) is mostly contributed from conformer **B**. This result is consistent with that of (2*S*,3*R*)-**7**, both in favor of conformer **B**. The crystal structure of (2*R*,3*R*)-**8** is shown in Fig. 5. It also adopts the conformer **B**. The two chromophores have a dihedral angle of –50.6° in the crystalline state.

Fig. 4. ORTEP drawing of bischromophoric (2*S*,3*R*)-**7** showing a dihedral angle of +75.0° between the two chromophores.

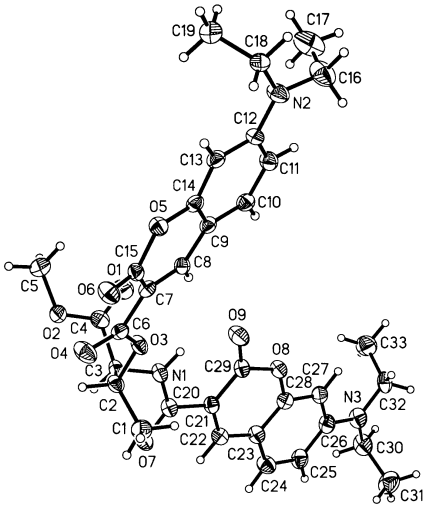
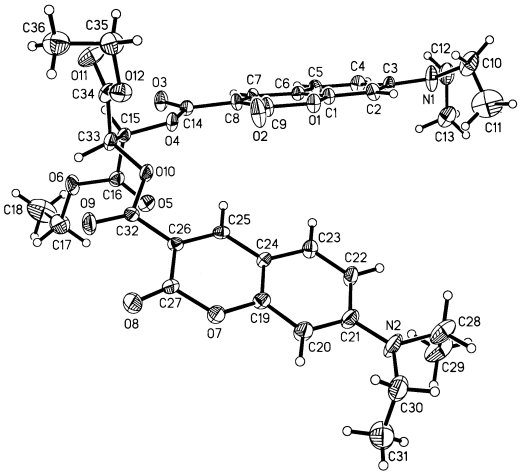


Fig. 5. ORTEP drawing of bischromophoric (2*R*,3*R*)-**8** showing a dihedral angle of –50.6° between the two chromophores.



When the two polar carboxylate substituents of compound **8** were replaced by non-polar methyl groups as in (2*R*,3*R*)-**9**, the preferred conformer changed. The CD of (2*R*,3*R*)-**9** exhibits a negative first CE at 432 nm (Δε -31.9) and a positive second CE at 395 nm (Δε +21.1), an *A* value of –53. This negative CD suggests that conformer **A** is dominant in (2*R*,3*R*)-**9**. This conformational preference is different from that of (2*S*,3*R*)-**7** and (2*S*,3*S*)-**8**. Although we were unable to measure the *vicinal* coupling constant for (2*R*,3*R*)-**9**, the preference of conformer **A** for non-polar substituents in internal *vic*-diol systems is in agreement with literature results.[13] This result further supports the point we obtained with the case of (*S*)-**2**; the polar carboxylate group of the β-hydroxy-α-amino acids will favor conformer **B**.

Conclusion:

A CD exciton chirality method using a red-shifted 7-diethylaminocoumarin-3-carboxylate (**1**) chromophore has been studied on the β-hydroxy-α-amino acids. Due to its red-shifted property, we were able to use CH_2Cl_2 as the solvent for CD and UV/vis measurements in this study. The polar carboxylate group in this class of compounds plays an important role in determining the population of each conformer. Data from NMR coupling constants reveal the preferred conformer **B**. By combining the information of preferred conformation with the resultant positive CD in (*S*)-**2** and (2*S*,3*R*)-**7**, we were able to determine the absolute configuration of these β-hydroxy-α-amino acids. We are currently investigating the effect on the CD of the compounds bearing aromatic substituents at C_b.

