行政院國家科學委員會專題研究計畫成果報告

生物催化劑篩選開發之研究

計畫類別：☑個別型計畫　□整合型計畫

計畫編號：NSC 89—2113—M—002-017

執行期間：88 年 8 月 1 日至 89 年 7 月 31 日

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共同主持人：

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註：整合型計畫總報告與子計畫成果報告請分開編印各成一冊，彙整一起繳送國科會。

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執行單位：台大化學系

中華民國 89 年 11 月 30 日

行政院國家科學委員會專題研究計畫成果報告 (NSC Project Report)
計畫名稱：生物催化劑篩選開發之研究
(Development of a Mechanism-Based Selection of Biocatalysts)
計畫編號：NSC 89-2113-M-002-017
執行期限：88 年 8 月 1 日 至 89 年 7 月 31 日
主持人：羅禮強　臺灣大學化學系副教授

摘要：我們在本計畫中利用相苴素-3-羧酸衍生物來製備其活化之 imidazolide 試劑，此新開發之螢光試劑可以應用於超微量立體化學之研究。針對含羥基之化合物進行微量反應，而來得到其螢光團衍生物，配合圓二色分光光譜儀即可判讀其絕對立體組態。本 imidazolide 試劑具有紅位移及高靈敏度之特點。

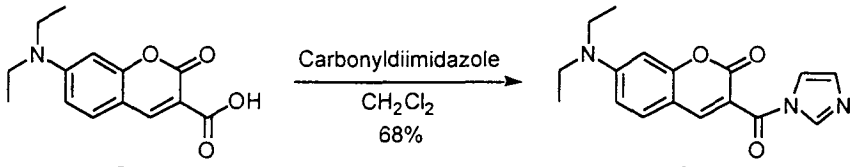
關鍵詞：紅位移，相苴素，圓二色光譜，微量，立體化學，螢光

Abstract: Imidazolide 1 has been prepared from 7-diethylaminocoumarin-3-carboxylic acid (2) and 1,1'-carbonyldiimidazole. This novel fluorescent derivatization reagent can be used in CD exciton chirality method for microscale structural determinations of hydroxyl-containing compounds. It features a red-shifted chromophore and offers the advantages of fluorescence and high sensitivity.

Keywords: red-shifted, coumarin, CD, microscale, stereochemistry, fluorescence

Many efforts in the development of red-shifted chromophores (λ_{max} 360-480 nm) for circular dichroic (CD) exciton chirality method have been made in the past few years.[1-5] Red-shifted chromophores provide the advantage of high sensitivity (ε values > 30,000)[6] and give distinct CD couplets in the region where no intrinsic chromophores on the parent compounds will interfere. Chromophores could be incorporated to the parent compounds via amide, imine or ester bond linkages. The resultant CD couplets of these derivatives thus reflect the stereochemistry of the parent molecules. However, some of these chromophores are highly conjugated double bond systems,[2,3,5] which require multiple synthetic steps to prepare and may suffer from isomerization on a prolonged exposure to light. Moreover, their long skeletons can adopt flexible conformations, possibly leading to ambiguous results on the determination of the chirality of parent compounds.[7,8] We report herein a convenient fluorescent reagent, 1-(7-diethylaminocoumarin-3-carbonyl)imidazole (**1**), for derivatizing hydroxyl groups at microscales.

Scheme 1

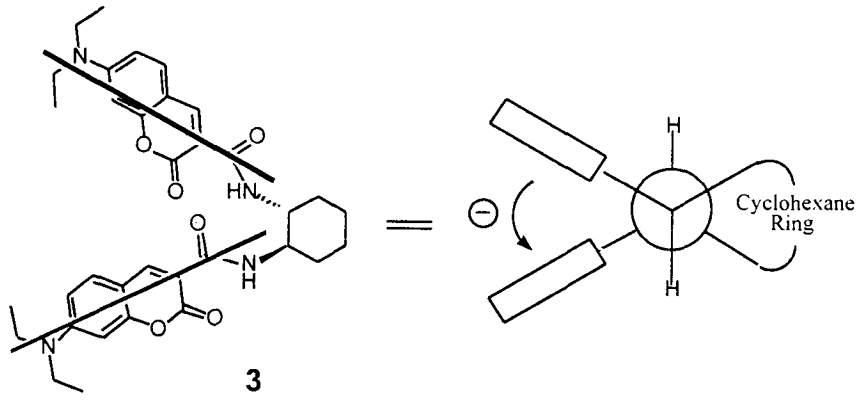


2 **1**

The crystalline imidazolide **1** was simply prepared by stirring its free acid **2** with 1,1'-carbonyldiimidazole in CH_2Cl_2 solution (Scheme 1).[9] The structure of **1** is confirmed by X-ray and NMR analyses. Imidazolide **1** reagent offers a red-shifted chromophore featuring fluorescence and high sensitivity (high ε), and is capable of microscale structural determinations. Coumarin derivative **2** has a skeleton resembling that of *p*-dimethylaminocinnamate.[10] But the exo double bond of this chromophore is locked by a lactone ring, making it absorb at a longer wavelength than its cinnamate counterpart (406 nm *vs.* 360 nm) in addition to its fluorescent property.[11] Its applicability in CD exciton chirality method is demonstrated as follows.

