Chiral *N*-(Coumarin-3-ylcarbonyl)-α-amino Acids: Fluorescent Markers for Amino Acids and Dipeptides

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Abstract: *N*-(Coumarin-3-ylcarbonyl)benzotriazole has been coupled with free amino acids, N-terminal-protected lysines and dipeptides to afford fluorescent amino acids and dipeptides (76–89% yield) with retention of original chirality. N^{α}- and N^{ε}-Coumarin-labeled lysines are obtained by simple, two-step procedures. N^{α}-Cbz-N^{ε}-(Coumarin-3-ylcarbonyl)-L-lysine is demonstrated to be an optically active fluorescent marker for labeling amino acids in solution-phase syntheses.

Key words: amino acids, peptides, coupling, coumarin fluorophore, benzotriazole methodology

The imaging of living cells is a big challenge in cell biology. Understanding the nature and dynamics of cellular events in living organisms is assisted by imaging cell components such as the nucleic acids, proteins and metabolites. Fluorescent markers are the visualization tools most commonly used, particularly fluorescent tagging with organic fluorophores[1] or green fluorescent protein (GFP).[2] Highly sensitive fluorescence derivatization techniques[3] are gaining an increasing share of the analytical world market, becoming competitive with, for example, radioimmunoassay.[4]

Radioactive labeling of peptides usually involves the introduction of a radiolabeled amino acid as part of the natural structure of the peptide. In contrast, fluorescent tags are introduced as an additional moiety to the molecule, by a variety of strategies.[5–10] Derivatives of rhodamine, fluorescein[11,12] and coumarin[13,14] are widely used as fluorescent markers for peptides and other biomolecules. Xanthenes such as rhodamines, fluoresceins and Alexa dyes are advantageously bright; however, drawbacks of existing derivatives in reporting protein conformational changes include (i) relatively long, flexible linkers between the probe and protein, which questions whether the probe motions faithfully mirror the motions of the residue to which it is attached, and (ii) multiple charges and relatively large surface areas, which can perturb local structure or motion, and inhibit labeling at certain positions.[7,13]

Coumarins (benzopyran-2-ones), the largest class of laser dyes for the blue-green region,[14–22] are highly sensitive. They have provided the most commercially acceptable categories of fluorescent derivatives with the advantages of an extended spectral range, high emission quantum yield, photostability and good solubility in many solvents.

The many bioanalytical applications of fluorescently labeled proteins include *in vitro* and *in vivo* imaging,[6,23] high-throughput screening,[24] proteomics,[24–26] diagnostics,[9,27,28] single biomolecule spectroscopy,[26,29] HPLC control[30] and exploring protein conformations.[7]

Probes for studying protein dynamics and electrostatics should be (i) sensitive to the state of hydrogen bonding in the solvent environment[31] and (ii) readily incorporated regiospecifically at the C- or the N-terminus of a protein. The use of an amino acid as a fluorescent chromophore offers a good possibility to synthesize fluorescent peptides by solid-phase peptide synthesis (SPPS).

Previously, enantioselective syntheses of coumarin-containing amino acids have been carried out by the diastereoselective alkylation of chiral glycine equivalents.[13,20,32] For example, the chiral auxiliaries of Oppolzer and co-workers and of Williams were used for the synthesis of L-(6,7-dimethoxycoumarin-4-yl)alanine (**1**) and L-(7-methoxycoumarin-4-yl)alanine, respectively. These methods produced optically pure amino acids; however, they required multiple steps (Scheme 1) and the products were obtained in poor overall yields. Recently, a synthesis of coumarin-containing amino acids was reported[33] utilizing protected aspartic and glutamic acids as chiral starting materials, which led to coumarinylalanines and coumarinylethylglycines in a two- or a three-step process.

Coumarin-labeled lysines are of considerable interest for the design and synthesis of fluorogenic triple-helical substrates for the analysis of matrix metalloproteinase family members. However, the attempted preparation of N^{α}-Fmoc-N^{ε}-[(7-methoxycoumarin-4-yl)acetyl]lysine (**10**),[18] by reaction of commercially available N^{α}-Fmoc-lysine in *N*,*N*-dimethylformamide with the preformed *N*-hydroxybenzotriazole ester of 7-methoxycoumarin-4-acetic acid, gave problems in (i) separation of **10** from unreacted fluorophore, (ii) side reactions and (iii) crystallization of the product. An alternative four-step synthetic approach (Scheme 2) using a copper-complexed intermediate[18] gave **10** in only 17% overall yield.

Berthelot and co-workers[19] labeled N^{α}-Fmoc-lysine (**11**) in three steps using the active coumarincarboxylic acid *N*-hydroxysuccinimide esters **13** (Scheme 3); the target compounds **14a** and **14b** were obtained in 64% and 50% yield, respectively.

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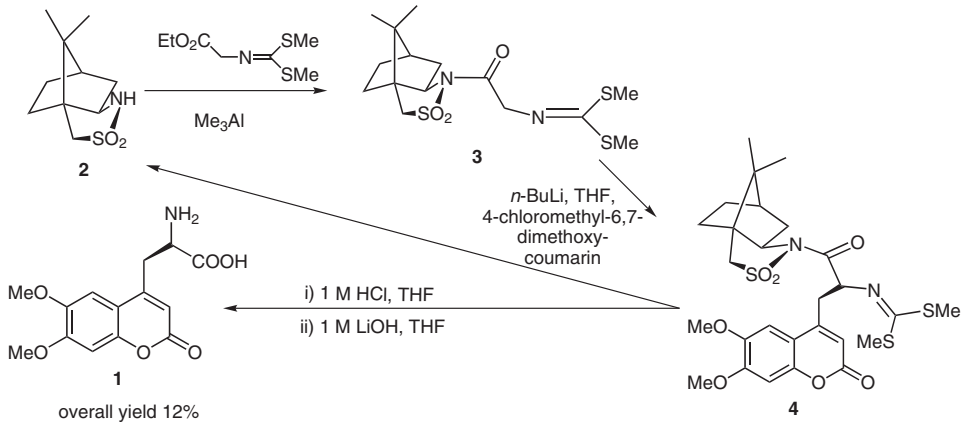
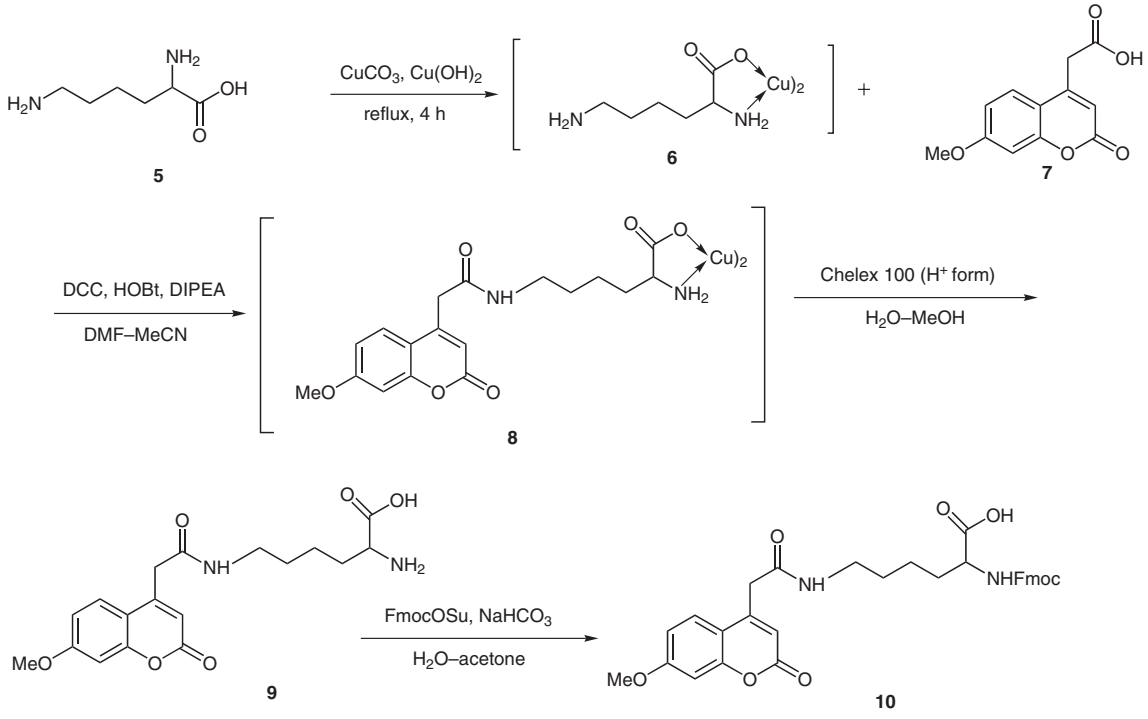
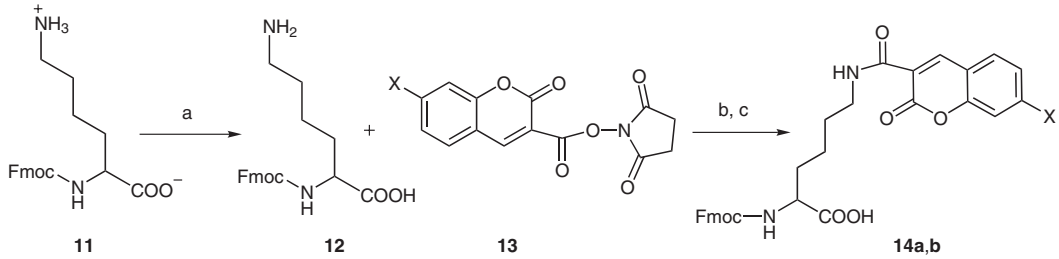


