ORGANIC LETTERS

2000
Vol. 2, No. 5
683−685

A Novel Coumarin-Type Derivatizing Reagent of Alcohols: Application in the CD Exciton Chirality Method for Microscale Structural Determination[†]

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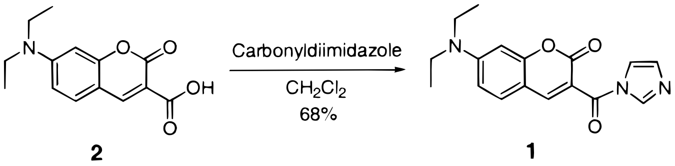
Received January 6, 2000

ABSTRACT



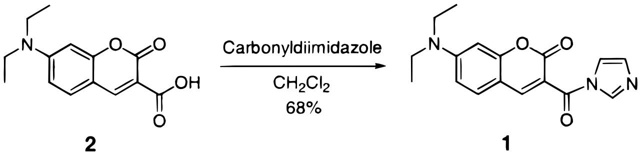
Imidazolide 1 has been prepared from 7-diethylaminocoumarin-3-carboxylic acid (2) and 1,1′-carbonyldiimidazole. This novel fluorescent derivatization reagent can be used in CD exciton chirality method for microscale structural determinations of hydroxyl-containing compounds. It features a red-shifted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

Many studies in the development of red-shifted chromophores (λ_{max} 360−480 nm) for circular dichroic (CD) exciton chirality method have been carried out in the past few years.[1−5] Red-shifted chromophores have the advantage of high sensitivity (ϵ values > 30 000)[6] and give distinct CD couplets in the region where no intrinsic chromophores in the parent compounds will interfere. Chromophores could be incorporated into 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 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 on a microscale.

Crystalline imidazolide **1** was simply prepared by stirring its free acid **2** with 1,1′-carbonyldiimidazole in a 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

Scheme 1



[†] This paper is dedicated to Professor Koji Nakanishi on the occasion of his 75th birthday.

(1) Cai, G.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1993**, *115*, 7192.

(2) Gargiulo, D.; Cai, G.; Ikemoto, N.; Bozhkova, N.; Odingo, J.; Berova, N.; Nakanishi, K. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 888.

(3) Gargiulo, D.; Ikemoto, N.; Odingo, J.; Bozhkova, N.; Iwashita, T.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1994**, *116*, 3760.

(4) Matile, S.; Berova, N.; Nakanishi, K.; Fleischhauer, J.; Woody, R. *J. Am. Chem. Soc.* **1996**, *118*, 5198.

(5) Ikemoto, N.; Estevez, I.; Nakanishi, K.; Berova, N. *Heterocycles* **1997**, *46*, 489−501.

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

10.1021/ol005514h CCC: $19.00

determinations. Coumarin derivative **2** has a skeleton resembling that of *p*-dimethylaminocinnamate.[10] However, 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 the 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 ($\Delta\epsilon$ −134) and 392 nm ($\Delta\epsilon$ +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 (Figure 1).

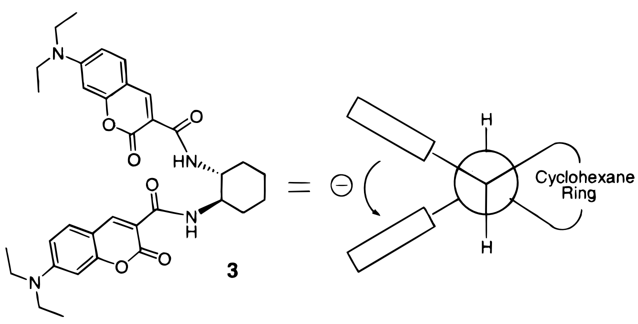


Figure 1. The structure of bischromophoric derivative **3** and its Newman projection showing negative handedness.

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 of stirring in CH_3CN solution), making this derivatization procedure well-suited for microscale reactions.[13] Compound **4** exemplifies the derivative from a simple chiral diol on a six-membered ring, compound **5** from a 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.

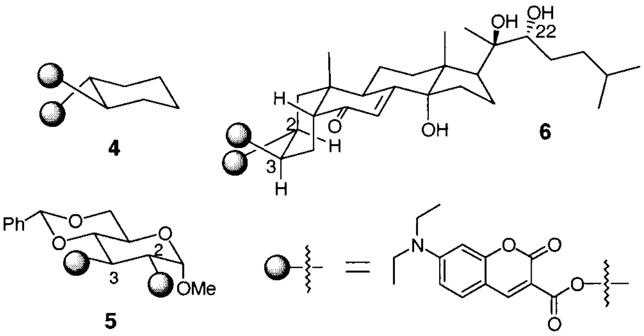


Figure 2. Structures of the bischromophoric derivatives **4**−**6**.

Table 1. UV/Vis, Fluorescence, and CD Data of Compounds **3**−**6** in Acetonitrile; Wavelengths in Nanometers (nm)

compd	UV/vis: λ_{max} (ϵ)	emission: λ_{max}	CD: λ_{ext} ($\Delta\epsilon$)	*A*
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 (−72), 394 (+38)	−110

All four bischromophoric derivatives (**3**−**6**) display fluorescence at λ_{em} 462−463 nm (λ_{ex} 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**. The effects of solvents (MeOH, MeCN, and CH_2Cl_2) on the UV/vis and CD spectra of compounds **3** and **4** were also studied. The results show only minor solvent dependence; wavelength shifts within 10 nm and *A* values within 15%. 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 the *p*-bromobenzoate (Glc***BBAA***, +69)[15] and *p*-methoxycinnamate (Glc***CCAA***, +95)[15] chromophores used in carbohydrate studies.[16]

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(7) Gargiulo, D.; Derguini, F.; Berova, N.; Nakanishi, K.; Harada, N. *J. Am. Chem. Soc.* **1991**, *113*, 7046.

