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Aust. J. Chem.
http://dx.doi.org/10.1071/CH14708

Full Paper

Synthesis, Absorption, and Fluorescence Studies of Coumaryl-Labelled Amino Acids and Dipeptides Linked Via Triazole Ring

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Fluorophores based on 4-triazolyl, 7-hydroxy-4-triazolylmethyl, 4-*O*-triazolylmethyl, and 7-*O*-triazolylmethyl coumaryl-tagged amino acids and dipeptides were synthesized by copper-catalyzed [3 + 2] cycloaddition reaction between azido- or alkynyl-functionalized coumarins with alkynyl- or azido-functionalized amino acid and dipeptides in good-to-excellent yields. Steady-state absorption and the fluorescence properties of the synthesized conjugates were studied. The chemical applicability of these amino acid and peptide-based fluorophores was successfully demonstrated by their linear elongation by further tagging them with appropriate *C*- or *N*-terminus amino acid.

Manuscript received: 11 December 2014.
Manuscript accepted: 9 February 2015.
Published online: 7 April 2015.

Introduction

Amino acids, peptides, and proteins are the most abundant molecules in living cells that exhibit various physiological functions in all living organisms. Impairing of amino acid metabolism in the body may lead to various disease conditions (e.g. phenylketonuria). Biologically active peptides serve as an interesting starting point in drug discovery processes. Several peptide-based pharmaceuticals have been developed as useful therapeutic agents, which offer advantages such as high potency and selectivity as well as low accumulation in tissues. Thus, investigation and understanding biologically relevant interactions and processes, such as receptor–ligand binding, protein structures, protein folding, protein–protein interactions, and enzyme activity using labelling studies, have become an established exercise in biochemical research.[1] For studying these interactions, tagging *C*- and *N*-terminal domains of a peptide with a fluorescent label and probe or incorporating a natural fluorescent amino acid, such as tyrosine (Tyr) or tryptophan (Trp) in peptide synthesis, are two interactive modes of detecting a peptide without allowing any changes in its conformational and biological characteristics.[2]

Coumarin is an excellent example of a fluorescent label with advantages of extended spectral ranges, high emission quantum yields, photo-stability, and good solubility in many solvents.[3] Coumarin derivatives are widely used as fluorescent probes,[4] labels[5] pigments,[6] and laser dyes[7] for the blue–green region and signalling unit in sensors and in sophisticated photophysical systems.[8] Excellent fluorescence quantum yields have been observed in coumarins bearing electron-donating substituents at the 7-position, such as 7-aminocoumarin, 7-(dialkylamino)coumarins, 7-hydoxycoumarin that makes them suitable candidates in the application of fluorescence labelling of living cells.[9] For example, α-L-(2-(7-hydroxycoumarin-4-yl)ethyl) glycine (Fig. 1, **I**) was genetically incorporated at a defined site in proteins in living organisms for the first time by Schultz and coworkers.[10] Coumarin-labelled lysines (Fig. 1, **II**) have also been designed for the synthesis of ñuorogenic triple-helical substrates for the analysis of matrix metalloproteinase family members.[11] Unnatural amino acid linked to 7-aminocoumarin substrate (Fig. 1, **III**) has been recently developed as a fluorescent ligase substrate for specific protein labelling in living cells.[12] Katritzky et al. have explored the application of benzotriazole methodology for the synthesis of fluorescent coumarin-labelled amino acids and dipeptides as building blocks for solid phase peptide synthesis (SPPS).[13]

Copper-catalyzed [3 + 2] cycloaddition reaction between an azide and an alkyne is an established and attractive labelling methodology for linking two chemical moieties via a triazole ring.[14] Triazoles themselves possess interesting chemical properties that include tolerance to acidic, basic, oxidative, and reductive conditions. A triazole ring has been considered as a non-classical bioisostere of the peptide bond.[15] Its function

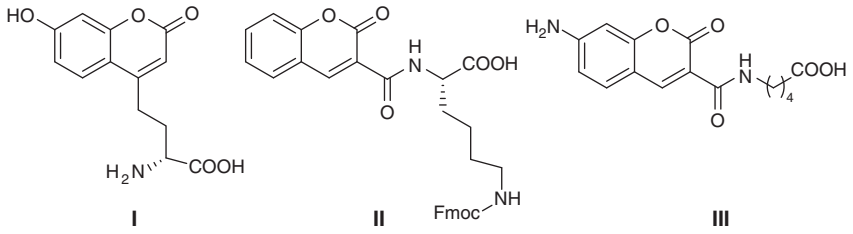
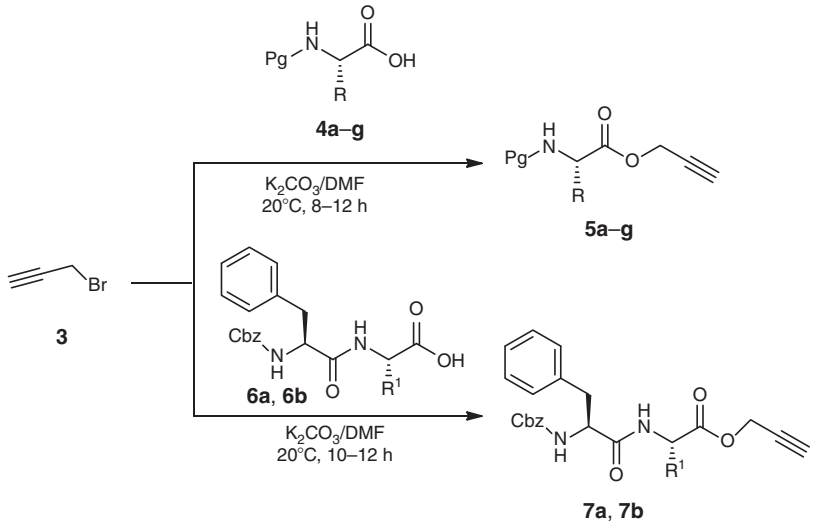


Fig. 1. Coumarin-tagged amino acids used in labelling studies.