Scheme 1 Synthesis of L-(6,7-dimethoxycoumarin-4-yl)alanine (**1**)[13]



DCC = *N,N*-dicyclohexylcarbodiimide
DIPEA = *N,N*-diisopropylethylamine
FmocOSu = *N*-(fluoren-9-ylmethoxycarbonyloxy)succinimide
HOBt = *N*-hydroxybenzotriazole

Scheme 2 Synthesis of N^{α}-Fmoc-N^{ε}-[(7-methoxycoumarin-4-yl)acetyl]-L-lysine (**10**)[18]



14a, X = OMe (64%)
14b, X = NEt_2 (50%)

(a) DIPEA, DMF, 0 °C, 30 min
(b) r.t., 2 h
(c) 2 M HCl, 4 °C, 12 h

Scheme 3 Synthesis of coumarin-labeled lysines **14a** and **14b**[19]

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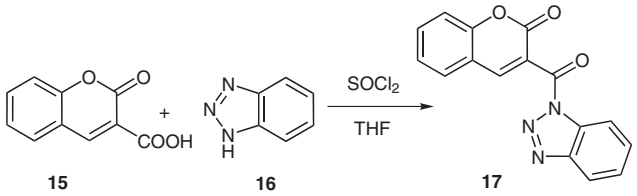
The quantitative analysis of peptides and proteins in complex biological matrixes is hindered by structural similarities between analyte, degradation products, impurities and endogenous components.[34] The development of derivatization strategies for the synthesis of suitable, optically pure fluorescent labeling probes remains a considerable challenge.

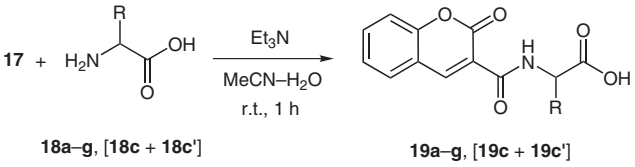
We now report straightforward syntheses of coumarin-labeled amino acids and dipeptides which afford enantiomerically pure fluorescent building blocks suitable for SPPS. Our simple, two-step synthetic route (i) converts coumarin-3-carboxylic acid (**15**) into its active benzotriazolide **17** and (ii) couples fluorophore with Cbz- and Fmoc-protected lysines and with the N-terminus of free amino acids, as well as with dipeptides, providing diverse optically pure fluorescent probes in 76–89% yield.

In the first step (Scheme 4), coumarin-3-carboxylic acid (**15**) was treated with 1*H*-benzotriazole (**16**) and thionyl chloride in tetrahydrofuran at 20 °C to give the corresponding 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-one (**17**) as a stable compound in 87% yield (all yields indicated in this work are calculated for pure products).



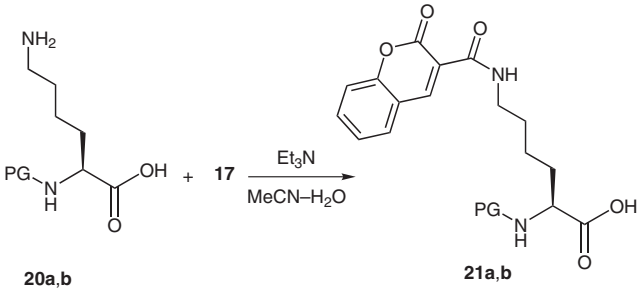
Scheme 4 Preparation of 3-(1*H*-benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-one (**17**)

Reaction of the coumarinoylbenzotriazole **17** with the amino group of peptides or proteins should allow the labeling of such biomolecules under the gentle conditions used in recent research in our laboratory.[35] Benzotriazole **17** was thus coupled with diverse L-amino acids **18a–f**, with the DL-amino acid [**18c + 18c′**][36] and with N^{ε}-Cbz-L-lysine (**18g**) in aqueous acetonitrile at 20°C (Scheme 5) to give the *N*-(coumarin-3-ylcarbonyl)-L-α-amino acids **19a–g** and racemate [**19c + 19c′**] in 76–89% yield (Table 1). Products **19a–g** retained the original chirality; each showed a single retention-time peak upon analytical HPLC. The racemate [**19c + 19c′**] displayed the expected two peaks.



Scheme 5 Synthesis of coumarin-labeled amino acids

N-Terminus-protected lysines are of great current interest[12,18,19] as versatile fluorescent building blocks for SPPS. In addition to N^{α}-coumarin-labeled N^{ε}-Cbz-L-lysine **19g**, with the free N^{ε}-amino group suitable for SPPS, we successfully synthesized the isomeric N^{ε}-coumarin-labeled N^{α}-Cbz-L-lysine **21a** (89% yield) and the Fmoc-protected analogue, N^{ε}-coumarin-labeled N^{α}-Fmoc-L-lysine **21b** (87% yield) (Scheme 6) utilizing the convenient two-step general procedure, as described above for the preparation of *N*-(coumarin-3-ylcarbonyl)-α-amino acids **19**. The enantiomeric purity of **21a** and of **21b** was demonstrated by NMR and HPLC analysis.



Scheme 6 Synthesis of coumarin-labeled lysines **21a** and **21b**

Table 1 Yields and HPLC Analysis of *N*-(Coumarin-3-ylcarbonyl)-α-amino Acids **19a–g** and Racemic Mixture [**19c + 19c′**]

Reactant **18**	R of coumarin-3-ylcarbonyl product **19**	Yield[a] (%)	t_R[b] (min)
L-Ala-OH (**18a**)	Me (**19a**)	82	3.96
L-Ser-OH (**18b**)	CH_2OH (**19b**)	76	4.33
L-Met-OH (**18c**)	$(CH_2)_2SMe$ (**19c**)	88	3.85
L-Phe-OH (**18d**)	CH_2Ph (**19d**)	88	4.03
L-Trp-OH (**18e**)	1*H*-indol-3-ylmethyl (**19e**)	78	4.04
L-Val-OH (**18f**)	*i*-Pr (**19f**)	81	3.64
N^{ε}-Cbz-L-Lys-OH (**18g**)	$(CH_2)_4NHCbz$ (**19g**)	77	3.93
DL-Met-OH ([**18c + 18c′**])	$(CH_2)_2SMe$ ([**19c + 19c′**])	89	3.69, 4.13

[a] Isolated yield.
[b] t_R = retention time. For conditions, see the experimental section.

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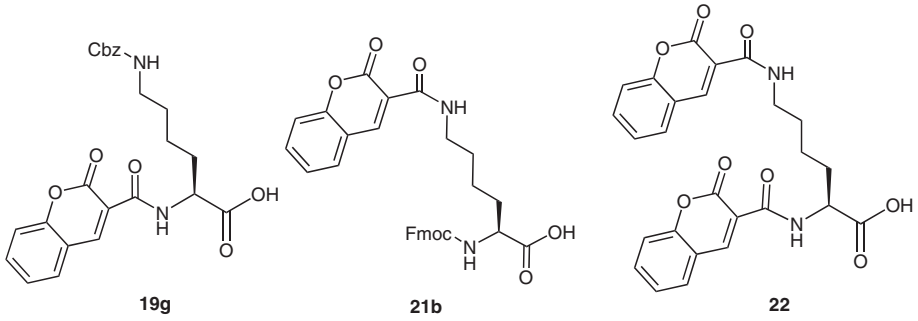


Figure 1

Coupling 2 equivalents of benzotriazole **17** with 1 equivalent of free lysine gave the bis(coumarin)-substituted lysine **22** (Figure 1) in 86% yield.

