JOC*Note*

Design of a Coumarin-Based Triketone as a Fluorescent Protecting Group for Primary Amines

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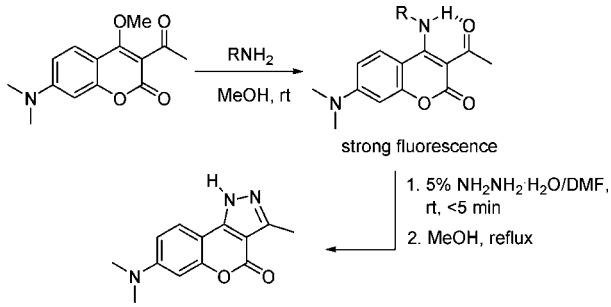

A series of 3-acetyl-4-hydroxycoumarin and its derivatives were prepared and evaluated for their potential to function as a fluorescent primary amine protection group. When primary amines or amino acids react with the protecting group 3-acetyl-4-methoxy-7-*N*,*N*-dimethylaminocoumarin, the resulting compounds emit blue fluorescence with a quantum yield of 0.25−0.50 in methylene chloride. These protected compounds display satisfactory acid/base stability, and the protecting group can be removed with 5% hydrazine hydrate in DMF within 5 min at ambient temperature.

Protecting groups are essential tools for the construction of complex organic molecules. The usefulness of a protecting group relies on its ease of synthesis and introduction, its stability to different reaction conditions, and its efficient cleavage under mild conditions. Among the various reported primary amine protection groups, the 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde, a triketone derivative)[1] has gained considerable attention. It is relatively stable in trifluoroacetic acid (TFA) and piperidine and can easily be removed from the amine with 2% hydrazine in DMF.

Thus, Dde has become a useful tool for the construction of branched, cyclic, side-chain modified peptides and polyamines by Fmoc/*t*-Bu solid-phase synthesis.[2] However, the Dde protection group itself lacks emission, which hampers in situ monitoring of the amine protection/deprotection progress by fluorescence spectroscopy. Recently, we have reported some unique properties of coumarin-based triketones.[3] These molecules have the potential to serve as a fluorescent amine protecting group, since coumarins have been widely used as fluorescent dyes.[4] In this paper, we report our efforts in design, synthesis, and evaluation of coumarin-based triketones as selective fluorescent protecting groups for primary amines.

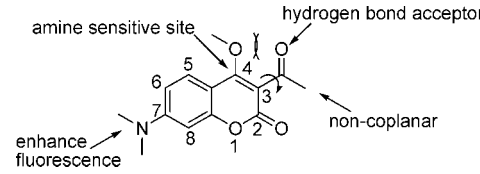

FIGURE 1. Structure of the designed potential fluorescent protecting group for primary amines.

Figure 1 shows our original design of the fluorescent protection group. The introduction of a methyl group at 4-hydroxycoumarin aims to make the resulting coumarin moiety sensitive to amine.[5] The oxygen atom at the 3-carbonyl group serves as a hydrogen bond acceptor to stabilize the protected amine. The incorporation of a *N*,*N*-dimethylamino group at the 7-position of coumarin enhances its fluorescence emission after protection. The relative stability of the protected amines in acidic and basic conditions and the ease of deprotection by hydrazine hydrate were also explored.

Scheme 1 depicts the synthesis of the potential coumarin-based primary amine protection compounds **2** and **3**. It began with the acetylation of the readily available 7-*N*,*N*-dimethylamino-4-hydroxycoumarin (**1**),[3] followed by the cyanide-catalyzed isomerization[6] of the enol ester using triethylamine as a base in methylene chloride to give the triketone **2**. Methylation of **2** with diazomethane generated from Diazald in diethyl ether under room temperature afforded the target compound **3** in a 72% yield. Condensation of **2** and **3** with different amino acid hydrochlorides (Val-OH, Leu-OH, Ile-OH, Phe-OH, Tyr-OH and Cys-OH) in the presence of triethylamine as a base in methanol gave the amine-protected derivatives **4a**−**d** and **5a**−**g**, respectively. Generally, the condensation reaction finished in 24 h at room temperature with the yield ranging