Experimental Section:

General methods. All reagents and starting materials were obtained from commercial suppliers (Acros, Aldrich and Merck) and were used without further purification. ^{1}H- and ^{13}C-NMR were recorded using a Bruker Avance 400 spectrometer. ^{1}H-NMR spectra in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.24 ppm and ^{13}C-NMR spectra to the central peak of $CDCl_3$ at 77.0 ppm. High resolution mass spectra were recorded on a JEOL-102A mass spectrometer. Analytical TLC (silica gel, 60F-54, Merck) and spots were visualized under UV light and/or phosphomolybdic acid-ethanol. Column chromatography was performed with Kiesegel 60 (70-230 mesh) silica gel (Merck). All samples were subjected to HPLC purification prior to UV/CD measurements. Spectrophotometric grade of CH_2Cl_2 and a Jasco J-720 spectropolarimeter were used for obtaining CD spectra. Melting points are reported without correction. The crystallographic data were collected on a NONIUS CAD4 diffractometer using graphite-monochromated MoKα radiation. Structural analysis was made by using SHELXTL program on *SiliconGraphics* computer.

General procedure for the preparation of bischromophoric derivatives: To an ice-cooled solution of starting diols or aminoalcohols (1.25 mmol), DMAP (3.0 mmol) and 7-diethylaminocoumarin-3-carboxylic acid (2.62 mmol) in CH_2Cl_2 (5 mL) was slowly added a solution of DCC (3.0 mmol) in CH_2Cl_2 (3 mL). The mixture was gradually allowed to return to rt and stirred overnight. It was quenched by adding a few drops of 5% citric acid and stirred for another 30 min. The CH_2Cl_2 was removed, and EtOAc was added. The DCU was first filtered off and the EtOAc filtrate was washed with 10% $NaHCO_3$ (x3), H_2O (x2), and finally with brine. The organic layer was dried over anhydrous Na_2SO_4. It was filtered, concentrated and subjected to silica gel column chromatography for purification. The desired product was eluted with $CHCl_3$/MeOH (9/1).

(*S*)-*N,O*-bis(7-Diethylaminocoumarin-3-carbonyl)-serine methyl ester (2): Yield 85%, R_f 0.67 ($CHCl_3$ /EtOAc = 1∶1), mp 102-105°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.57 (d, *J*

= 8.0 Hz, 1 H, NH), 8.62 (s, 1 H), 8.44 (s, 1 H), 7.39 (d, *J* = 9.0 Hz, 1 H), 7.36 (d, *J* = 9.0 Hz, 1 H), 6.61-6.55 (m, 2 H), 6.43 (d, *J* = 2.3 Hz, 1 H), 6.36 (d, *J* = 2.3 Hz, 1 H), 5.09 (ddd, *J* = 8.0, 4.0, 3.7 Hz, 1 H), 4.77 (dd, *J* = 11.2, 3.7 Hz, 1 H), 4.53 (dd, *J* = 11.2, 4.0 Hz, 1 H), 3.77 (S, 3 H), 3.43-3.37 (m, 8 H), 1.20-1.16 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 169.9 (C), 163.1 (C), 162.6 (C), 162.4 (C), 158.4 (C), 158.0 (C), 157.7 (C), 153.0 (C), 152.7 (C), 149.4 (CH), 148.3 (CH), 131.6 (CH), 131.2 (CH), 109.9 (CH), 109.4, 108.2 (C), 107.6 (C), 107.5 (C), 96.5 (CH), 96.5 (CH), 64.1 (CH_2), 52.8 (CH_3), 51.8 (CH), 45.0 (CH_3), 12.3 (CH_3); FAB-HRMS calcd for $C_{32}H_{36}N_3O_9$ (M + H^+) 606.2451, found 606.2457.