We synthesized dipeptides containing a coumarin fluorophore by two routes: (i) stepwise elongation of *N*-coumarinoyl-α-amino acids from the C-terminus and (ii) direct introduction of the coumarin-containing fluorophore into free dipeptides at the N-terminus.

In the stepwise elongation procedure [route (i)], the key step was the synthesis of the stable *N*-acylbenzotriazole intermediates **23a** and [**23b** + **23b′**] (Table 2). These were prepared from **19a** and [**19c** + **19c′**] by a procedure (Scheme 7) similar to that described in Scheme 4 for the formation of coumarinoylbenzotriazole **17**.

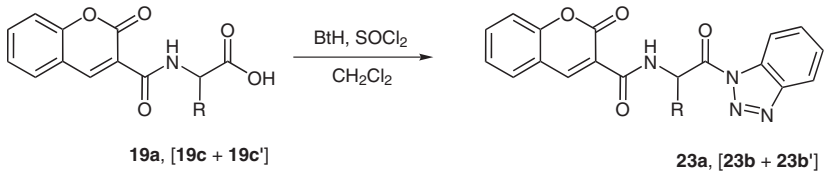
Subsequent coupling of *N*-(coumarin-3-ylcarbonyl-α-aminoacyl)benzotriazoles **23a** and [**23b** + **23b′**] with unprotected L-phenylalanine (**24**) in aqueous acetonitrile (Scheme 8) afforded enantiomerically pure **25a** in 74% yield and the diastereomeric mixture [**25b** + **25b′**] in 68% yield, respectively (Table 3). The ^{13}C NMR spectrum of [**25b** + **25b′**] showed duplication of almost all the aliphatic and carbonyl carbon signals. As anticipated, dipeptide **25a** showed no duplication of carbon signals and the ^{1}H NMR spectrum in DMSO-d_6 displayed a single prominent doublet at 1.30 ppm for the methyl group of the L-alanine fragment. These data, along with HPLC analysis, demonstrate the enantiomeric purity of **25a**.

N^{ε}-Coumarin-labeled N^{α}-Cbz-L-lysine **21a** proved to be a convenient starting material in the stepwise elongation route (i) for the preparation of fluorescent dipeptides in solution. In future work we plan to use coumarin-labeled lysines **21a** and **21b** as fluorescent building blocks for labeling peptides in SPPS. The benzotriazole derivative **26** (Figure 2) was synthesized from **21a** using a methodology similar to that described in Scheme 7 for the formation of derivative **23a**. Benzotriazole **26** is stable and able to perform efficient acylation of amino groups in aqueous acetonitrile at ambient temperature without side reactions. Coupling **26** with L-aspartic acid gave the multifunctional

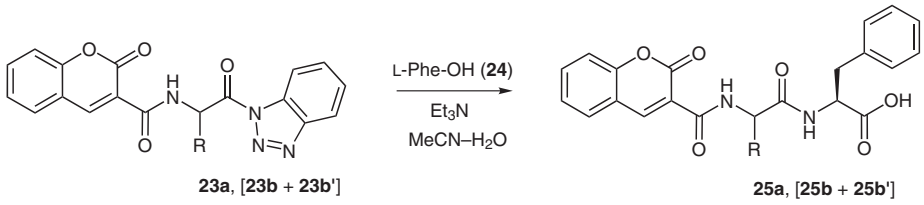
Table 2 Yields of *N*-(Coumarin-3-ylcarbonyl-α-aminoacyl)benzotriazoles **23a** and [**23b** + **23b′**]

R of coumarin-3-ylcarbonyl reactant **19**	Benzotriazole product **23**	Yield[a] (%)
Me (**19a**)	*N*-(coumarin-3-ylcarbonyl)-L-Ala-Bt (**23a**)	78
$(CH_2)_2SMe$ ([**19c** + **19c′**])	*N*-(coumarin-3-ylcarbonyl)-DL-Met-Bt ([**23b** + **23b′**])	74

[a] Isolated yield.



Scheme 7 Synthesis of the *N*-acylbenzotriazole intermediates **23a** and [**23b** + **23b′**]



Scheme 8 Synthesis of coumarin-labeled dipeptides by stepwise elongation

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Table 3 Yields and HPLC Analysis of Coumarin-Labeled Dipeptides **25a** and [**25b** + **25b′**]

Reactant **23**	Coumarin-labeled dipeptide product **25**	Yield[a] (%)	t_R[b] (min)
23a	*N*-(coumarin-3-ylcarbonyl)-L-Ala-L-Phe-OH (**25a**)	74	4.13
[**23b** + **23b′**]	*N*-(coumarin-3-ylcarbonyl)-DL-Met-L-Phe-OH ([**25b** + **25b′**])	68	5.67, 6.77

[a] Isolated yield.
[b] t_R = retention time. For conditions, see the experimental section.

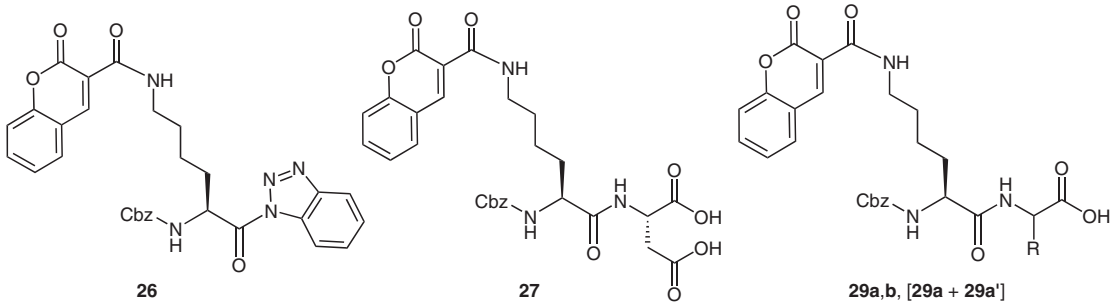

Figure 2

Table 4 Yields and HPLC Analysis of Coumarin-Labeled, Lysine-Containing Dipeptides **29a**,**b** and [**29a** + **29a′**] Derived from **26**

Amino acid reactant	Coumarin-labeled dipeptide product **29**	Yield[a] (%)	t_R[b] (min)
L-Ala-OH (**28a**)	N^{ε}-(coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-L-Ala-OH (**29a**)	91	5.57
L-Phe-OH (**28b**)	N^{ε}-(coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-L-Phe-OH (**29b**)	89	7.43
DL-Ala-OH ([**28a** + **28a′**])	N^{ε}-(coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-DL-Ala-OH ([**29a** + **29a′**])	94	5.51, 6.92

[a] Isolated yield.
[b] t_R = retention time. For conditions, see the experimental section.

fluorescent building block **27** in 85% yield; L- and DL-amino acids **28a**,**b**, [**28a** + **28a′**] gave targets **29a**,**b**, [**29a** + **29a′**] in 89–94% yield (Table 4).

All couplings proceeded with complete retention of chirality: comparison of the ^{1}H and ^{13}C NMR spectra of **29a** with those of [**29a** + **29a′**] demonstrated that **29a** is a single stereoisomer. Thus, the ^{1}H NMR spectrum of **29a** in DMSO-d_6 showed a doublet at 1.28 ppm (J = 7.3 Hz) characteristic of the methyl group of the L-alanine part of the molecule, while the corresponding signal for the diastereomeric mixture [**29a** + **29a′**] gave a multiplet at 1.21–1.31 ppm. The ^{13}C NMR spectrum showed a duplication of carbon peaks corresponding to the carbonyl groups and the aliphatic portion of the molecule for [**29a** + **29a′**], while single peaks were observed for each carbon of enantiomerically pure **29a**. The enantiopurity of dipeptides **27** and **29a**,**b** was confirmed by HPLC analysis using a chiral column: dipeptides **27**, **29a** and **29b** each gave a single retention-time peak (4.17 min for **27**), whereas the diastereomeric mixture [**29a** + **29a′**] showed two peaks (Table 4).