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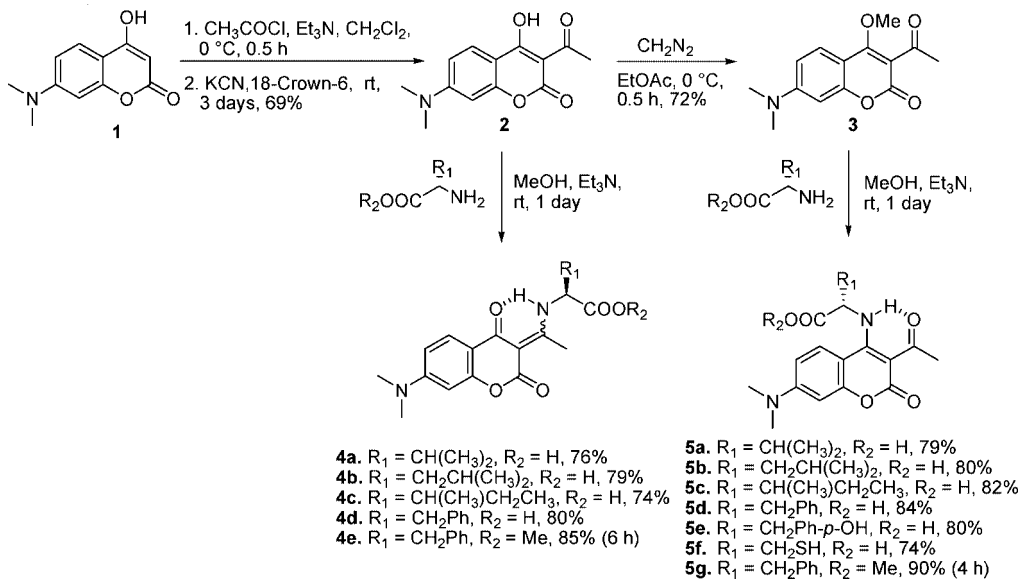
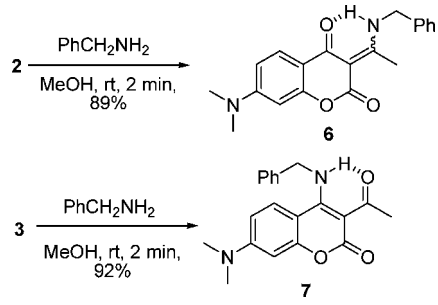
SCHEME 1



SCHEME 2



from 74 to 90%. However, when an amino acid ester hydrochloride (for example, Phe-OMe) was used, the reaction went much faster and the product (**4e** or **5g**) precipitated out from the solution in 4−6 h. Furthermore, this protecting reaction can be carried out smoothly without the presence of any base if the free amine is used instead of the corresponding hydrochloride salt. For instance, the condensation of **2** and **3** with benzyl amine in MeOH gave **6** and **7** within 10 min in 89 and 92% yield, respectively (Scheme 2).

The unsymmetrical nature of the triketone **2** predisposed the resulting protected compounds to undergo a geometrical isomerism. The *Z*:*E* ratios of **4a**−**e** were determined from their ^{1}H NMR spectra and are shown in Table 1. It has been documented[7] that NH protons in *Z*-isomers of **4a**−**e** have higher δ values than *E*-isomers, due to the stronger intramolecular hydrogen bonding of the ester oxygen atom (*Z*-form) than the carbonyl oxygen atom (*E*-form). The X-ray crystal structure of **5g**[8] unambiguously confirmed that the amine nitrogen attached to the expected C-4 position of coumarin moiety. The detection of a significantly downfield signal of 4-hydroxyl hydrogen absorption peak between 16.7−17.8 ppm on ^{1}H NMR spectra of triketone **2** clearly indicates that the C-3 carbonyl moiety of **2** is coplanar and conjugated with the coumarin ring system by an intramolecular hydrogen bond of the C-4 hydroxyl hydrogen to the oxygen atom of C-3 carbonyl group. For compound **3**, conversely, the dipolar repulsions between the C-3 carbonyl group and the other two oxygen atoms on the coumarin ring system caused deformation of **3** from planarity and resulted in its high susceptibility to a nucleophilic 1,4-addition and subsequent elimination reaction.[5] The preference of **3** to form stable derivatives with primary amines is attributed to, as with Dde, the strong intramolecular hydrogen bond in the resultant protected derivatives.

While no emission was observed for the synthesized series **4a**−**e** in methylene chloride, compounds **5a**-**g** exhibited blue fluorescence under the same conditions. Table 2 lists the fluorescence parameters of **3** and **5a**−**g** in methylene chloride with the quantum yields between 0.25 and 0.50. Derivatives **4a**−**e** and **5a**−**g** were then subjected to 50% TFA in CH_2Cl_2 and 20% piperidine in DMF at room temperature to investigate their relative stability under both acidic and basic conditions. The results are listed in Table 1. Most of the compounds showed good acid stability, but some decomposed in 20% piperidine in DMF within 1 min. Among the compounds tested, compounds **5a**−**g** all displayed excellent acid/base stability. No sign of decomposition was detected after exposure to trifluoroacetic acid and piperidine for more than 37 h. The reasons that **5a**−**g** are more stable than the corresponding **4a**−**e** in both acidic and basic conditions are currently unclear.