Table 1. Synthesis of protected aminoacyl and dipeptidoyl *O*-propargyl esters 5 and 7



Serial no.	Protected amino acid and dipeptide	Product	Yield [%]	mp [°C]
1	Boc-L-Phe-OH (**4a**)	**5a**	90	Oil[24]
2	Cbz-L-Ala-OH (**4b**)	**5b**	86	58–60[25]
3	Cbz-L-Phe-OH (**4c**)	**5c**	88	64–66[24]
4	Fmoc-L-Phe-OH (**4d**)	**5d**	96	90–92[25]
5	Fmoc-L-Leu-OH (**4e**)	**5e**	98	96–97[25]
6	Fmoc-L-Ile-OH (**4f**)	**5f**	97	102–103
7	Boc-L-Ala-OH (**4g**)	**5g**	87	Oil[26]
8	Cbz-L-Phe-L-Trp-OH (**6a**)	**7a**	98	120–122
9	Cbz-L-Phe-L-Ala-OH (**6b**)	**7b**	96	126–128

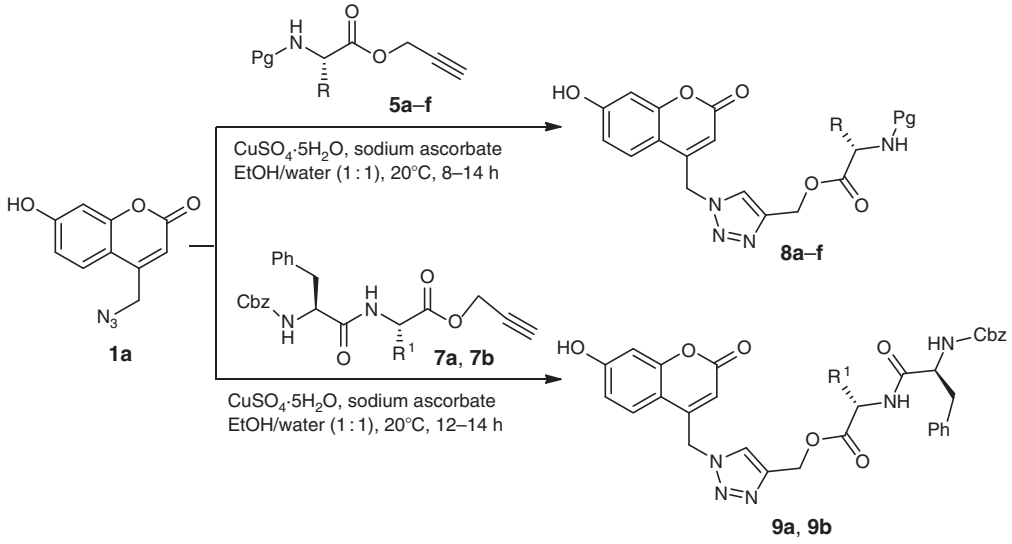
to act as a helical component,[16] a β-turn unit,[17] and a *cis* or *trans*-prolyl ratio modifier[18] in linear peptidomimetics has been studied by few researchers. Construction of a triazole ring has facilitated an increase in the emission intensity and variation in emission wavelength properties, desirable for molecules to act as fluorogenic probes.[19] Moreover, the beneficial effects on fluorescence properties of molecules acting as selective chemosensors have been produced by attaching triazole moiety as a linker[20] and as a coordinating ligand.[21] Thus, it was envisaged that augmenting triazole moiety to coumaryl-amino acid labels will yield candidates with fluorescence-emission properties that may be used for studying various biological interactions in labelling studies.

Results and Discussion

We plan to explore the application of click chemistry for synthesizing targeted molecules via two strategies. The first strategy involves clicking alkynyl-functionalized amino acids with coumarin-based azides. The second strategy involves clicking azide-functionalized amino acids with coumarin-based alkyne derivatives. Thus, azido-functionalized coumarins viz. 7-hydroxy-4-azidomethyl-2*H*-chromen-2-one (**1a**) and 4-azido-2*H*-chromen-2-one (**1b**) were synthesized by the reaction of NaN_3/DMF with 7-hydroxy-4-chloromethyl-2*H*-chromen-2-one and 4-chloro-2*H*-chromen-2-one, respectively, according to literature procedures.[22,23]

In order to employ the first strategy, alkynyl-functionalized amino acids-based clickable agents were prepared by esterification of different protected amino acids **4a**–**g** with propargyl bromide (**3**) using K_2CO_3 in DMF at 20°C to yield protected amino acyl *O*-propargyl esters **5a**–**g** (Table 1). Two novel dipeptidoyl *O*-propargyl esters, **7a** and **7b**, were envisaged and synthesized using the same method starting from their corresponding *Z*-protected dipeptides **6a** and **6b**, respectively (Table 1).

Table 2. Synthesis of 7-hydroxy-4-triazolylmethyl coumaryl-labelled amino acids and dipeptides 8 and 9



Serial no.	Protected amino acyl-*O*-propargyl esters	Product	Yield [%][A]	mp [°C]
1	Boc-L-Phe-$OCH_2C{\equiv}CH$ (**5a**)	**8a**	92	143–145
2	Cbz-L-Ala-$OCH_2C{\equiv}CH$ (**5b**)	**8b**	87	147–149
3	Cbz-L-Phe-$OCH_2C{\equiv}CH$ (**5c**)	**8c**	86	150–152
4	Fmoc-L-Phe-$OCH_2C{\equiv}CH$ (**5d**)	**8d**	85	148–150
5	Fmoc-L-Leu-$OCH_2C{\equiv}CH$ (**5e**)	**8e**	72	146–148
6	Fmoc-L-Ile-$OCH_2C{\equiv}CH$ (**5f**)	**8f**	74	143–146
7	Cbz-L-Phe-L-Trp-$OCH_2C{\equiv}CH$ (**7a**)	**9a**	85	155–158
8	Cbz-L-Phe-L-Ala-$OCH_2C{\equiv}CH$ (**7b**)	**9b**	85	153–155

[A]Isolated yield (before recrystallization).