In route (ii), involving direct introduction of the coumarin-containing fluorophore, the free dipeptides Gly-Gly and Gly-L-Leu were directly labeled with benzotriazole **17** to give the coumarin-labeled dipeptides **30** and **31** (Figure 3) in 81 and 85% yield, respectively, by a procedure similar to that adopted for the preparation of *N*-(coumarin-3-ylcarbonyl)-α-amino acids **19** (Scheme 5). This direct approach [route (ii)] is preferred for the synthesis of fluorescent dipeptides compared to the stepwise elongation approach [route (i)] described above.

Absorption ($\lambda_{Abs.}$) and fluorescence ($\lambda_{Em.}$) wavelength maxima and extinction coefficients were measured for most of the labeled compounds (Figure 4, Table 5). The coumarin-labeled α-amino acids and dipeptides (except for **21b**) had higher emission wavelength values and high-

Figure 3

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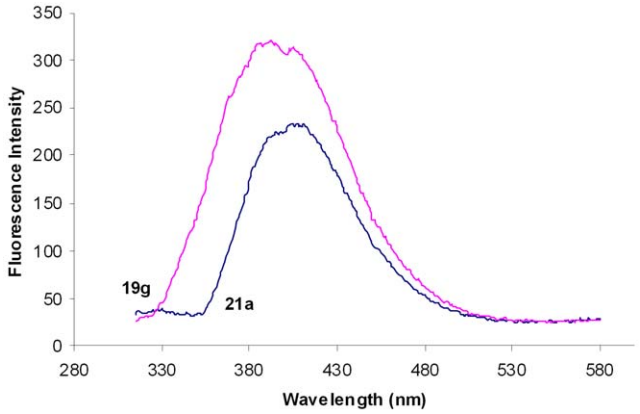

Figure 4 Fluorescence emission spectra of N^{α}-(coumarin-3-ylcarbonyl)-N^{ε}-Cbz-L-lysine (**19g**), $\lambda_{Ex.} = 295$ nm, and N^{α}-Cbz-N^{ε}-(coumarin-3-ylcarbonyl)-L-lysine (**21a**), $\lambda_{Ex.} = 293$ nm, in methanol

Table 5 Absorption and Fluorescence Data for Coumarin-Labeled α-Amino Acids and Dipeptides

Compound	$\lambda_{Abs.}$[a] (nm)	$\lambda_{Em.}$[a] (nm)	ε[b] ($cm^{-1}M^{-1}$)
19a	295	408	–[c]
19b	294	407	–
19c	294	409	–
19d	295	408	–
19e	290	–[d]	–
19f	295	408	–
19g	295	392	17600
21a	293	407	17900
21b	265	405	27500
22	293	409	–
25a	297	408	–
27	294	408	–
29a	294	409	–
29b	294	407	–
30	294	406	–
31	294	408	–

[a] Determined in HPLC-grade methanol.
[b] Extinction coefficient.
[c] Not determined.
[d] Not detected.

er absorption values than tryptophan ($\lambda_{Abs.} = 278$ nm, $\lambda_{Em.} = 352$ nm) or tyrosine ($\lambda_{Abs.} = 274$ nm, $\lambda_{Em.} = 303$ nm).

In conclusion, N^{α}- and N^{ε}-coumarin-labeled Cbz- and Fmoc-L-lysines **19g**, **21a** and **21b**, chiral fluorescent probes for amino acids and peptides, have been readily synthesized in high yields. The *N*-acylbenzotriazoles **17**, **23a**, [**23b** + **23b′**] and **26** are efficient acylation reagents for the fluorescent labeling of amino acids and dipeptides in solution phase because of their stability and ability to react with amino groups in aqueous acetonitrile at ambient temperature without side reactions. They also have the potential to be applied for labeling using solid-phase synthetic methods. The protocols which have been employed should be adaptable for the syntheses of many similar fluorescent markers.

Melting points were determined on a Mel-Temp II capillary point apparatus equipped with a digital thermometer. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on an Oxford 300 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as an internal reference. Free amino acids and *N*-Cbz- and *N*-Fmoc-amino acids were purchased from Fluka and Acros and were used without further purification. Elemental analyses were performed on a Carlo Erba-1106 instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter using the sodium D line. HPLC analyses were performed on a Beckman System Gold® programmable solvent module 126, using a Chirobiotic™ T chiral column (4.6 × 250 mm), detection at 254 nm, flow rate of 1.0 mL/min and MeOH as the eluting solvent. UV and fluorescence measurements were recorded on Cary 100 UV–Vis and FluoroMax spectrophotometers, respectively.

3-(1*H*-Benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-one (17)

Thionyl chloride (7.5 mmol) was added to a soln of 1*H*-benzotriazole (**16**; 25 mmol) in anhyd THF (30 mL) at r.t. and the reaction mixture was stirred for 20 min. Coumarin-3-carboxylic acid (**15**; 5 mmol) was added to the reaction mixture which was stirred for 4 h at 25 °C. The white precipitate which formed during the reaction was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with EtOAc (150 mL) and the solution was washed with sat. Na_2CO_3 soln (3 × 50 mL) and sat. NaCl soln (50 mL), and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave **17**, which was recrystallized from CH_2Cl_2–hexanes for elemental analysis.

Yield: 87%; white microcrystals; mp 186–187 °C.

^{1}H NMR ($CDCl_3$): δ = 7.41 (t, *J* = 7.6 Hz, 1 H), 7.46 (d, *J* = 8.2 Hz, 1 H), 7.58 (t, *J* = 8.2 Hz, 1 H), 7.64–7.80 (m, 3 H), 8.16 (d, *J* = 8.2 Hz, 1 H), 8.34 (s, 1 H), 8.36 (d, *J* = 8.4 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 114.4, 117.2, 117.6, 120.5, 121.9, 125.3, 126.8, 129.6, 130.9, 131.2, 134.5, 146.2, 147.0, 154.9, 157.4, 162.6.

Anal. Calcd for $C_{16}H_9N_3O_3$: C, 65.98; H, 3.11; N, 14.43. Found: C, 65.67; H, 3.10; N, 14.22.

***N*-(Coumarin-3-ylcarbonyl)-α-amino Acids 19a–g, 21a,b, 22 and Racemate [19c + 19c′]; General Procedure**

3-(1*H*-Benzotriazol-1-ylcarbonyl)-2*H*-chromen-2-one (**17**; 1 mmol) was added to a soln of the appropriate α-amino acid **18a**–**g**, **20a**,**b**, [**18c** + **18c′**] (1 mmol) in MeCN–H_2O (10 mL:5 mL) in the presence of Et_3N (1.1 mmol) [**17** (2 mmol) and L-lysine (1 mmol) were used to obtain **22**]. The reaction mixture was stirred at 20 °C for about 1 h (until TLC showed the absence of **17**). Aq 4 N HCl (1 mL) was then added and the MeCN was removed under reduced pressure. The residue obtained was dissolved in EtOAc (150 mL), washed with 4 N HCl (3 × 50 mL) and sat. NaCl soln (50 mL), and dried ($MgSO_4$). After evaporation of the solvent, the residue was crystallized from EtOAc–hexanes or CH_2Cl_2–hexanes.

(*S*)-2-[(2-Oxo-2*H*-chromen-3-ylcarbonyl)amino]propanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Ala-OH, 19a]

Yield: 82%; white microcrystals; mp 203–204 °C; $[\alpha]_D^{23}$ +12.0 (*c* 1.92, DMF).

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^{1}H NMR (DMSO-d_6):[37] δ = 1.43 (d, *J* = 7.1 Hz, 3 H), 4.49 (quin, *J* = 7.0 Hz, 1 H), 7.46 (t, *J* = 7.5 Hz, 1 H), 7.53 (d, *J* = 8.4 Hz, 1 H), 7.77 (td, *J* = 8.5, 1.5 Hz, 1 H), 8.01 (dd, *J* = 7.8, 1.4 Hz, 1 H), 8.91 (s, 1 H), 9.11 (d, *J* = 6.9 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.9, 48.2, 116.2, 118.3, 118.4, 125.2, 130.4, 134.3, 148.0, 154.0, 160.5, 160.6, 173.6.

Anal. Calcd for $C_{13}H_{11}NO_5$: C, 59.77; H, 4.24; N, 5.36. Found: C, 59.66; H, 4.40; N, 5.49.