The bischromophoric derivative of (1*R*,2*R*)-1,2-diaminocyclohexane (**3**)[12] displays an absorption maximum at 406 nm (ε 73,400) and an emission at 462 nm in acetonitrile. Its CD spectrum shows an intense couplet at 429 nm (Δε -134) and 392 nm (Δε +69). This gives rise to a high *A* value of -203. The sign of this CD couplet is consistent with the chirality of the starting chiral diamine. It supports that the direction of the electronic transition dipole moment corresponding to 406 nm is along the long axis of the

chromophore and is parallel to the N-C bond on the parent molecule, as shown by the bold lines on top of the chromophores (Figure 1).



3

Figure 1. The structure of bischromophoric derivative **3** and its Newman projection showing negative handedness.

High isolated yields of bischromophoric derivatives **4-6** (Figure 2) are achieved when the parent hydroxyl compounds are subjected to imidazolide reagent **1** in the presence of DBU (5 h stirring in CH_3CN solution), making this derivatization procedure well-suited for microscale reactions.[13] Compound **4** exemplies the derivative from a simple chiral diol on a six-membered ring, compound **5** from 2,3-diol of a glucopyranoside derivative and compound **6** from ponasterone A.[14] Their UV/Vis, fluorescence and CD data are shown in Table 1.

Table 1. UV/Vis, fluorescence and CD data of compounds **3-6** in acetonitrile; Wavelengths in nanometer (nm).

Compound	UV/Vis: λ_{max} (ε)	Emission: λ_{max}	CD: λ_{ext} ($\Delta\varepsilon$)	*A* value
3	406 (73,400)	462	429 (-134), 392 (+69)	-203
4	414 (79,300)	462	431 (-100), 394 (+61)	-161
5	417 (80,900)	463	435 (+118), 398 (-77)	+195
6	412 (78,900)	462	432 (-75), 394 (+38)	-110

All four bischromophoric derivatives (**3-6**) display fluorescence at λ_{em} 462-463 nm (λ_{ext} 406-417 nm), which is an important feature for highly sensitive detection of biological samples. The CD Cotton effects resulting from these bischromophoric derivatives faithfully represent the stereochemistry of the parent compounds with no ambiguity in the structural determination. For example, bis-ester **4** gives a negative Cotton effect (*A* = -

161) similar to that of the bis-amide **3**. Compound **5**, derived from methyl 4,6-*O*-benzylidene-α-D-glucopyranoside, has a strongly positive couplet (A = +195). This A value is much higher than those from *p*-bromobenzoate (Glc***BBAA***, +69)[15] and *p*-methoxycinnamate (Glc***CCAA***, +95)[15] chromophores used in carbohydrate studies.[16]

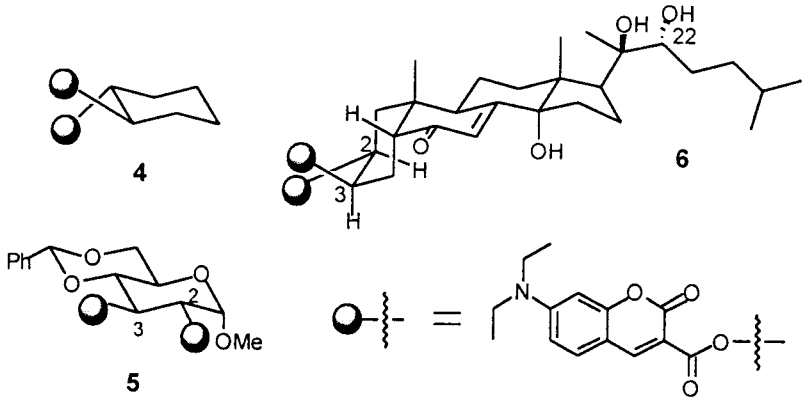


Figure 2. Structures of the bischromophoric derivatives **4**, **5**, and **6**.