We attempted the copper-catalyzed azide–alkyne cycloaddition (CuAAC) with clickable alkynyl-functionalized amino acid and dipeptide and azido-functionalized coumarin reagents. Due to the high fluorescence quantum yield, relatively large Stokes shift, small size, and pH sensitivity, the methodology was attempted on 7-hydroxycoumarin moiety. Thus, 7-hydroxy-4-azidomethyl-2*H*-chromen-2-one (**1a**) on cycloaddition with protected α-amino acyl *O*-propargyl esters **5a**–**f**, using $CuSO_4{\cdot}5H_2O$ and sodium ascorbate, yielded a series of 7-hydroxy-4-triazolylmethyl coumaryl-labelled amino acids **8a**–**f** in 72–92 % yield (Table 2). Furthermore, 7-hydroxy-4-azidomethyl-2*H*-chromen-2-one (**1a**) on cycloaddition with dipeptidoyl *O*-propargyl ester **7a** or **7b** using $CuSO_4{\cdot}5H_2O$ and sodium ascorbate yielded 7-hydroxy-4-triazolylmethyl coumaryl-labelled dipeptide **9a** or **b**, respectively (Table 2).

In order to make a comparison on the fluorescent behaviour of 7-substituted coumaryl derivatives with unsubstituted derivatives, we extended the methodology for synthesizing few derivatives of **1b**. Thus, 4-azido-2*H*-chromen-2-one (**1b**) on cycloaddition with few protected amino acyl *O*-propargyl esters, **5a**–**d** and **5g**, using $CuSO_4{\cdot}5H_2O$ and sodium ascorbate yielded a series of 4-triazolyl coumaryl-labelled amino acids, **10a**–**d** and **10g**, respectively, in 81–93 % yields (Table 3). 4-Azido-2*H*-chromen-2-one (**1b**) on cycloaddition with dipeptidoyl *O*-propargyl ester **7a** under similar conditions yielded 4-triazolyl coumaryl-labelled dipeptide **11a** in 78 % yield (Table 3).

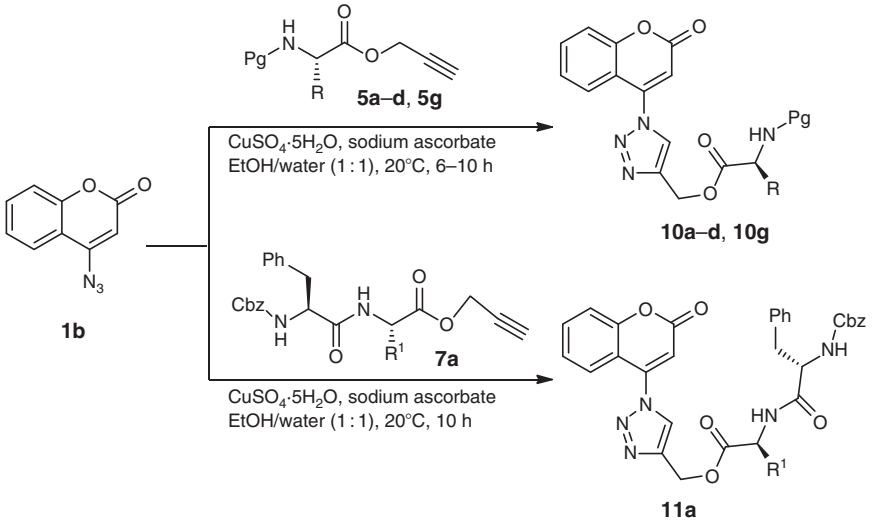
In the second strategy, 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one (**2a**) and 4-(prop-2-ynyloxy)-2*H*-chromen-2-one (**2b**) were prepared by *O*-alkylation of 4-methyl-7-hydroxy-2*H*-chromen-2-one and 4-hydroxy-2*H*-chromene-2-one, respectively, with propargyl bromide using K_2CO_3/CH_3CN following literature procedures.[27] α-Azido esters **12a**–**c** were also synthesized by the reaction of triflic azide (prepared in situ) with α-amino esters following literature procedures.[28–30]

Copper-catalyzed cycloaddition was then attempted with clickable alkynyl-functionalized coumarin and azido-functionalized amino acids. The cycloaddition of 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one (**2a**) and 4-(prop-2-ynyloxy)-2*H*-chromen-2-one (**2b**) and with clickable α-azido esters **12a**–**c** using $CuSO_4{\cdot}5H_2O$ and sodium ascorbate yielded two series of 7-*O*-triazolylmethyl and 4-*O*-triazolylmethyl coumaryl-labelled amino acids, **13a**–**f**, in 82–90 % yields (Table 4).

All the products were characterized by their detailed spectral studies (^{1}H NMR, ^{13}C NMR, and high-resolution mass spectrometry (HRMS)). The absence of any minor or duplicate signals in the ^{1}H NMR spectra of triazolyl-linked coumaryl-labelled dipeptides, **9a**, **9b**, **11a**, and their univocal correspondence to the expected structure showed them not to be a mixture of any possible diastereoisomers (that might have resulted due to two chiral centres of dipeptide).

The steady-state absorption and emission parameters of several newly synthesized derivatives of coumarins have been studied thoroughly in polar aprotic tetrahydrofuran (THF) solvent. As coumarin molecules are fluorescent probes, they are quite sensitive to their environment and display solvatochromism. Therefore, some sort of comparison with a more polar solvent than THF would be important. However, in the current investigation, these derivatives are insoluble in water. Thus, we have recorded UV-visible absorption and fluorescence spectra of some representative conjugates in polar protic methanol (MeOH) solvent. The absorption maxima (λ_{Abs}), emission

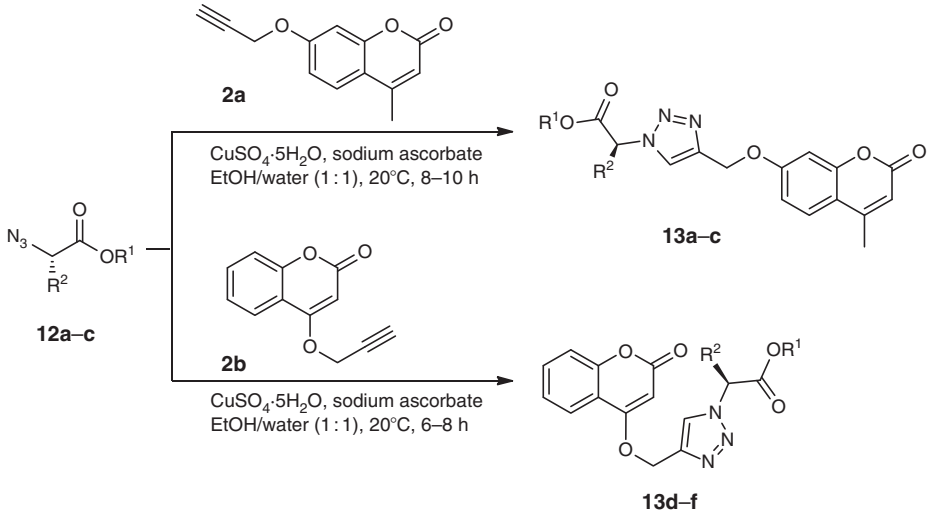
Table 3. Synthesis of 4-triazolyl-linked coumaryl-labelled amino acids and dipeptide 10 and 11