(*S*)-3-Hydroxy-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]propanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Ser-OH, 19b]

Yield: 76%; white microcrystals; mp 225–226 °C; $[\alpha]_D^{23}$ +20.65 (*c* 1.85, DMF).

^{1}H NMR (DMSO-d_6): δ = 3.76 (dd, *J* = 10.8, 3.2 Hz, 1 H), 3.89 (dd, *J* = 10.8, 3.2 Hz, 1 H), 4.48–4.58 (m, 1 H), 5.29 (br s, 1 H), 7.46 (t, *J* = 7.5 Hz, 1 H), 7.54 (d, *J* = 8.4 Hz, 1 H), 7.78 (t, *J* = 7.5 Hz, 1 H), 8.03 (d, *J* = 7.5 Hz, 1 H), 8.96 (s, 1 H), 9.27 (d, *J* = 7.5 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 54.9, 61.1, 116.2, 118.1, 118.4, 125.3, 130.5, 134.4, 148.3, 154.0, 160.6, 160.7, 171.6.

Anal. Calcd for $C_{13}H_{11}NO_6 \cdot H_2O$: C, 52.89; H, 4.44; N, 4.74. Found: C, 53.16; H, 4.31; N, 4.67.

(*S*)-4-(Methylsulfanyl)-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]butanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Met-OH, 19c]

Yield: 88%; white microcrystals; mp 115–116 °C; $[\alpha]_D^{23}$ +1.33 (*c* 1.73, DMF).

^{1}H NMR ($CDCl_3$): δ = 2.13 (s, 3 H), 2.16–2.26 (m, 1 H), 2.27–2.42 (m, 1 H), 2.64 (t, *J* = 7.5 Hz, 2 H), 4.86–4.98 (m, 1 H), 7.37–7.47 (m, 2 H), 7.65–7.75 (m, 2 H), 8.93 (s, 1 H), 9.35 (d, *J* = 7.3 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 15.3, 30.0, 31.1, 52.0, 116.6, 117.5, 118.4, 125.4, 130.0, 134.4, 149.2, 154.5, 161.2, 162.0, 175.1.

Anal. Calcd for $C_{15}H_{15}NO_5S$: C, 56.07; H, 4.70; N, 4.36. Found: C, 55.76; H, 4.75; N, 4.25.

(*S*)-2-[(2-Oxo-2*H*-chromen-3-ylcarbonyl)amino]-3-phenylpropanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Phe-OH, 19d]

Yield: 88%; white microcrystals; mp 178–179 °C; $[\alpha]_D^{23}$ +41.53 (*c* 1.78, DMF).

^{1}H NMR (DMSO-d_6): δ = 3.12 (dd, *J* = 13.8, 6.7 Hz, 1 H, B part of AB system), 3.21 (dd, *J* = 13.7, 5.3 Hz, 1 H, A part of AB system), 4.73–4.83 (m, 1 H), 7.18–7.34 (m, 5 H), 7.45 (t, *J* = 7.6 Hz, 1 H), 7.51 (d, *J* = 8.4 Hz, 1 H), 7.77 (t, *J* = 7.8 Hz, 1 H), 8.00 (d, *J* = 7.4 Hz, 1 H), 8.91 (s, 1 H), 9.05 (d, *J* = 7.4 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 36.8, 53.7, 116.2, 117.9, 118.4, 125.2, 126.7, 128.3 (2 C), 129.3 (2 C), 130.4, 134.4, 136.6, 148.3, 154.0, 160.5, 160.6, 172.1.

Anal. Calcd for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 67.34; H, 4.34; N, 4.10.

(*S*)-3-(1*H*-Indol-3-yl)-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]propanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Trp-OH, 19e]

Yield: 78%; yellow microcrystals; mp 201–202 °C; $[\alpha]_D^{23}$ +33.14 (*c* 1.91, DMF).

^{1}H NMR (DMSO-d_6): δ = 3.23–3.41 (m, 2 H), 4.80 (q, *J* = 6.1 Hz, 1 H), 6.95 (t, *J* = 7.4 Hz, 1 H), 7.06 (t, *J* = 7.5 Hz, 1 H), 7.19 (d, *J* = 2.2 Hz, 1 H), 7.33 (d, *J* = 8.1 Hz, 1 H), 7.44 (t, *J* = 7.5 Hz, 1 H), 7.51 (t, *J* = 8.7 Hz, 2 H), 7.75 (td, *J* = 7.1, 1.5 Hz, 1 H), 7.99 (dd, *J* = 7.5, 1.4 Hz, 1 H), 8.93 (s, 1 H), 9.10 (d, *J* = 7.3 Hz, 1 H), 10.94 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 27.1, 54.9, 108.8, 111.4, 116.2, 118.0, 118.3, 118.4 (2 C), 121.0, 123.9, 125.2, 127.3, 130.4, 134.3, 136.1, 148.2, 154.0, 160.5, 160.6, 172.6.

Anal. Calcd for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44. Found: C, 66.79; H, 4.13; N, 7.25.

(*S*)-3-Methyl-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]butanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Val-OH, 19f]

Yield: 81%; white microcrystals; mp 211–212 °C; $[\alpha]_D^{23}$ + 5.58 (*c* 2.04, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.97 (d, *J* = 6.7 Hz, 6 H), 2.15–2.31 (m, 1 H), 4.47 (dd, *J* = 8.3, 4.7 Hz, 1 H), 7.47 (t, *J* = 7.5 Hz, 1 H), 7.54 (d, *J* = 8.2 Hz, 1 H), 7.78 (t, *J* = 7.8 Hz, 1 H), 8.02 (d, *J* = 7.6 Hz, 1 H), 8.93 (s, 1 H), 9.09 (d, *J* = 8.2 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.6, 19.1, 30.5, 57.3, 116.2, 118.2, 118.5, 125.3, 130.4, 134.4, 148.1, 154.0, 160.9, 161.0, 172.4.

Anal. Calcd for $C_{15}H_{15}NO_5$: C, 62.28; H, 5.23; N, 4.84. Found: C, 62.20; H, 5.11; N, 4.74.

(*S*)-6-(Benzyloxycarbonylamino)-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoic Acid [N^{α}-(Coumarin-3-ylcarbonyl)-N^{ε}-Cbz-L-Lys-OH, 19g]

Yield: 77%; white microcrystals; mp 149–150 °C; $[\alpha]_D^{23}$ + 6.27 (*c* 2.08, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.25–1.51 (m, 4 H), 1.68–1.95 (m, 2 H), 2.94–3.06 (m, 2 H), 4.43–4.58 (m, 1 H), 4.98 (s, 2 H), 7.22–7.40 (m, 6 H), 7.46 (t, *J* = 7.6 Hz, 1 H), 7.53 (d, *J* = 8.4 Hz, 1 H), 7.77 (t, *J* = 7.5 Hz, 1 H), 8.01 (d, *J* = 7.3 Hz, 1 H), 8.91 (s, 1 H), 9.08 (d, *J* = 7.4 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 22.2, 29.1, 31.2, 52.3, 65.1, 116.2, 118.3, 118.4, 125.2 (2 C), 127.7 (2 C), 128.3 (2 C), 130.4, 134.3, 137.3, 148.0, 154.0, 156.1, 160.6, 160.8, 173.0 (one aliphatic carbon is merged in the DMSO-d_6 peak).

Anal. Calcd for $C_{24}H_{24}N_2O_7$: C, 63.71; H, 5.35; N, 6.19. Found: C, 63.82; H, 5.32; N, 6.11.

4-(Methylsulfanyl)-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]butanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-DL-Met-OH, [19c + 19c′]]

Yield: 89%; white microcrystals; mp 136–137 °C.

^{1}H NMR ($CDCl_3$): δ = 2.13 (s, 3 H), 2.20 (quin, *J* = 7.3 Hz, 1 H), 2.27–2.42 (m, 1 H), 2.65 (t, *J* = 7.4 Hz, 2 H), 4.87–4.98 (m, 1 H), 7.36–7.47 (m, 2 H), 7.65–7.75 (m, 2 H), 8.94 (s, 1 H), 9.36 (d, *J* = 7.4 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 15.4, 30.0, 31.1, 52.0, 116.7, 117.5, 118.4, 125.4, 130.0, 134.5, 149.2, 154.5, 161.3, 162.1, 175.4.

Anal. Calcd for $C_{15}H_{15}NO_5S$: C, 56.07; H, 4.70; N, 4.36. Found: C, 55.74; H, 4.70; N, 4.27.