(8) Berova, N.; Gargiulo, D.; Derguini, F.; Nakanishi, K.; Harada, N. *J. Am. Chem. Soc.* **1993**, *115*, 4769.

(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 rt for 14 h and 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, 12.4; FAB-HRMS for $C_{17}H_{18}O_3N_3$ ($M^+ + 1$) calcd 312.1348, found 312.1319.

(10) Verdine, G. L.; Nakanishi, K. *Chem. Commun.* **1985**, 1093.

(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 the carbodiimide coupling method (EDC/HOBt): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.82 (bs, 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 (bs, 2 H), 3.39 (q, $J = 7.0$ Hz, 8 H), 2.15 (bs, 2 H), 1.74 (bs, 2 H), 1.42 (bs, 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 a 0.5 M DBU/CH_3CN solution. After being stirred at rt for 5 h, the mixture was concentrated and the desired product was purified by silica gel column chromatography.

(14) Nakanishi, K.; Koreeda, M.; Sasaki, S.; Chang, M. L.; Hsu, H. Y. *Chem. Commun.* **1966**, 915.

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** as the major product. Acylation at the C-22 hydroxyl group is impeded due to steric hindrance. 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. A similar result was also observed when ouabain was acylated with naphthoylimidazole, leaving the sterically hindered C-11 OH of ouabain untouched.[17] The Cotton effects of the enone group in ponasterone A are weak (λ_{ext} 327 nm, $\Delta\epsilon = +1.8$; λ_{ext} 248 nm, $\Delta\epsilon = -3.9$)[18] and far away from those of the bischromophoric derivative **6** (Figure 3). The *A* value of compound **6** ($A = -110$) is three times higher than that from the benzoate chromophore originally used for its structural study.[18]

In summary, imidazolide **1** represents a convenient derivatizing reagent with desirable properties for hydroxyl groups. Its λ_{max} of 406 nm makes it one of the most red-shifted chromophores synthesized and utilized in CD studies to date. It can expand the scope of the reagents used for hydroxyl groups, such as naphthoylimidazole,[19] which has its absorption maximum in the UV region (λ_{max} 234 nm). Since this coumarin fluorophore (ethyl ester of compound **2**) displays a high quantum yield in ethyl acetate ($\Phi = 0.73$) relative to that of the standard 7-diethylamino-4-methylcoumarin, its application in fluorescence detection circular dichroism (FDCD)[20] is currently underway.

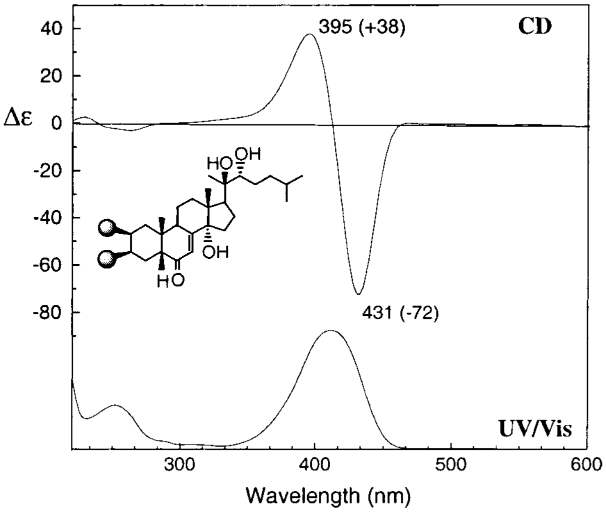

Figure 3. The CD and UV/vis curves of bischromophoric ponasterone A (**6**) in acetonitrile.

Acknowledgment. This work was supported by the National Science Council, Taiwan (NSC-89-2113-M-002-017 to L.-C.L. and NSC-88-2113-M-002-018 to C.-T.C.). We also thank Professor Jim-Min Fang for helpful discussions.

Supporting Information Available: Experimental procedures and characterization for compounds **3**−**6** and a microscale reaction using 50 μg of (1*R*,2*R*)-*trans*-1,2-cyclohexanediol together with its HPLC profile. This material is available free of charge via the Internet at http://pubs.acs.org.

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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).

(16) Wiesler, W. T.; Berova, N.; Ojika, M.; Meyers, H. V.; Chang, M.; Zhou, P.; Lo, L.-C.; Niwa, M.; Takeda, R.; Nakanishi, K. *Helv. Chim. Acta* **1990**, *73*, 509.

(17) Zhao, N.; Lo, L.-C.; Berova, N.; Nakanishi, K.; Tymiak, A. A.; Ludens, J. H.; Haupert, G. T., Jr. *Biochemistry* **1995**, *34*, 9893.

(18) Harada, N.; Nakanishi, K. *J. Am. Chem. Soc.* **1969**, *91*, 3989.

(19) Ikemoto, N.; Lo, L.-C.; Nakanishi, K. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 890.

(20) Dong, J.-G.; Wada, A.; Takakuwa, T.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc.* **1997**, *119*, 12024.