Serial no.	Protected amino acyl-*O*-propargyl esters	Product	Yield [%][A]	mp [°C]
1	Boc-L-Phe-$OCH_2C{\equiv}CH$ (**5a**)	**10a**	85	138–140
2	Cbz- L-Ala-$OCH_2C{\equiv}CH$ (**5b**)	**10b**	91	139–142
3	Cbz-L-Phe-$OCH_2C{\equiv}CH$ (**5c**)	**10c**	93	143–145
4	Fmoc-L-Phe-$OCH_2C{\equiv}CH$ (**5d**)	**10d**	89	134–136
5	Boc-L-Ala-$OCH_2C{\equiv}CH$ (**5g**)	**10g**	81	133–135
6	Cbz-L-Phe-L-Trp-$OCH_2C{\equiv}CH$ (**7a**)	**11a**	78	150–152

[A]Isolated yield (before recrystallization).

Table 4. Synthesis of 7-*O*-triazolylmethyl and 4-*O*-triazolylmethyl coumaryl-labelled amino acids 13



Serial no.	α-Azido ester (Reactant 1)	Reactant 2	Product	Yield [%][A]	mp [°C]
1	N_3-L-Gly-OEt (**12a**)	**2a**	**13a**	90	128–130
2	N_3-L-Phe-OMe (**12b**)	**2a**	**13b**	87	130–132
3	N_3-L-Trp-OMe (**12c**)	**2a**	**13c**	85	134–136
4	N_3-L-Gly-OEt (**12a**)	**2b**	**13d**	88	129–132
5	N_3-L-Phe-OMe (**12b**)	**2b**	**13e**	85	133–135
6	N_3-L-Trp-OMe (**12c**)	**2b**	**13f**	82	138–140

[A]Isolated yield (before recrystallization).

Table 5. Spectroscopic steady-state data of 7-hydroxy-4-triazolylmethyl and 4-triazolyl coumaryl-labelled amino acids and dipeptide

Compound	Solvent	λ_{Abs} (nm)	λ_{Em} (nm)	$\bar{\nu}_a - \bar{\nu}_f$ [cm^{-1}]	ε [M^{-1} cm^{-1}]	Quantum yield, Φ
8a	THF	323	388	5186.6	7860	0.136
8b	THF	323	388	5199.9	18500	0.140
8c	THF	323	387	5120.0	9120	0.162
8d	THF	266	389	5252.8	16460	0.184
	MeOH	265	411	6250.1	13840	0.220
8e	THF	266	388	5119.9	20860	0.150
8f	THF	266	387	5119.9	19700	0.143
9a	THF	324	388	5120.0	4960	0.190
9b	THF	275	388	5186.6	13780	0.161
10a	THF	280	383	5003.3	27580	0.018
10b	THF	280	383	5071.9	9620	0.025
10c	THF	280	383	5101.2	26900	0.048
10d	THF	267	381	5042.9	12860	0.091
	MeOH	265	411	6343.1	5820	0.140
10g	THF	280	413	7036.9	17640	0.009
11a	THF	281	383	5476.8	9400	0.096

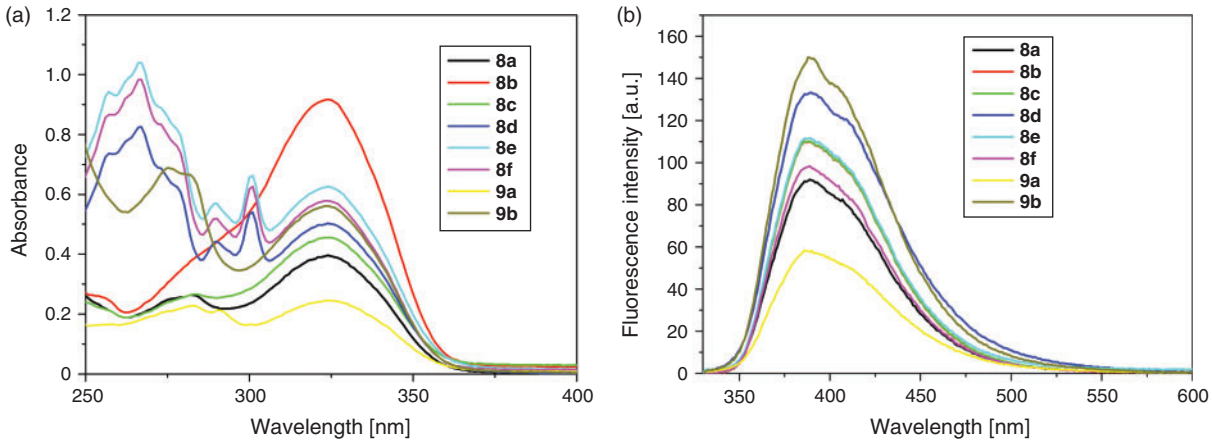


Fig. 2. (a) Absorption spectra and (b) emission spectra of compounds **8a**–**f**, **9a**, **9b** (5×10^{-5} M) in THF; $\lambda_{ex} = 325$ nm.