(*S*)-2-(Benzyloxycarbonylamino)-6-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoic Acid [N^{α}-Cbz-N^{ε}-(Coumarin-3-ylcarbonyl)-L-Lys-OH, 21a]

Yield: 89%; white microcrystals; mp 144–145 °C; $[\alpha]_D^{23}$ –8.54 (*c* 1.68, DMF).

^{1}H NMR ($CDCl_3$): δ = 1.37–1.56 (m, 2 H), 1.58–1.74 (m, 2 H), 1.75–2.40 (m, 2 H), 3.33–3.58 (m, 2 H), 4.34–4.45 (m, 1 H), 5.09 (s, 2 H), 5.75 (d, *J* = 8.0 Hz, 1 H), 7.27–7.42 (m, 7 H), 7.52–7.72 (m, 2 H), 8.92 (s, 1 H), 8.95–9.04 (m, 1 H).

^{13}C NMR ($CDCl_3$): δ = 22.3, 28.9, 31.5, 39.3, 53.6, 66.9, 116.5 (2 C), 117.9, 118.5, 125.3, 128.0, 128.1, 128.4 (2 C), 130.0, 134.1, 136.2, 148.8, 154.3, 156.3, 161.3, 162.1, 175.3.

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Anal. Calcd for $C_{24}H_{24}N_2O_7$: C, 63.71; H, 5.35; N, 6.19. Found: C, 63.82; H, 5.09; N, 6.04.

(*S*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-6-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoic Acid [N^{α}-Fmoc-N^{ε}-(Coumarin-3-ylcarbonyl)-L-Lys-OH, 21b]
Yield: 87%; white microcrystals; mp 110–111 °C; $[\alpha]_D^{23}$ –1.62 (*c* 1.85, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.32–1.50 (m, 2 H), 1.50–1.62 (m, 2 H), 1.62–1.85 (m, 2 H), 3.26–3.38 (m, 2 H), 3.92–4.01 (m, 1 H), 4.17–4.36 (m, 3 H), 7.22–7.54 (m, 6 H), 7.60–7.80 (m, 4 H), 7.87 (d, *J* = 7.4 Hz, 2 H), 7.96 (d, *J* = 7.4 Hz, 1 H), 8.73 (t, *J* = 5.5 Hz, 1 H), 8.84 (s, 1 H), 12.62 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 23.2, 28.6, 30.5, 46.7, 53.8, 65.6, 116.1, 118.5, 119.0, 120.1, 125.1, 125.3 (2 C), 127.1 (2 C), 127.7 (2 C), 130.2, 134.0, 140.7 (2 C), 143.8 (2 C), 147.3, 153.8, 156.2, 160.4, 161.0 (2 C), 174.0 (one aliphatic carbon is merged in the DMSO-d_6 peak).

Anal. Calcd for $C_{31}H_{28}N_2O_7$: C, 68.88; H, 5.22; N, 5.18. Found: C, 68.59; H, 5.11; N, 5.16.

(*S*)-2,6-Bis[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoic Acid [N^{α},N^{ε}-Bis(coumarin-3-ylcarbonyl)-L-Lys-OH, 22]
Yield: 86%; white microcrystals; mp 220–221 °C; $[\alpha]_D^{23}$ +14.13 (*c* 1.86, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.30–1.48 (m, 2 H), 1.50–1.65 (m, 2 H), 1.72–2.00 (m, 2 H), 3.25–3.40 (m, 2 H), 4.47–4.57 (m, 1 H), 7.37–7.52 (m, 4 H), 7.68–7.80 (m, 2 H), 7.95 (t, *J* = 8.0 Hz, 2 H), 8.68 (t, *J* = 5.6 Hz, 1 H), 8.81 (s, 1 H), 8.88 (s, 1 H), 9.06 (d, *J* = 7.4 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 18.0, 22.1, 28.4, 31.0, 52.1, 116.1, 116.2, 118.3 (2 C), 118.4, 119.0, 125.1, 125.2, 130.2, 130.4, 134.0, 134.3, 147.3, 148.0, 153.8, 153.9, 160.4, 160.6, 160.7, 161.0, 173.0.

Anal. Calcd for $C_{26}H_{22}N_2O_8$: C, 63.67; H, 4.52; N, 5.71. Found: C, 63.43; H, 4.42; N, 5.69.

***N*-(Coumarin-3-ylcarbonyl-α-aminoacyl)benzotriazoles 23a, [23b + 23b′] and 26; General Procedure**
Thionyl chloride (1.2 mmol) was added to a soln of 1*H*-benzotriazole (**16**; 4 mmol) in anhyd CH_2Cl_2 (15 mL) at 20 °C and the reaction mixture was stirred for 20 min. To the reaction mixture was added the *N*-(coumarin-3-ylcarbonyl)-α-amino acid **19a**, [**19c** + **19c′**], **21a** (1 mmol) and the relevant mixture was stirred for 1 h at r.t. to obtain **23a**, **26** and for 15 min to obtain the diastereomeric mixture [**23b** + **23b′**]. The white precipitate which formed during the reaction was filtered off, the filtrate was diluted with additional CH_2Cl_2 (80 mL), and the solution was washed with 4 N HCl (3 × 50 mL) [sat. Na_2CO_3 soln (3 × 50 mL) to obtain **26**] and sat. NaCl soln (50 mL), and dried ($MgSO_4$). Removal of the solvent under reduced pressure gave the desired product, which was recrystallized from CH_2Cl_2–hexanes.

***N*-[(1*S*)-2-(1*H*-1,2,3-Benzotriazol-1-yl)-1-methyl-2-oxoethyl]-2-oxo-2*H*-chromene-3-carboxamide [*N*-(Coumarin-3-ylcarbonyl)-L-Ala-Bt, 23a]**
Yield: 78%; white microcrystals; mp 178–179 °C.

^{1}H NMR ($CDCl_3$): δ = 1.85 (d, *J* = 7.3 Hz, 3 H), 6.13 (quin, *J* = 7.0 Hz, 1 H), 7.39 (t, *J* = 7.4 Hz, 1 H), 7.45 (d, *J* = 8.7 Hz, 1 H), 7.54 (t, *J* = 7.5 Hz, 1 H), 7.64–7.76 (m, 3 H), 8.16 (d, *J* = 8.2 Hz, 1 H), 8.31 (d, *J* = 8.2 Hz, 1 H), 8.90 (s, 1 H), 9.49 (d, *J* = 6.2 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 18.3, 49.6, 114.5, 116.7, 117.7, 118.4, 120.3, 125.4, 126.4, 129.9, 130.7, 131.2, 134.4, 146.0, 148.9, 154.6, 161.3, 161.5, 171.4.

Anal. Calcd for $C_{19}H_{14}N_4O_4$: C, 62.98; H, 3.89; N, 15.46. Found: C, 62.65; H, 3.81; N, 15.26.

***N*-[1-(1*H*-1,2,3-Benzotriazol-1-ylcarbonyl)-3-(methylsulfanyl)propyl]-2-oxo-2*H*-chromene-3-carboxamide [*N*-(Coumarin-3-ylcarbonyl)-DL-Met-Bt, [23b + 23b′]]**
Yield: 74%; white microcrystals; mp 123–124 °C.

^{1}H NMR ($CDCl_3$): δ = 2.13 (s, 3 H), 2.30–2.45 (m, 1 H), 2.52–2.69 (m, 1 H), 2.77 (t, *J* = 7.0 Hz, 2 H), 6.23–6.35 (m, 1 H), 7.40 (t, *J* = 7.6 Hz, 1 H), 7.50 (d, *J* = 8.7 Hz, 1 H), 7.55 (t, *J* = 7.6 Hz, 1 H), 7.64–7.78 (m, 3 H), 8.16 (d, *J* = 8.2 Hz, 1 H), 8.30 (d, *J* = 8.2 Hz, 1 H), 8.91 (s, 1 H), 9.60 (d, *J* = 7.1 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 15.3, 30.2, 31.8, 52.9, 114.4, 116.7, 117.6, 118.4, 120.3, 125.4, 126.5, 130.0, 130.8, 131.2, 134.5, 146.0, 149.1, 154.6, 161.4, 161.8, 170.5.

Anal. Calcd for $C_{21}H_{18}N_4O_4S$: C, 59.70; H, 4.29; N, 13.26. Found: C, 59.93; H, 4.17; N, 12.88.