Since it is essential to demonstrate that the protecting group can be removed smoothly from amino acids before applying in synthetic procedures or solid state peptide synthesis, compounds **5a**−**e** were treated with 5% hydrazine hydrate in DMF at ambient temperature. Amino acids/esters were released quantitatively within 5 min with concomitant formation of hydrazine derivative **8** (Scheme 3). Subsequent heating of **8** in methanol formed the cyclized pyrazole derivative **9**, whose structure was confirmed by single-crystal X-ray diffraction analysis[8] (see the Supporting Information). Finally, the absence of detectable racemization at the α-carbon of the protected amino esters during the protection and deprotection steps was established by

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(8) Crystallographic data (excluding structure factors) for compounds **5g** and **9** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-665112 and -665113, respectively. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

TABLE 1. Relative Stability of 4a−e, 5a−g, 6, and 7 in Acidic and Basic Conditions

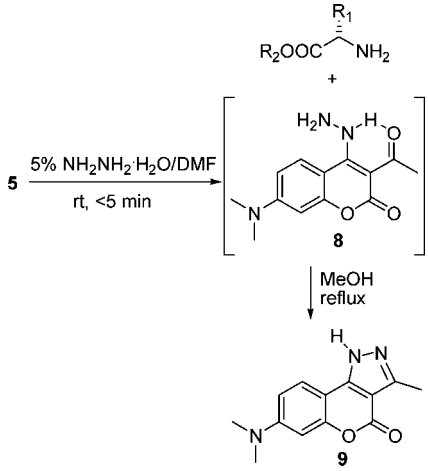
entry	compd	R_1	R_2	*Z*:*E*	time (h)[a] 50%TFA/CH_2Cl_2	time (h)[a] 20% piperidine/ DMF
1	**4a**	$CH(CH_3)_2$	H	1:37	17	1 min
2	**4b**	$CH_2CH(CH_3)_2$	H	1:23	19	1 min
3	**4c**	$CH(CH_3)CH_2CH_3$	H	1:4	20	1 min
4	**4d**	CH_2Ph	H	1:16	26	1 min
5	**4e**	CH_2Ph	Me	1:9	14	1 min
6	**5a**	$CH(CH_3)_2$	H		>37	>37
7	**5b**	$CH_2CH(CH_3)_2$	H		>37	>37
8	**5c**	$CH(CH_3)CH_2CH_3$	H		>37	>37
9	**5d**	CH_2Ph	H		>37	>37
10	**5e**	CH_2Ph-*p*-OH	H		>37	>37
11	**5f**	CH_2SH	H		>37	>37
12	**5g**	CH_2Ph	Me		35	30
13	**6**			1:36	14	3 min
14	**7**				30	>37

[a] Time of new spots detected by TLC analysis.

TABLE 2. Fluorescence Parameters for 3 and 5a−g in CH_2Cl_2

compd	λ_{ex} (nm)	λ_{em} (nm)	Stokes shift (cm^{-1})	quantum yield (%)
3	373	419	2943	0.18
5a	371	446	4533	0.40
5b	372	446	4460	0.27
5c	371	445	4482	0.31
5d	374	450	4516	0.26
5e	368	415	3078	0.50
5f	372	424	3297	0.25
5g	373	449	4538	0.43

SCHEME 3



specific rotation comparisons of the amino esters released from hydrazine deprotection with the authentic compounds.

In summary, we have developed a fluorescent coumarin-based primary amine protection group from readily available 7-*N*,*N*-dimethylamino-4-hydroxycoumarin. The protected amines emit strong blue fluorescence in methylene chloride and are resistant to a range of acidic and basic conditions. The amines can be released quantitatively with 5% hydrazine hydrate in DMF within 5 min.

Experimental Section

Preparation of 3-Acetyl-7-*N*,*N*-dimethylamino-4-methoxychromen-2-one (3). To a stirred mixture of **2** (1 g, 4.04 mmol) in EtOAc (10 mL) was added an ethereal solution of diazomethane at 0 °C for 0.5 h. After completion of the reaction, the solution was evaporated and the residue was purified by column chromatography (1:6 EtOAc/hexanes) to give a yellow solid in a 72% yield: mp 134−135 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (d, J = 9.0 Hz, 1H), 6.60 (dd, J = 9.0, 2.4 Hz, 1H), 6.42 (d, J = 2.4 Hz, 1H), 3.95 (s, 3H), 3.07 (s, 6H), 2.67 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 199.5, 166.8, 161.9, 154.6, 153.5, 125.1, 108.8, 105.1, 104.5, 96.4, 61.6, 39.6, 31.9; IR ν (KBr) 1711, 1578, 1348, 821, 764 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{14}H_{15}NO_4$ 261.1001, found 261.1007 (M^+).