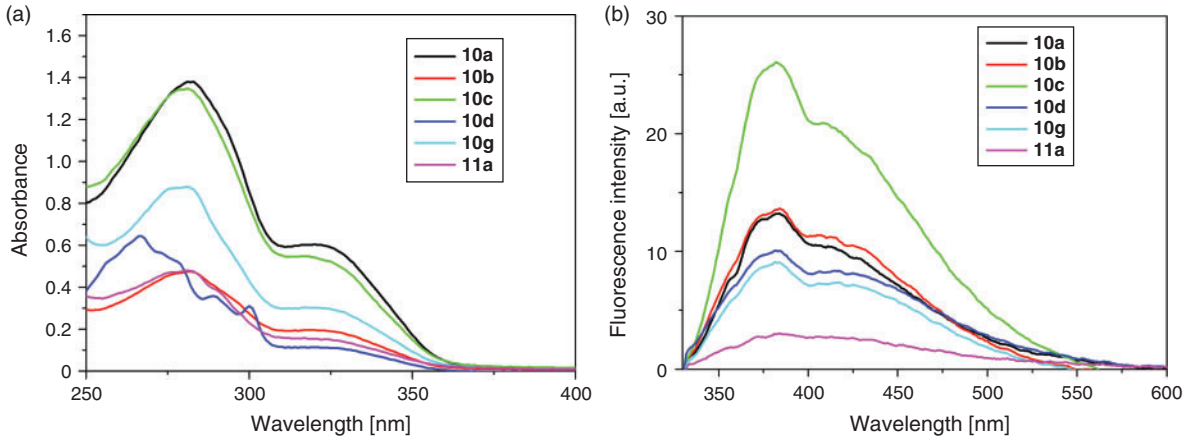


Fig. 3. (a) Absorption spectra and (b) emission spectra of compound **10a**–**d**, **10g**, **11a** (5×10^{-5} M) in THF; $\lambda_{ex} = 325$ nm.

maxima (λ_{Em}), Stokes shift ($\bar{\nu}_a - \bar{\nu}_f$), extinction coefficient (ε), and quantum yield (Φ) of compounds **8a**–**f**, **9a**, **9b**, **10a**–**d**, **10g**, **11a** in THF are listed in Table 5. The corresponding absorption and emission spectra are shown in Figs 2 and 3.

In general, 7-hydroxytriazolylmethyl- and 4-triazolyl-linked coumaryl amino acids and dipeptide conjugates (**8**, **9**, **10**, **11**) showed absorption maxima in the wavelength region of 265–324 nm and are shown in Table 5. The well-resolved absorption band around 325 nm is due to a $\pi \rightarrow \pi^*$ transition to the S_1 state. The absorbance of 4-triazolyl-linked coumaryl-labelled amino acids and dipeptides was less than that for 7-hydroxytriazolylmethyl-linked coumaryl amino acids and

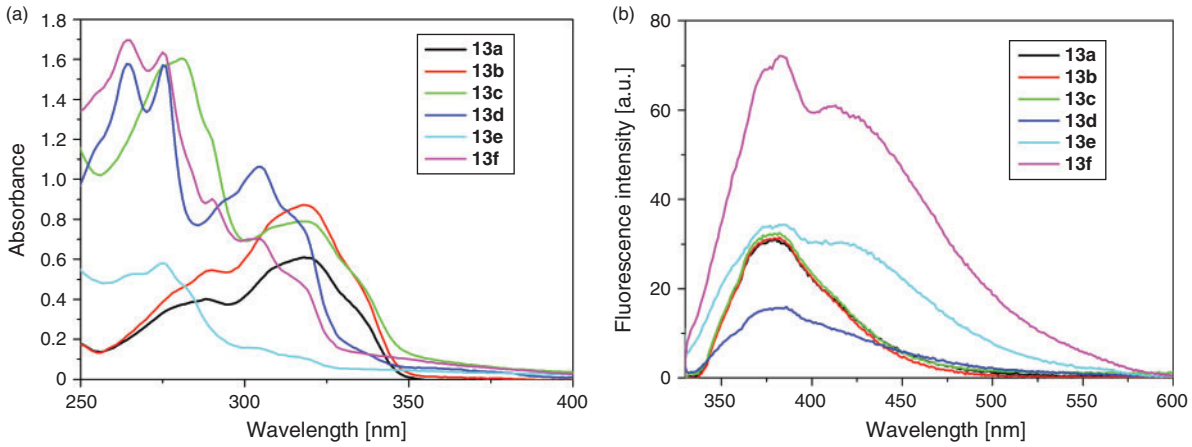


Fig. 4. (a) Absorption spectra and (b) emission spectra of compounds **13a**–**f** (5×10^{-5} M) in THF; $\lambda_{ex} = 325$ nm.

Table 6. Spectroscopic steady-state data of 7-*O*- triazolylmethyl and 4-*O*-triazolylmethyl coumaryl-labelled amino acids

Compound	Solvent	λ_{Abs} [nm]	λ_{Em} [nm]	$\bar{\nu}_a - \bar{\nu}_f$ [cm^{-1}]	ε [M^{-1} cm^{-1}]	Quantum yield, Φ
13a	THF	318	380	4992.7	6360	0.201
13b	THF	318	380	5061.3	17500	0.240
	MeOH	316	392	6134.8	23600	0.302
13c	THF	280	381	5336.8	32000	0.280
13d	THF	275	382	5090.7	31200	0.038
13e	THF	275	382	5535.9	11600	0.082
	MeOH	265	401	6004.5	5640	0.120
13f	THF	275	382	5535.9	32400	0.096

dipeptide conjugates. The absorbance maxima of 7-hydroxy-triazolylmethyl-linked coumaryl amino acids are clearly red shifted (50 nm) than that for 4-triazolyl-linked coumaryl-labelled amino acids and dipeptides. The ($\bar{\nu}_a - \bar{\nu}_f$) is expressed in wave number and are calculated by taking the difference between last absorption maximum and first emission maximum of each derivative studied. Given the different nature of the various absorption bands due to difference in structural aspects of the conjugates (amino acids: Gly (Glycine), Ala (Alanine), Phe (Phenyl alanine), Trp (Tryptophan), Ile (Isoleucine), Leu (Leucine), and protecting groups: Fmoc (Fluorenylmethyloxy-carbonyl), Cbz (carboxybenzyl), Boc (t-butoxycarbonyl)), the excitation wavelength in fluorescence measurements is crucial and it will make sense to compare the various fluorescence quantum yields of the conjugates only if excitation is performed in the charge-transfer band region. So, the fluorescence spectrum measurements in the present study have been performed by exciting at 325 nm.