Benzyl{(*S*)-1-(1*H*-1,2,3-Benzotriazol-1-ylcarbonyl)-5-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]pentyl}carbamate [N^{α}-Cbz-N^{ε}-(Coumarin-3-ylcarbonyl)-L-Lys-Bt, 26]
Yield: 79%; white microcrystals; mp 156–157 °C.

^{1}H NMR ($CDCl_3$): δ = 1.50–1.80 (m, 4 H), 1.96–2.12 (m, 1 H), 2.13–2.28 (m, 1 H), 3.36–3.48 (m, 1 H), 3.48–3.64 (m, 1 H), 5.13 (s, 2 H), 5.69–5.83 (m, 1 H), 6.12 (d, *J* = 7.8 Hz, 1 H), 7.26–7.47 (m, 8 H), 7.52 (t, *J* = 7.6 Hz, 1 H), 7.60–7.74 (m, 2 H), 8.13 (d, *J* = 8.2 Hz, 1 H), 8.27 (d, *J* = 8.2 Hz, 1 H), 8.86 (s, 1 H), 8.82–8.97 (m, 1 H).

^{13}C NMR ($CDCl_3$): δ = 22.3, 28.9, 31.6, 38.5, 54.6, 67.1, 114.4, 116.5, 118.2, 118.6, 120.3, 125.2, 126.4, 128.0 (2 C), 128.1, 128.5 (2 C), 129.8, 130.6, 131.1, 134.0, 136.2, 145.9, 148.6, 154.3, 156.2, 161.4, 162.0, 171.7.

Anal. Calcd for $C_{30}H_{27}N_5O_6$: C, 65.09; H, 4.92; N, 12.65. Found: C, 64.91; H, 4.76; N, 12.59.

Coumarin-Labeled Dipeptides 25a, [25b + 25b′], 27, 29a,b and [29a + 29a′]; General Procedure for Route (i)
A *N*-(coumarin-3-ylcarbonyl-α-aminoacyl)benzotriazole **23a**, [**23b** + **23b′**], **26** (0.5 mmol) was added to a soln of an α-amino acid **24**, **28a**,**b**, [**28a** + **28a′**] (0.5 mmol), or L-aspartic acid (to obtain **27**), in MeCN–H_2O (10 mL:5 mL) in the presence of Et_3N (1 mmol). The reaction mixture was stirred at 20 °C for 1 h. Aq 4 N HCl (1 mL) was then added and the MeCN was removed under reduced pressure. The obtained residue was dissolved in EtOAc (100 mL), washed with 4 N HCl (3 × 50 mL) and sat. NaCl soln (50 mL), and dried ($MgSO_4$). Evaporation of the solvent gave the desired product, which was recrystallized from CH_2Cl_2–hexanes.

(*S*)-2-{(*S*)-2-[(2-Oxo-2*H*-chromen-3-ylcarbonyl)amino]propionylamino}-3-phenylpropanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-L-Ala-L-Phe-OH, 25a]
Yield: 74%; white microcrystals; mp 208–209 °C; $[\alpha]_D^{23}$ +19.46 (*c* 1.67, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.30 (d, *J* = 6.9 Hz, 3 H), 2.91 (dd, *J* = 13.7, 9.5 Hz, 1 H), 3.10 (dd, *J* = 13.9, 4.8 Hz, 1 H), 4.44–4.50 (m, 1 H), 4.55 (quin, *J* = 7.0 Hz, 1 H), 7.13–7.32 (m, 5 H), 7.45 (t, *J* = 7.6 Hz, 1 H), 7.52 (d, *J* = 8.2 Hz, 1 H), 7.76 (t, *J* = 7.3 Hz, 1 H), 8.00 (d, *J* = 7.4 Hz, 1 H), 8.51 (d, *J* = 8.0 Hz, 1 H), 8.90 (s, 1 H), 9.13 (d, *J* = 7.3 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 19.3, 36.5, 48.3, 53.6, 116.2, 118.3, 118.5, 125.2, 126.5, 128.2 (2 C), 129.2 (2 C), 130.4, 134.3, 137.6, 147.9, 154.0, 160.1, 160.5, 171.5, 172.7.

Anal. Calcd for $C_{22}H_{20}N_2O_6$: C, 64.70; H, 4.94; N, 6.86. Found: C, 64.66; H, 4.96; N, 6.74.

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(*S*)-2-{4-(Methylsulfanyl)-2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]butyrylamino}-3-phenylpropanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-DL-Met-L-Phe-OH, [25b + 25b′]]
Yield: 68%; white microcrystals; mp 184–185 °C; $[\alpha]_D^{23}$ –1.02 (*c* 1.96, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.60–2.05 (m, 2 H), 1.92 (s, 1.5 H), 2.01 (s, 1.5 H), 2.03–2.20 (m, 1 H), 2.43 (t, *J* = 8.0 Hz, 1 H), 2.78–2.98 (m, 1 H), 3.13 (td, *J* = 13.6, 4.4 Hz, 1 H), 4.42–4.58 (m, 1 H), 4.59–4.70 (m, 1 H), 7.12–7.32 (m, 5 H), 7.44 (t, *J* = 7.4 Hz, 1 H), 7.51 (d, *J* = 8.4 Hz, 1 H), 7.75 (t, *J* = 7.8 Hz, 1 H), 7.98 (d, *J* = 7.6 Hz, 1 H), 8.54 (d, *J* = 7.8 Hz, 0.5 H), 8.64 (d, *J* = 8.4 Hz, 0.5 H), 8.88 (s, 1 H), 9.09 (d, *J* = 7.8 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 14.5, 14.6, 28.7, 28.8, 32.9, 36.4, 36.7, 51.9, 52.0, 53.3, 53.6, 116.2, 118.4, 125.2, 126.5, 128.2, 128.2, 129.1, 130.3, 134.3, 137.5, 137.5, 147.8, 153.9, 160.5, 160.5, 170.1, 170.2, 172.7, 172.8.

Anal. Calcd for $C_{24}H_{24}N_2O_6S$: C, 61.53; H, 5.16; N, 5.98. Found: C, 61.27; H, 5.20; N, 6.00.

(*S*)-2-{(*S*)-2-(Benzyloxycarbonylamino)-6-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoylamino}succinic Acid [N^{ε}-(Coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-L-Asp-OH, 27]
Yield: 85%; white microcrystals; mp 206–207 °C; $[\alpha]_D^{23}$ –10.48 (*c* 0.83, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.27–1.42 (m, 2 H), 1.43–1.73 (m, 4 H), 2.60 (dd, *J* = 16.5, 6.6 Hz, 1 H), 2.68 (dd, *J* = 16.8, 5.6 Hz, 1 H), 3.20–3.38 (m, 2 H), 3.94–4.09 (m, 1 H), 4.48–4.58 (m, 1 H), 5.00 (s, 2 H), 7.25–7.38 (m, 5 H), 7.40–4.47 (m, 2 H), 7.50 (d, *J* = 8.2 Hz, 1 H), 7.75 (td, *J* = 7.8, 1.4 Hz, 1 H), 7.97 (d, *J* = 7.7 Hz, 1 H), 8.23 (d, *J* = 8.0 Hz, 1 H), 8.70 (t, *J* = 5.6 Hz, 1 H), 8.86 (s, 1 H), 12.58 (br s, 2 H).

^{13}C NMR (DMSO-d_6): δ = 22.9, 28.7, 31.7, 36.0, 48.5, 54.3, 65.4, 116.2, 118.5, 119.1, 125.2, 127.7 (2 C), 127.8, 128.4 (2 C), 130.3, 134.1, 134.3, 137.0, 147.4, 153.9, 156.0, 160.4, 161.0, 171.7, 171.9, 172.4.

Anal. Calcd for $C_{28}H_{29}N_3O_{10}$: C, 59.26; H, 5.15; N, 7.40. Found: C, 59.44; H, 5.14; N, 7.24.