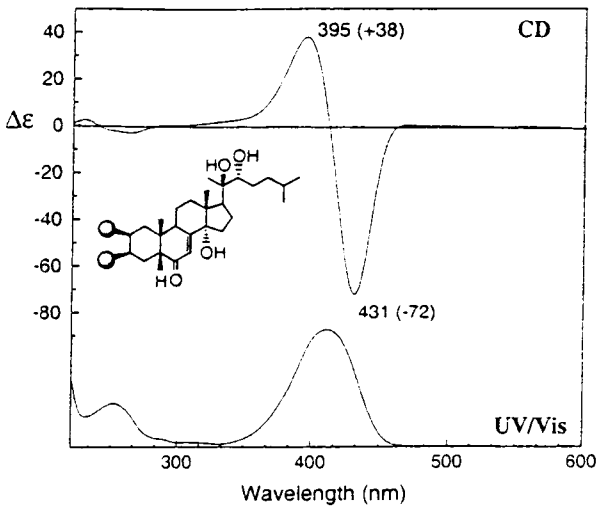


Figure 3. The CD and UV/Vis curves of bischromophoric ponasterone A (**6**).

When reacting with ponasterone A, imidazolide reagent **1** selectively acylates the C-2 and C-3 hydroxyl groups to give 2,3-bischromophoric product **6**. The downfield shifts of the proton signals of compound **6** (H-2 at 5.68 and H-3 at 5.36 ppm) reveal the positions where the chromophores attached. The Cotton effects of the enone group in ponasterone A are weak (λ_{ext} 327 nm, $\Delta\varepsilon$ = +1.8; λ_{ext} 248 nm, $\Delta\varepsilon$ = -3.9)[17] and far away from those of

the bischromophoric derivative **6** (Figure 3). The *A* value of compound **6** (*A* = -113) is three times higher than that resulted from the benzoate chromophore originally used for its structural study.[17]

In summary, imidazolide **1** represents a convenient derivatizing reagent with desirable properties for hydroxyl groups. Its λ_{max} of 406 nm is one of the most red-shifted chromophores that have been synthesized and utilized in CD studies. It can expand the scope of the reagents in use for hydroxyl groups, such as naphthoylimidazole[18] which has the absorption maximum in the UV region (λ_{max} 234 nm). Further investigations of its application in fluorescence detected circular dichroism (FDCD)[19] are currently underway.

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9. To a stirred solution of 7-diethylaminocoumarin-3-carboxylic acid (**2**, 1.00 g, 3.83 mmol) in CH_2Cl_2 (35 mL) was slowly added a solution of 1,1'-carbonyldiimidazole in CH_2Cl_2 (5 mL). The reaction mixture was stirred at room temperature for 14 h, then concentrated at reduced pressure to remove most of the solvent. The desired imidazolide **1** was obtained after recrystallization from benzene (805 mg, 68%). mp 128-130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (*s*, 1 H), 8.08 (*s*, 1 H), 7.47 (*s*, 1 H), 7.36 (*d*, *J* = 9.0 Hz, 1 H), 7.06 (*s*, 1 H), 6.64 (*dd*, *J* = 9.0, 2.4 Hz, 1 H), 6.48 (*d*, *J* = 2.4 Hz, 1 H), 3.45 (*q*, *J* = 7.1 Hz, 4 H), 1.23 (*t*, *J* = 7.1 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.5, 158.6, 158.5, 153.4, 149.4, 137.8, 131.3, 130.4, 117.4, 110.7, 110.1,

107.5, 96.9, 45.2 (2x), 12.4 (2x). FAB-HRMS for $C_{17}H_{18}O_3N_3$ (M^++1) calcd 312.1348, found 312.1319.

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11. 7-Diethylaminocoumarin-3-carboxylic acid (**2**) is a commercially available reagent widely used in the fluorescent labeling of bioconjugates with the surface accessible amino acid side chains. Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes: Eugene, OR 1996.
12. Compound **3** was prepared by carbodiimide coupling method (EDC/HOBt). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (*br*, 2 H), 8.63 (*s*, 2 H), 7.34 (*d*, *J* = 9.0 Hz, 2 H), 6.55 (*dd*, *J* = 9.0, 2.1 Hz, 2 H), 6.41 (*d*, *J* = 2.1 Hz, 2 H), 4.05 (*br*, 2 H), 3.39 (*q*, *J* = 7.0 Hz, 8 H), 2.15 (*br*, 2 H), 1.74 (*br*, 2 H), 1.42 (*br*, 4 H), 1.18 (*t*, *J* = 7.0 Hz, 12 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.9, 162.3, 157.5, 152.2, 147.9, 131.0, 110.8, 109.6, 108.4, 96.6, 52.7, 45.0, 32.3, 24.6, 12.4.
13. General procedure for derivatization of diols using imidazolide **1**: To a solution of the diol substrate (0.1 mmol) and imidazolide **1** (0.25 mmol) in anhydrous CH_3CN (5 mL) was slowly added 0.5 mL of 0.5 M DBU/CH_3CN solution. After stirred at room temperature for 5 h, the mixture was concentrated and the desired product was purified by silica gel column chromatography.
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15. Wiesler, W. T.; Vazquez, J. T.; Nakanishi, K. *J. Am. Chem. Soc.* **1987**, *109*, 5586.; Glc***BBAA*** denotes methyl α-D-glucopyranoside 4,6-diacetate-2,3-bis(*p*-bromobenzoate) and Glc***CCAA*** denotes methyl α-D-glucopyranoside 4,6-diacetate-2,3-bis(*p*-methoxycinnamate).
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Supporting Information