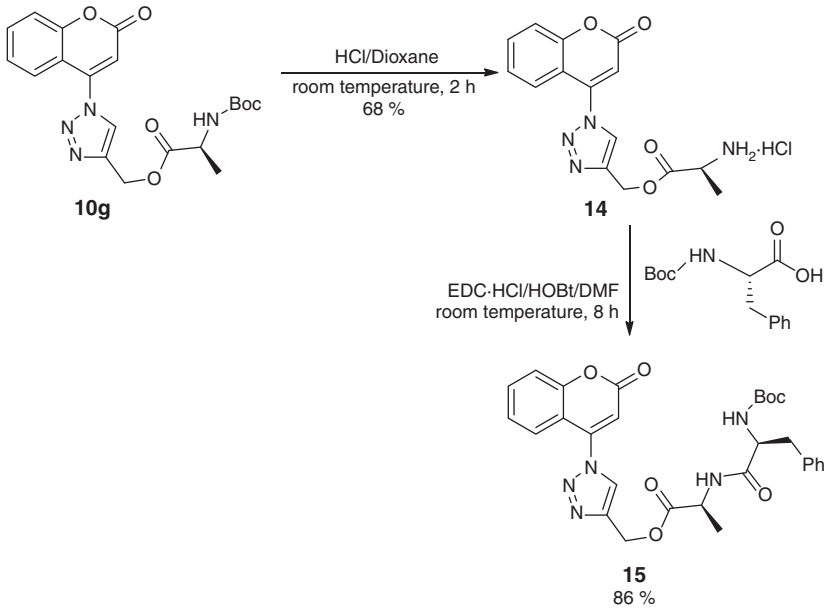
Both the locally excited (LE) band around 380 nm and intermolecular charge transfer (ICT) shoulder around 415 nm have been observed in the fluorescence spectra of all the coumarin derivatives **10a**–**d** and **10g** studied upon excitation at 325 nm. The quantum yields of 7-hydroxy-4-triazolylmethyl coumaryl-labelled amino acids and dipeptide, **8a**–**d** and **9a**, were found be greater than the corresponding 4-triazolyl coumaryl derivatives **10a**–**d** and **11a**.

4-*O*-Triazolyl- and 7-*O*-triazolylmethyl-linked coumaryl amino acids **13a**–**f** showed absorption maxima in the wavelength region of 264–326 nm and are shown in Fig. 4. The corresponding spectroscopic data are given in Table 6. The absorbance maxima of 7-*O*-triazolylmethyl-linked coumaryl amino acids **13a**–**c** are clearly red shifted (50 nm) than that for 4-*O*-triazolyl-linked coumaryl-labelled amino acids **13d**–**f**. The conjugate **13c** possessing the natural fluorescent amino acid tryptophan showed the maximum value of quantum yield (0.280) as compared with all others in THF.

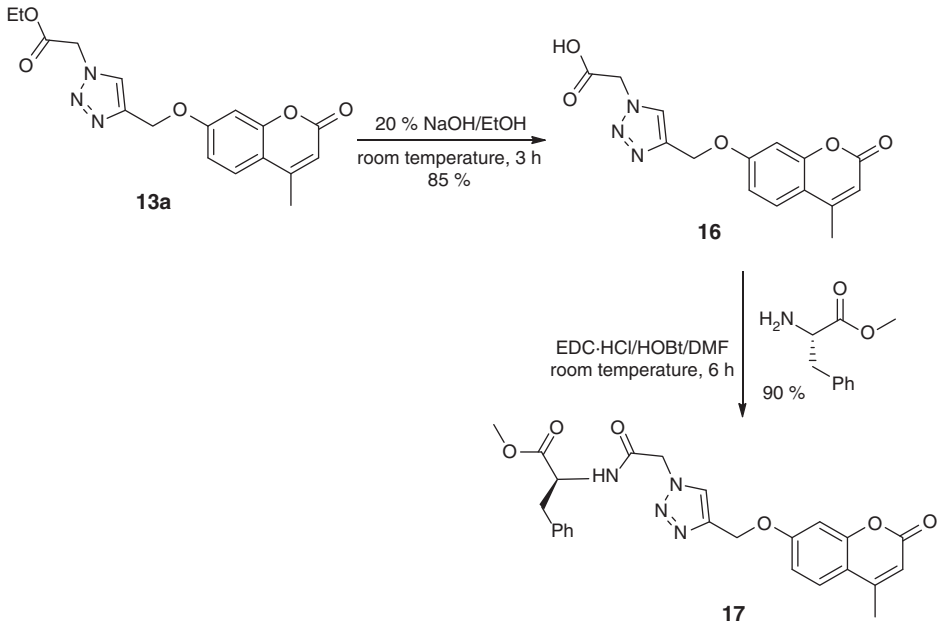
The electron-donating functionalities cause an increase in the quantum yield of molecules. The observed bathochromic shift of fluorescence maxima and increase in quantum yield could be possibly due to the electron-donating hydroxyl group at position 7 of coumaryl derivatives. These effects are more prominent for coumarin derivatives with *O*-substituted triazolylmethyls than that for unsubstituted derivatives and possibly due to the intermolecular hydrogen bonding in the former. The natural tryptophan amino acid emits in the wavelength range of 330–350 nm, whereas the different amino acid derivatives studied in the present manuscript emit in the range of 340–560 nm.

To further demonstrate the chemical utility of the synthesized triazolyl-linked coumaryl-labelled amino acid conjugates as fluorescent labels, their linear elongation by coupling them with appropriate *C*- or *N*-terminus amino acid in solution phase was attempted. Thus, **10g** on reaction with dioxane/HCl yielded unprotected 4-triazolyl-linked coumaryl-labelled amino acid hydrochloride salt **14** in 68 %, which on further reaction with Boc-L-phenylalanine using EDC (*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide)·HCl/HOBt (Hydroxybenzotriazole) in DMF yielded **15** in 86 % yield (Scheme 1).

The absence of any minor or duplicate signals in the ^{1}H NMR spectrum of **15** and their univocal correspondence to the expected structure revealed the absence of any possible diastereoisomers or epimers mixtures.



Scheme 1. Linear elongation of 4-triazolyl-linked coumaryl amino acid **10g** with *C*-terminus amino acid.



Scheme 2. Linear elongation of 7-*O*-triazolylmethyl-linked coumaryl amino acid **13a** with *N*-terminus amino acid.

Similar chemical applicability of 7-*O*-triazolylmethyl coumaryl-labelled amino acid conjugates as fluorescent labels was demonstrated on one of its derivatives viz. **13a**. Hydrolysis of **13a** with 20 % NaOH solution in ethanol followed by acidic workup gave **16** in 85 % yield, which on reaction with L-phenylalanine methyl ester using EDC·HCl/HOBt in DMF gave **17** in 90 % yield (Scheme 2).