(*S*)-1,2-bis(7-Diethylaminocoumarin-3-carbonyl)-2-amino-1-propanol (3): Yield 91%, R_f 0.28 ($CHCl_3$ /EtOAc = 1∶1), mp 172-174°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.97 (d, *J* = 8.1 Hz, 1 H, NH), 8.67 (s, 1 H), 8.48 (s, 1 H), 7.39 (d, *J* = 9.0 Hz, 2 H), 6.63-6.57 (m, 2 H), 6.47 (d, *J* = 2.3 Hz, 1 H), 6.41 (d, *J* = 2.3 Hz, 1 H), 4.56 (m, 1 H), 4.38 (dd, *J* = 11.0, 4.6 Hz, 1 H), 4.30 (dd, *J* = 11.0, 4.9 Hz, 1 H), 3.45-3.39 (m, 8 H), 1.36 (d, *J* = 6.8 Hz, 3 H), 1.23-1.19 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 163.4 (C), 162.7 (C), 162.6 (C), 158.5 (C), 158.0 (C), 157.7 (C), 152.9 (C), 152.5 (C), 149.3 (CH), 148.1 (CH), 131.3 (CH), 131.1 (CH), 110.3 (C), 109.9 (CH), 109.4 (CH), 108.4 (C), 108.3 (C), 107.7 (C), 96.7 (CH), 96.6 (CH), 67.3 (CH_2), 45.1 (CH_2), 45.0 (CH_2), 44.4 (CH), 17.8 (CH_3), 12.4 (CH_3); FAB-HRMS calcd for $C_{31}H_{36}N_3O_7$ (M + H^+) 562.2553, found 562.2509.

(*R*)-1,2-bis(7-Diethylaminocoumarin-3-carbonyl)-2-amino-1-butanol (4): Yield 91%, R_f 0.38 ($CHCl_3$ /EtOAc = 1∶1), mp 91-93°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.96 (d, *J* = 8.3 Hz, 1 H, NH), 8.67 (s, 1 H), 8.47 (s, 1 H), 7.38 (m, 2 H), 6.60 (m, 2 H), 6.46-6.40 (m, 2 H), 4.43 (dd, *J* = 10.5, 4.0 Hz, 1 H), 4.38 (m, 1 H), 4.31 (dd, *J* = 10.5, 4.2 Hz, 1 H), 3.42 (m, 8 H), 1.80-1.69 (m, 3 H), 1.22-1.18 (m, 12 H), 1.01 (t, *J* = 7.4 Hz, 3 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 163.3 (C), 162.9 (C), 162.7 (C), 158.4 (C), 158.1 (C), 157.6 (C), 152.9 (C), 152.5 (C), 149.2 (CH), 148.1 (CH), 131.3 (CH), 131.1 (CH), 110.2 (C), 109.9 (CH), 109.4 (CH), 108.4 (C), 108.3 (C), 107.7 (C), 96.6 (CH), 96.5 (CH), 65.8 (CH_2), 49.8 (CH), 45.0 (CH_2), 45.0 (CH_2), 24.8 (CH_2), 12.4 (CH_3), 10.5 (CH_3); FAB-HRMS calcd for $C_{32}H_{37}N_3O_7$ (M + H^+) 576.2710, found 576.2717.

(*S*)-1,2-bis(7-Diethylaminocoumarin-3-carbonyl)-2-amino-3-phenyl-1propanol (5): Yield 67%, R_f 0.50 ($CHCl_3$ /EtOAc = 1∶1), mp 84-85°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.14 (d, *J* = 8.6 Hz, 1 H, NH), 8.64 (s, 1 H), 8.42 (s, 1 H), 7.39 (d, *J* = 8.7 Hz, 1 H), 7.37 (d, *J* = 8.7 Hz, 1 H), 7.30-7.16 (m, 5 H), 6.62-6.59 (m, 2 H), 6.45 (d, *J* = 2.3 Hz, 1 H), 6.41 (d, *J* = 2.3 Hz, 1 H), 4.69 (m, 1 H), 4.37 (dd, *J* = 11.1, 3.9 Hz, 1 H), 4.29 (dd, *J* = 11.1, 4.6 Hz, 1 H), 3.44-3.39 (m, 8 H), 3.05 (m, 2 H), 1.22-1.07 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 163.4 (C), 162.7 (C), 162.6 (C), 158.5 (C), 158.1 (C), 157.6 (C), 152.9 (C), 152.5 (C), 149.3 (CH), 148.1 (CH), 137.6 (C), 131.4 (CH), 131.1 (CH), 129.5 (CH), 128.5 (CH), 126.5 (CH), 110.1 (C), 109.9 (CH), 109.4 (CH), 108.3 (C), 108.3 (C), 107.7 (C), 96.6 (CH), 96.5 (CH), 65.0 (CH_2), 49.8 (CH), 45.0 (CH_2), 45.0 (CH_2), 38.0 (CH_2), 12.4 (CH_3), 12.4 (CH_3); FAB-HRMS calcd for $C_{37}H_{40}N_3O_7$ (M + H^+) 638.2867, found 638.2877.