General Procedure for Preparation of Compounds 4a−d. To a solution of **2** (100 mg, 0.40 mmol) in methanol (5 mL) were added amino acid (0.40 mmol) and triethylamine (41 mg, 0.40 mmol) at room temperature for 1 day. After completion of the reaction, the solvent was concentrated in vacuo. This mixture was poured into water, 2 N NaOH was added to the aqueous solution until pH ∼12, and the aqueous solution was washed with methylene chloride. A HCl solution (6 N) was added to the aqueous solution until pH ∼2, and the aqueous solution was extracted with methylene chloride. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the pure product.

2- (1-[7-*N*,*N*-Dimethylamino-2,4-dioxochroman-(3*E*)-ylidene-]ethylamino)-3-methyl butyric acid (4a): yellow solid; yield 76%; mp 157−158 °C; $[\alpha]^{28}_D$ = 32.26 (*c* 0.006, DMSO). Only the data of the major isomer (*E* form) is given: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 14.63 (s, 1H), 7.92 (d, J = 9.0 Hz, 1H), 7.06 (bs, 1H), 6.57 (dd, J = 9.0, 2.4 Hz, 1H), 6.30 (d, J = 2.4 Hz, 1H), 4.40 (dd, J = 8.1, 4.8 Hz, 1H), 3.04 (s, 6H), 2.67 (s, 3H), 2.50−2.40 (m, 1H), 1.16 (d, J = 6.9 Hz, 3H), 1.11 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 176.1, 171.4, 155.2, 154.6, 127.1, 126.5, 110.0, 108.8, 96.4, 95.6, 61.8, 40.0, 31.1, 29.6, 19.0, 18.8, 17.7; IR ν (KBr) 1754, 1669, 1602, 1476, 1193, 777, 698 cm^{-1}; HRMS (EI) *m*/*z* calcd for $C_{18}H_{22}N_2O_5$ 346.1529, found 346.1525 (M^+).

General Procedure for Preparation of Compounds 5a−f. To a solution of **3** (100 mg, 0.38 mmol) in methanol (5 mL) were added amino acid (0.38 mmol) and triethylamine (39 mg, 0.38 mmol) at room temperature for 1 day. This mixture was poured into water, 2 N NaOH was added to the aqueous solution until pH ∼12, and the aqueous solution was washed with with methylene chloride. HCl (6 N) was added o the aqueous solution until pH ∼2, and the aqueous solution was extracted with methylene chloride. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the pure product.

2-(3-Acetyl-7-*N*,*N*-dimethylamino-2-oxo-2*H*-chromen-4-ylamino)-3-methylbutyric acid (5a): yellow solid; yield 79%; mp 178−180 °C; $[\alpha]^{29}_D$ = −76.92 (*c* 0.004, DMSO); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.83 (d, J = 8.7 Hz, 1H), 7.58 (d, J = 9.6 Hz, 1H), 6.50 (dd, J = 9.6, 2.4 Hz, 1H), 6.32 (d, J = 2.4 Hz, 1H), 5.22 (bs, 1H), 4.59 (dd, J = 8.1, 5.4 Hz, 1H), 3.03 (s, 6H), 2.67 (s, 3H), 2.46−2.38 (m, 1H), 1.14 (d, J = 6.9 Hz, 1H), 1.04 (d, J = 6.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.4, 173.6, 162.7, 161.8, 156.2, 153.8, 128.0, 108.4, 101.4, 97.9, 95.8, 65.7, 39.8,

33.0, 32.9, 19.1, 17.8; IR ν (KBr) 1672, 1595, 1471, 1391, 823 cm^{-1}; HRMS (EI) *m/z* calcd for $C_{18}H_{22}N_2O_5$ 346.1529, found 346.1530 (M^+).

Acknowledgment. The financial assistance provided by National Science Council of Republic of China is thankfully acknowledged.

Supporting Information Available: Synthesis of compounds **3**, **4a**−**e**, **5a**−**g**, **6**, **7**, and **9**, experimental details, and additional spectra. X-ray structure details for compounds **5g** and **9** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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