The methodology used for the linear elongation of glycine-based 7-*O*-triazolylmethyl coumaryl-labelled amino acid conjugate **13a** (Scheme 2) was very similar to that followed in Scheme 1 and thus it was assumed that epimerization will not be a problem if the linear elongation were to be carried on chiral substrates.

The absorption and emission spectra of **15** and **17** were also recorded in THF in order to compare and contrast different spectroscopic data with **10g** and **13a**. The absorption and emission data **15** and **17** are summarized in Table 7. Clearly, there is a significant increase in quantum yields of **15** and **17** when compared with that of **10g** and **13a**. This observation suggests that incorporation of another amino acid into triazolyl-based fluorescent conjugates makes these significantly more fluorescent.

However, biological interactions in peptides are usually studied in aqueous solutions. Because the conjugates are water insoluble, a comparative study using a polar protic solvent (MeOH) of some representative conjugates (**8d**, **10d**, **13b**, and

Table 7. Spectroscopic steady-state data of 4- triazolyl and 7-*O*-triazolylmethyl coumaryl-linked amino acids with *C*-terminus amino acid and *N*-terminus amino acid

Compound	Solvent	λ_{Abs} [nm]	λ_{Em} [nm]	$\bar{\nu}_a - \bar{\nu}_f$ [cm^{-1}]	ε [$M^{-1}\,cm^{-1}$]	Quantum yield, Φ
10g	THF	280	384	7036.9	17640	0.009
15	THF	281	381	5071.9	10800	0.110
13a	THF	318	380	4962.7	6360	0.201
17	THF	265	382	5306.3	19500	0.340

13e) indicated a red shift in the absorption and emission maxima in the UV-visible absorption and fluorescence spectra. The shift of fluorescence maxima is more pronounced than the absorption maxima. This behaviour is indicative of an emitting state that is more polar than the ground state. Interestingly, the quantum yield values in MeOH are significantly higher than that in THF and exhibited larger Stokes shifts. The Stokes shift is considerably larger than that expected on the basis of solvent polarity alone. We believe that the hydrogen bonding reaction of the fluorophore with the hydroxyl MeOH is responsible for the large Stokes-shifted fluorescence. The observation of polarity-dependent enhancement of quantum yield and bathochromic shift of absorption spectra is consistent with our assignment of absorption band to transition to the first excited state in these derivatives.

In addition, the water soluble version **14** of one of the conjugates **10g** (λ_{Em} (THF) = 413 nm, $\Phi = 0.009$) was characterized by a significant red shift of its fluorescence emission in water ($\lambda_{Em} = 430$ nm, $\Phi = 0.046$) when compared with THF ($\lambda_{Em} = 383$ nm, $\Phi = 0.016$).

Conclusion

In summary, we have reported an efficient synthesis of triazolyl-conjugated coumaryl–amino acid conjugates by introducing fluorophores on the *C*- and *N*-termini of natural amino acids and few dipeptides. Spectroscopic studies showed that 7-triazolyl-linked conjugates showed better fluorescence behaviour than 4-triazolyl-linked conjugates. On removing the protecting group, hydrosoluble coumaryl-tagged amino acids could be obtained, to which amino acids and peptides could be coupled. Thus, our methodology presents a useful tool for the synthesis of environment-sensitive fluorescent amino acid conjugates, with potential applications in labelling studies.

Experimental

General

All chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in open capillary tubes on a MPA120-automated melting point apparatus and are uncorrected. Reactions were monitored using thin layer chromatography (TLC) on 0.2 mm silica gel F254 plates (Merck). The chemical structures of final products and intermediates were characterized by nuclear magnetic resonance spectra (^{1}H NMR, 13C NMR) determined on a Bruker NMR spectrometer (300 (or 400) MHz, 75 (or 101 MHz). ^{13}C NMR spectra are fully decoupled. Chemical shifts were reported in parts per million (ppm) using deuterated solvent peak or tetramethylsilane as internal standard. The chemical structures of final products were confirmed on a high-resolution Biosystems QStar Elite time-of-flight electrospray mass spectrometer. HRMS was performed on an Agilent 6210 instrument using time-of-flight mass spectrometry (TOF-MS) with electrospray ionization (ESI). Electronic absorption spectra were taken using dual beam Thermo Evolution 201 UV/Vis/NIR spectrophotometer and fluorescence spectra were recorded using Shimadzu RF-5301PC spectrofluorometer. The data were analyzed using the related software. The concentration of the coumarin derivatives in all the solutions prepared in solvents THF and MeOH was 5×10^{-5} M. The fluorescence quantum yields of all the coumarin derivatives in THF and MeOH were estimated by using quinine sulfate in 0.1N sulfuric acid ($\Phi = 0.55$) as the standard reference.[31–32]

Synthesis

Typical Procedure for the Synthesis of Protected Amino Acyl or Dipeptidoyl O-Propargyl Esters ***5***, ***7***

N-Protected amino acid or dipeptide **4a**–**g** or **6a**, **6b** (1 equiv.) was dissolved in anhydrous DMF (10 mL) and the solution was cooled to −10°C. Anhydrous K_2CO_3 (1.5 equiv.) was added to the solution and the stirring continued until a syrup-like solution formed. Propargyl bromide (2 equiv., 80 % solution in toluene) was added dropwise to the reaction mixture, and the stirring continued at −10°C for 1 h. The reaction mixture was then stirred at room temperature for 8–12 h and monitored via TLC until the disappearance of the starting material. The reaction mixture was then added dropwise in an ice bath, and the precipitated white solid was collected by filtration and washed with cold water (3 × 20 mL) to yield pure **5** or **7**.

Prop-2-yn-1-yl((benzyloxy)carbonyl)-L-phenylalanyl-L-tryptophanate (**7a**) (0.110 g, 98 %), white solid, mp 120–122°C. δ_H ([D6]DMSO + $CDCl_3$, 400 MHz) 10.84 (1H, s), 8.44 (1H, br s), 7.52 (1H, s), 7.41–7.12 (13H, m), 7.11–6.95 (2H, m), 5.02–4.85 (2H, m), 4.75–4.58 (3H, m), 4.34 (1H, br s), 3.38 (1H, s), 3.18 (2H, d, *J* 20.1), 3.01 (1H, d, *J* 11.9), 2.74 (1H, s). δ_C ([D6]DMSO + $CDCl_3$, 101 MHz) 171.7, 170.8, 155.7, 137.9, 136.8, 136.0, 129.1, 128.1, 127.8, 127.5, 127.4, 127.0, 126.1, 123.7, 120.9, 118.4, 117.9, 111.3, 108.9, 77.7, 77.5, 65.3, 55.8, 52.9, 52.2, 37.5, 26.8.