Preparation of Compound 3: To a cooled solution of 7-diethylaminocoumarin-3-carboxylic acid **2** (265 mg, 1.018 mmol), 1-hydroxybenzotriazole (14 mg, 0.103 mmol) and (1*R*,2*R*)-1,2-cyclohexanediamine (58 mg, 0.508 mmol) in CH_2Cl_2 (10 mL) was added triethylamine (108 mg, 1.067 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDCI) (205 mg, 1.069 mmol). The reaction mixture was stirred at rt for 13 h, then concentrated to residue. It was purified by silica gel column chromatography eluted with CH_2Cl_2/ethyl acetate (3/2) to give the desired product **3** (272 mg, 89%). mp 244-246°C, R_f = 0.46 (CH_2Cl_2/ethyl acetate = 1/1). IR (neat) 3329, 1705, 1621, 1585, 1515, 1420, 1354 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 8.82 (*br*, 2 H), 8.63 (*s*, 2 H), 7.34 (*d*, *J* = 9.0 Hz, 2 H), 6.55 (*dd*, *J* = 9.0, 2.1 Hz, 2 H), 6.41 (*d*, *J* = 2.1 Hz, 2 H), 4.05 (*br*, 2 H), 3.39 (*q*, *J* = 7.0 Hz, 8 H), 2.15 (*br*, 2 H), 1.74 (*br*, 2 H), 1.42 (*br*, 4 H), 1.18 (*t*, *J* = 7.0 Hz, 12 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.9, 162.3, 157.5, 152.2, 147.9, 131.0, 110.8, 109.6, 108.4, 96.6, 52.7, 45.0, 32.3, 24.6, 12.4. FAB-HRMS for $C_{34}H_{41}N_4O_6$ (M^++1) calcd 601.3011, found 601.3026.

Preparation of Compound 4: To a solution of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol (11.6 mg, 0.1 mmol) and imidazolide **1** (79 mg, 0.254 mmol) in CH_3CN (5 mL) was added a 0.5 M solution of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.5 mL, 0.25 mmol) in CH_3CN at rt. The reaction mixture was stirred for 5 h. It was then concentrated and subjected to silica gel column chromatography. The desired product **4** was obtained (48 mg, 80%) by eluting with CH_2Cl_2/ethyl acetate (1/1). mp 96-98°C, R_f = 0.49 (CH_2Cl_2/ethyl acetate = 1/1). IR (neat) 1761, 1622, 1588, 1515, 1224, 1192 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.28 (*s*, 2 H), 7.35 (*d*, *J* = 9.0 Hz, 2 H), 6.53 (*dd*, *J* = 9.0, 2.4 Hz, 2 H), 6.32 (*d*, *J* = 2.4 Hz, 2 H), 5.12 (*t*, *J* = 4.3 Hz, 2 H), 3.36 (*q*, *J* = 7.1 Hz, 8 H), 2.22-2.12 (*m*, 2 H), 1.82-1.70 (*m*, 2 H), 1.60-1.48 (*m*, 2 H), 1.42-1.36 (*m*, 2 H), 1.15 (*t*, *J* = 7.1 Hz, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 158.2, 152.8, 148.8, 131.5, 109.5, 108.1, 107.5, 96.5, 74.2, 45.0, 30.3, 23.6, 12.3. FAB-HRMS for $C_{34}H_{39}N_2O_8$ (M^++1) calcd 603.2706, found 603.2690.