(*S*)-2-{(*S*)-2-(Benzyloxycarbonylamino)-6-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoylamino}propanoic Acid [N^{ε}-(Coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-L-Ala-OH, 29a]
Yield: 91%; white microcrystals; mp 156–157 °C; $[\alpha]_D^{23}$ –8.43 (*c* 0.83, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.28 (d, *J* = 7.3 Hz, 3 H), 1.32–1.76 (m, 6 H), 3.25–3.48 (m, 2 H), 3.95–4.10 (m, 1 H), 4.20 (quin, *J* = 7.2 Hz, 1 H), 5.01 (s, 2 H), 7.26–7.38 (m, 5 H), 7.41 (d, *J* = 8.4 Hz, 1 H), 7.46 (d, *J* = 7.6 Hz, 1 H), 7.51 (d, *J* = 8.4 Hz, 1 H), 7.70–7.82 (m, 1 H), 7.98 (d, *J* = 7.7 Hz, 1 H), 8.21 (d, *J* = 7.1 Hz, 1 H), 8.71 (t, *J* = 5.5 Hz, 1 H), 8.86 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.1, 22.9, 28.7, 31.6, 47.4, 54.2, 65.4, 116.2, 118.5, 119.1, 125.2, 127.7 (2 C), 127.8, 128.3 (2 C), 130.3, 134.1, 137.1, 147.4, 153.9, 156.0, 160.4, 161.0, 171.8, 174.1 (one aliphatic carbon is merged in the DMSO-d_6 peak).

Anal. Calcd for $C_{27}H_{29}N_3O_8$: C, 61.94; H, 5.58; N, 8.03. Found: C, 61.64; H, 5.65; N, 7.66.

(*S*)-2-{(*S*)-2-(Benzyloxycarbonylamino)-6-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoylamino}-3-phenylpropanoic Acid [N^{ε}-(Coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-L-Phe-OH, 29b]
Yield: 89%; white microcrystals; mp 170–171 °C; $[\alpha]_D^{23}$ –5.71 (*c* 1.75, DMF).

^{1}H NMR (DMSO-d_6): δ = 1.20–1.80 (m, 6 H), 2.92 (dd, *J* = 13.7, 8.8 Hz, 1 H), 3.06 (dd, *J* = 13.7, 4.8 Hz, 1 H), 3.22–3.40 (m, 2 H), 3.90–4.10 (m, 1 H), 4.38–4.51 (m, 1 H), 5.02 (s, 2 H), 7.16–7.48 (m, 12 H), 7.51 (d, *J* = 8.2 Hz, 1 H), 7.75 (t, *J* = 7.7 Hz, 1 H), 7.98 (d, *J* = 7.6 Hz, 1 H), 8.15 (d, *J* = 7.7 Hz, 1 H), 8.66–8.78 (m, 1 H), 8.87 (s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 22.9, 28.7, 31.7, 36.7, 39.1, 53.4, 54.5, 65.4, 116.2, 118.5, 119.1, 125.2, 126.5, 127.7 (3 C), 127.8, 127.8, 128.2, 128.4 (2 C), 129.2, 130.3, 134.1, 137.1, 137.4, 147.4, 153.9, 155.9, 160.5, 161.0, 172.0, 172.9.

Anal. Calcd for $C_{33}H_{33}N_3O_8$: C, 64.10; H, 5.55; N, 7.01. Found: C, 64.37; H, 5.51; N, 6.61.

2-{(*S*)-2-(Benzyloxycarbonylamino)-6-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]hexanoylamino}propanoic Acid [N^{ε}-(Coumarin-3-ylcarbonyl)-N^{α}-Cbz-L-Lys-DL-Ala-OH, [29a + 29a′]]
Yield: 94%; white microcrystals; mp 131–132 °C.

^{1}H NMR (DMSO-d_6): δ = 1.21–1.31 (m, 3 H), 1.31–1.80 (m, 6 H), 3.27–3.34 (m, 2 H), 3.85–4.08 (m, 1 H), 4.09–4.30 (m, 1 H), 5.02 (s, 2 H), 7.24–7.48 (m, 7 H), 7.51 (d, *J* = 8.6 Hz, 1 H), 7.76 (t, *J* = 7.7 Hz, 1 H), 7.97 (d, *J* = 8.0 Hz, 1 H), 8.21 (d, *J* = 6.9 Hz, 1 H), 8.65–8.76 (m, 1 H), 8.86 (s, 1 H), 12.58 (br s, 1 H).

^{13}C NMR (DMSO-d_6): δ = 17.1, 17.5, 22.9, 28.6, 28.7, 31.6, 32.0, 39.1, 47.4, 54.2, 65.3, 116.1, 118.5, 119.1, 125.2, 127.7, 127.7, 127.8, 128.3, 130.3, 134.1, 137.1, 147.4, 153.9, 155.9, 156.0, 160.4, 161.0, 171.7, 171.8, 174.0, 174.1.

Anal. Calcd for $C_{27}H_{29}N_3O_8$: C, 61.94; H, 5.58; N, 8.03. Found: C, 61.64; H, 5.59; N, 7.65.

Coumarin-Labeled Dipeptides 30 and 31; General Procedure for Route (ii)
Benzotriazole **17** (1 mmol) was added to a soln of a dipeptide in MeCN–H_2O (10 mL:5 mL) in the presence of Et_3N (1.1 mmol). The reaction mixture was stirred at 20 °C for 1 h. Aq 4 N HCl (1 mL) was then added and the solvent was removed under reduced pressure. The residue obtained was dissolved in CH_2Cl_2 (80 mL), and the organic extract was washed with 4 N HCl (3 × 40 mL) and sat. NaCl soln (30 mL), and dried ($MgSO_4$). Evaporation of the solvent gave the pure product.

2-{2-[(2-Oxo-2*H*-chromen-3-ylcarbonyl)amino]acetylamino}acetic Acid [*N*-(Coumarin-3-ylcarbonyl)-Gly-Gly-OH, 30]
Yield: 81%; white microcrystals; mp 243–244 °C.

^{1}H NMR (DMSO-d_6): δ = 3.82 (d, *J* = 5.8 Hz, 2 H), 4.06 (d, *J* = 5.2 Hz, 2 H), 7.46 (t, *J* = 7.5 Hz, 1 H), 7.52 (d, *J* = 8.2 Hz, 1 H), 7.77 (td, *J* = 7.5, 1.2 Hz, 1 H), 8.01 (d, *J* = 6.9 Hz, 1 H), 8.44 (t, *J* = 5.8 Hz, 1 H), 8.92 (s, 1 H), 9.11 (t, *J* = 5.2 Hz, 1 H).

^{13}C NMR (DMSO-d_6): δ = 40.7, 42.5, 116.2, 118.4, 125.2, 130.4 (2 C), 134.3, 147.8, 154.0, 160.4, 161.1, 168.6, 171.1.

Anal. Calcd for $C_{14}H_{12}N_2O_6 \cdot H_2O$: C, 52.18; H, 3.75; N, 8.69. Found: C, 52.25; H, 3.86; N, 8.36.

(*S*)-4-Methyl-2-{2-[(2-oxo-2*H*-chromen-3-ylcarbonyl)amino]acetylamino}pentanoic Acid [*N*-(Coumarin-3-ylcarbonyl)-Gly-L-Leu-OH, 31]
Yield: 85%; white microcrystals; mp 194–195 °C; $[\alpha]_D^{23}$ –1.02 (*c* 1.96, DMF).

^{1}H NMR (DMSO-d_6): δ = 0.86 (d, *J* = 6.3 Hz, 3 H), 0.91 (d, *J* = 6.5 Hz, 3 H), 1.48–1.58 (m, 2 H), 1.58–1.74 (m, 1 H), 4.06 (d, *J* = 5.1 Hz, 2 H), 4.28 (q, *J* = 7.6 Hz, 1 H), 7.46 (t, *J* = 7.5 Hz, 1 H), 7.52 (d, *J* = 8.2 Hz, 1 H), 7.77 (t, *J* = 7.8 Hz, 1 H), 8.01 (d, *J* = 7.7 Hz, 1 H), 8.37 (d, *J* = 7.8 Hz, 1 H), 8.92 (s, 1 H), 9.09 (t, *J* = 4.9 Hz, 1 H).

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^{13}C NMR (DMSO-d_6): δ = 21.4, 22.9, 24.3, 42.4, 50.3, 116.2, 118.4, 118.5, 125.2, 130.4 (2 C), 134.3, 147.8, 154.0, 160.4, 161.0, 168.1, 174.0.

Anal. Calcd for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.59; H, 5.77; N, 7.52.

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(36) Two compound numbers given as a combination within square brackets represent a racemate or a diastereomeric mixture; all single compound numbers represent enantiomers.
(37) No signal was seen in the proton NMR spectra for the acid proton (COOH) for most of the free carboxylic acids; this signal is reported only for compounds **21b**, **27** and [**29a** + **29a′**].

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