(2*S*,3*S*)-1,2-bis(7-Diethylaminocoumarin-3-carbonyl)-2-amino-1-phenylpropane-1,3-diol (6): Yield 56%, R_f 0.38 ($CHCl_3$ /EtOAc = 1∶1), mp 127-129°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.49 (d, *J* = 7.9 Hz, 1 H, NH), 8.55 (s, 1 H), 8.43 (s, 1 H), 7.46 (d, *J* = 7.4 Hz, 2 H), 7.35-7.17 (m, 5 H), 6.59-6.56 (m, 2 H), 6.55-6.40 (m, 2 H), 5.16 (s, 1 H, OH), 4.63 (dd, *J* = 11.1, 4.7 Hz, 1 H), 4.57 (m, 1 H), 4.52 (d, *J* =3.4 Hz, 1 H), 4.40 (dd, *J* = 11.1, 3.6 Hz, 1 H), 3.44-3.37 (m, 8 H), 1.22-1.16 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 164.1 (C), 163.7 (C), 162.3 (C), 158.6 (C), 158.5 (C), 157.6 (C), 153.1 (C), 152.5 (C), 150.0 (CH), 148.0 (CH), 141.5 (C), 131.3 (CH), 131.0 (CH), 128.2 (CH), 127.4 (CH), 126.1 (CH), 109.9 (C), 109.8 (CH), 109.7 (CH), 108.2 (C), 107.8 (C), 107.8 (C), 96.5 (CH), 96.5 (CH), 73.4 (CH), 65.5 (CH_2), 54.5 (CH), 45.0 (CH_2), 45.0 (CH_2), 12.4 (CH_3), 12.4 (CH_3); FAB-HRMS calcd for $C_{37}H_{40}N_3O_8$ (M + H^+) 654.2815, found 654.2797.

(2*S*,3*R*)-*N,O*-bis(7-Diethylaminocoumarin-3-carbonyl)-threonine methyl ester (7): Yield 94%, R_f 0.69 ($CHCl_3$/EtOAc = 1 : 1), mp 193-197°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 9.62 (d, *J* = 9.2 Hz, 1 H, NH), 8.67 (s, 1 H), 8.61 (s, 1 H), 7.52 (d, *J* = 9.2 Hz, 1 H) 7.40 (d, *J* = 8.8 Hz, 1 H), 6.64-6.59 (m, 2 H), 6.49 (d, *J* = 2.4 Hz, 1 H), 6.39 (d, *J* = 2.4 Hz, 1 H), 5.72 (m, 1 H), 4.98 (dd, *J* = 9.2, 2.0 Hz, 1 H), 3.71 (s, 3 H), 3.47-3.39 (m, 8 H), 1.41 (d, *J* = 6.8 Hz, 3 H), 1.24-1.18 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 170.3 (C), 163.7 (C), 162.6 (C), 161.5 (C), 158.4 (C), 158.2 (C), 157.9 (C), 153.1 (C), 152.8 (C), 149.6 (CH), 148.5 (CH), 131.9 (CH), 131.3 (CH), 110.0 (CH), 109.6 (C), 109.5 (C), 108.3 (C), 107.9 (C), 107.6 (C), 96.6 (CH), 96.5 (CH), 70.5 (CH), 56.0 (CH), 52.7 (CH_3), 45.1 (CH_2), 17.5 (CH_3), 12.4 (CH_3); FAB-HRMS calcd for $C_{33}H_{38}N_3O_9$ (M + H^+) 620.2608, found 620.2596. A crystal of dimension 0.30 x 0.20 x 0.15 mm was selected for X-ray analysis. The compound crystallized from dioxane/hexane in the space group $P2_1$, monoclinic, a = 9.0165(2) Å, b = 14.0230(3) Å, c = 14.6431(3) Å, β = 100.21°, V = 1822.11(7) Å^3, Z = 2, λ = 0.71073 Å, ρ(calcd) = 1.283 g/cm^3, μ(MoKα) = 0.091 mm^{-1}, F(000) = 752 and T = 150 K. The structure was solved by the direct method and refined by full-matrix least squares on F^2 values. The final indices were R = 0.063, Rw = 0.158 with goodness-of-fit = 1.110.