Preparation of Compound 5: To a solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (29 mg, 0.102 mmol) and imidazolide **1** (79 mg, 0.254 mmol) in CH_3CN (5 mL) was added a 0.5 M solution of DBU (0.5 mL, 0.25 mmol) in CH_3CN at rt. The reaction mixture was stirred for 12 h. It was then concentrated and subjected to silica gel column chromatography. The desired product **5** was obtained (61 mg, 78%) by eluting with hexane/ethyl acetate (2/3). mp 154-156°C, R_f = 0.18 (hexane/ethyl acetate = 2/3). IR (neat) 1764, 1622, 1589, 1516, 1224, 1194 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (*s*,

1 H), 8.25 (*s*, 1 H), 7.43 (*m*, 3 H), 7.28-7.18 (*m*, 4 H), 6.51 (*dd*, *J* = 9.0, 2.4 Hz, 1 H), 6.45 (*dd*, *J* = 9.0, 2.4 Hz, 1 H), 6.26 (*d*, *J* = 2.4 Hz, 1 H), 6.23 (*d*, *J* = 2.4 Hz, 1 H), 5.94 (*t*, *J* = 9.7 Hz, 1 H), 5.57 (*s*, 1 H), 5.18 (*d*, *J* = 3.6 Hz, 1 H), 5.05 (*dd*, *J* = 9.7, 3.6 Hz, 1 H), 4.30 (*dd*, *J* = 10.2, 4.6 Hz, 1 H), 3.98 (*dd*, *J* = 10.2, 4.6 Hz, 1 H), 3.90 (*t*, *J* = 9.5 Hz, 1 H), 3.82 (*t*, *J* = 10.2 Hz, 1 H), 3.38 (*s*, 3 H), 3.36-3.20 (*m*, 8 H), 1.16-1.00 (*m*, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4 (2x), 158.4 (3x), 157.9, 153.3, 153.0, 150.2, 148.9, 137.0, 132.3, 131.3, 129.0, 128.2, 126.3, 109.8, 109.6, 107.7, 107.4 (2x), 106.3, 101.7, 97.9, 96.4, 96.3, 79.0, 72.7, 69.2, 68.9, 62.6, 55.4, 45.1 (2x), 12.4 (2x). FAB-HRMS for $C_{42}H_{45}N_2O_{12}$ (M^++1) calcd 769.2973, found 769.2927.

Preparation of Compound 6: Ponasterone A (3.0 mg, 6.4 μmol) was first dissolved in 2 drops of pyridine, then added imidazolide **1**.(10 mg) in CH_3CN (5 mL). After 0.5 M DBU solution (0.32 mL) was injected, the reaction mixture was stirred at rt for 1.5 h. It was then concentrated and purified by silica gel column chromatography eluted with CH_2Cl_2/MeOH (19/1). It gives compound **6** as the major product (2.5 mg, 58%). R_f = 0.20 (CH_2Cl_2/MeOH = 19/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.43 (*s*, 1 H), 8.40 (*s*, 1 H), 7.47 (*d*, *J* = 9.0 Hz, 1 H), 7.41 (*d*, *J* = 9.0 Hz, 1 H), 6.61 (*dd*, *J* = 9.0, 2.3 Hz, 1 H), 6.56 (*dd*, *J* = 9.0, 2.3 Hz, 1 H), 6.45 (*d*, *J* = 2.3 Hz, 1 H), 6.38 (*d*, *J* = 2.3 Hz, 1 H), 5.90 (*d*, *J* = 2.0 Hz, 1 H), 5.68 (*s*, 1 H), 5.36 (*m*, 1 H), 3.47-3.37 (*m*, 9 H), 3.26 (*t*, *J* = 8.0 Hz, 1 H), 2.58 (*dd*, *J* = 12.6, 3.8 Hz, 1 H), 2.35 (*m*, 1 H), 2.18-1.41 (*m*, 19 H), 1.08 (*s*, 12 H), 0.92-0.86 (*m*, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 163.2, 162.7, 158.5, 158.4, 158.2, 153.0, 152.9, 150.0, 148.8, 132.0, 131.3, 121.6, 109.6, 109.0, 108.0, 107.8, 107.7, 96.8, 96.5, 84.7, 69.6, 68.1, 51.3, 49.0, 47.4, 45.1 (2x), 38.7, 36.4, 34.4, 33.7, 31.9, 31.0, 29.4, 28.0, 23.8, 23.1, 22.3, 20.8, 20.4, 20.3, 17.5, 12.4 (2x). FAB-HRMS for $C_{55}H_{71}N_2O_{13}$ (M^++1) calcd 951.5007, found 951.4977. A minor product was also observed during the reaction, which has R_f = 0.28 (CH_2Cl_2/MeOH = 19/1) and was considered as 2,3,22-trichromophoric derivatives. But we didn't secure enough material for its characterization.