(2*R*,3*R*)-Diethyl tartrate-2,3-bis(7-diethylaminocoumarin-3-carboxylate) (8): Yield 80%, R_f 0.50 ($CHCl_3$/EtOAc = 1 : 1), mp 200-203°C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 2 H), 7.38 (d, *J* = 9.2 Hz, 2 H), 6.57 (dd, *J* = 8.8, 2.0 Hz, 2 H), 6.38 (d, *J* = 2.0 Hz, 2 H), 5.91 (s, 2 H), 4.26-4.16 (m, 4 H), 3.44-3.38 (m, 8 H), 1.28-1.17 (m, 18 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 166.1 (C), 162.0 (C), 158.6 (C), 157.7 (C), 153.3 (C), 150.2 (CH), 131.6 (CH), 109.6 (CH), 107.6 (C), 106.6 (C), 96.5 (CH), 71.1 (CH), 62.2 (CH_2), 45.1 (CH_2), 14.0 (CH_3), 12.3 (CH_3); FAB-HRMS calcd for $C_{36}H_{41}N_2O_{12}$ (M + H^+) 693.2660, found 693.2654. A crystal of dimension 0.25 x 0.20 x 0.10 mm was selected for X-ray analysis. The compound crystallized from dioxane/hexane in the space group $P2_12_12_1$, orthorhombic, a = 8.6608(5) Å, b = 12.4842(7) Å, c = 32.259(2) Å, β = 90°, V = 3488.0(3) Å^3, Z = 4, λ = 0.71073 Å, ρ(calcd) = 1.319 g/cm^3, μ(MoKα) = 0.100 mm^{-1}, F(000) = 1464 and T = 295 K. The structure was solved by the direct method and refined by full-matrix least squares on F^2 values. The final indices were R = 0.093, Rw = 0.167 with goodness-of-fit = 1.008.

(2*R*,3*R*)-2,3-bis(7-Diethylaminocoumarin-3-carbonyl)-2,3-butanediol (9): Yield 94%, R_f 0.71 ($CHCl_3$/EtOAc = 1 : 1), mp 201-202°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.38 (s, 2 H), 7.29 (d, *J* = 9.0 Hz, 2 H), 6.53 (dd, *J* = 9.0, 2.0 Hz, 2 H), 6.33 (d, *J* = 2.0 Hz, 2 H), 5.27-5.20 (m, 2 H), 3.39-3.34 (m, 8 H), 1.32 (d, *J* = 6.8 Hz, 6 H), 1.20-1.13 (m, 12 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 162.8 (C), 158.3 (C), 158.1 (C), 152.8 (C), 149.1 (CH), 131.4 (CH), 109.4 (CH), 108.1 (C), 107.5 (C), 96.4 (CH), 72.0 (CH), 44.9 (CH_2), 16.4 (CH_3), 12.3 (CH_3); FAB-HRMS calcd for $C_{32}H_{37}N_2O_8$ (M + H^+) 577.2550, found 577.2535.

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