Prop-2-yn-1-yl((benzyloxy)carbonyl)-L-phenylalanyl-L-alaninate (**7b**) (0.105 g, 96 %), white solid, mp 126–128°C. δ_H ([D6]DMSO, 300 MHz) 8.54 (1H, d, *J* 6.0), 7.48 (1H, d, *J* 8.1), 7.37–7.09 (10H, m), 4.90 (2H, s), 4.70 (2H, s), 4.30 (2, dd, *J* 13.1, 6.8), 3.55 (1H, s), 3.00 (1H, d, *J* 13.2), 2.71 (1H, t, *J* 12.1), 1.31 (3H, d, *J* 6.5). δ_C ([D6]DMSO, 75 MHz) 172.3, 156.4, 138.7, 137.5, 129.7, 128.8, 128.6, 128.2, 128.0, 126.8, 78.7, 78.5, 65.7, 56.4, 52.9, 48.1, 37.9, 17.2.

Typical Procedure for the Synthesis of Triazolyl-Linked Coumaryl-Labelled Amino Acids or Peptides ***8a–f***, ***9a***, ***9b***, ***10a–d***, ***10g***, ***11a***

To a solution of 4-azidomethyl-7-hydroxycoumarin (**1a**) or 4-azido coumarin (**1b**) (0.100 g, 1 equiv.) in ethanol/H_2O (1 : 1)

was added protected amino acyl *O*-propargyl ester **5** or **7** (1 equiv.), followed by addition of $CuSO_4$ (0.01 equiv.) and sodium ascorbate (0.1 equiv.). The reaction mixture was stirred at room temperature for 6–14 h and monitored via TLC. After completion of the reaction, the reaction mixture was evaporated and the residue was diluted with water and ammonium hydroxide (2–3 mL) and extracted into dichloromethane. Organic layer was separated, dried over anhydrous sodium sulfate to evaporate the volatiles. The crude compound was recrystallized from ethanol to yield pure **8a**–**f**, **9a**, **9b**, **10a**–**d**, **10g**, **11a**.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl(tert-butoxycarbonyl)-*L*-phenylalaninate* (**8a**) (0.235 g, 85 %), white solid, mp 143–145°C. δ_H ([D6]DMSO, 400 MHz) 10.65 (1H, s), 8.21 (1H, s), 7.71 (1H, d, *J* 8.7), 7.27–7.16 (6H, m), 6.83 (1H, dd, *J* 8.7, 7.1), 6.76 (1H, s), 5.90 (2H, s), 5.62 (1H, s), 5.25–5.12 (2H, m), 4.24–4.14 (1H, m), 2.98 (1H, dd, *J* 13.8, 5.0), 2.86 (1H, dd, *J* 13.5, 10.1), 1.29 (9H, s). δ_C ([D6]DMSO + $CDCl_3$, 101 MHz) 171.8, 161.6, 159.8, 155.3, 155.1, 150.2, 142.1, 137.3, 129.0, 128.0, 126.3, 125.9, 125.7, 113.1, 109.4, 109.3, 102.5, 78.2, 57.6, 55.2, 49.1, 36.3, 28.0. *m*/*z* (HRMS ESI) 543.1837; $[M + Na]^+$ requires 543.1850.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl((benzyloxy)carbonyl)-*L*-alaninate* (**8b**) (0.200 g, 92 %), white solid, mp 147–149°C. δ_H ($CDCl_3$, 300 MHz) 7.77 (1H, s), 7.30 (1H, d, *J* 7.5), 7.22–7.14 (6H, m), 6.70 (1H, d, *J* 7.6), 6.61 (1H, s), 5.62–5.55 (2H, m), 5.51 (2H, s), 5.20–5.13 (1H, m), 5.01–4.90 (2H, m), 4.27–4.17 (1H, m), 1.26 (3H, d, *J* 6.5). δ_C ($CDCl_3$, 75 MHz) 173.0, 161.5, 161.18, 156.1, 155.2, 148.9, 143.1, 136.0, 128.6, 128.3, 128.0, 125.5, 125.0, 114.0, 110.5, 109.7, 103.5, 67.1, 58.1, 50.3, 49.8, 18.0. *m*/*z* (HRMS ESI) 501.1390; $[M + Na]^+$ requires 501.1381.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl((benzyloxy)carbonyl)-*L*-phenylalaninate* (**8c**) (0.220 g, 87 %), white solid, mp 150–152°C. δ_H ([D6]DMSO, 400 MHz) 8.27 (1H, s), 7.87 (1H, d, *J* 8.1), 7.73 (1H, d, *J* 8.8), 7.36–7.29 (3H, m), 7.28–7.21 (8H, m), 6.86 (1H, dd, *J* 8.7, 2.3), 6.78 (1H, d, *J* 2.3), 5.94 (2H, s), 5.61 (1H, s), 5.23 (2H, s), 5.02–4.95 (2H, m), 4.30 (1H, ddd, *J* 10.0, 8.1, 5.2), 3.03 (1H, dd, *J* 13.8, 5.1), 2.88 (1H, dd, *J* 13.7, 10.1). δ_C ([D6]DMSO, 101 MHz) 171.6, 161.8, 159.9, 155.9, 155.1, 150.4, 142.1, 142.0, 137.2, 137.1, 136.8, 129.0, 128.3, 128.2, 127.7, 127.5, 127.0, 126.5, 126.1, 125.9, 113.2, 109.3, 102.5, 65.4, 57.7, 55.5, 49.2, 36.3. *m*/*z* (HRMS ESI) 577.1693; $[M + Na]^+$ requires 577.1694.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-phenylalaninate* (**8d**) (0.250 g, 86 %), white solid, mp 148–150°C. δ_H ($CDCl_3$, 300 MHz) 7.72 (2H, d, *J* 7.5), 7.61 (1H, s), 7.53–7.47 (3H, m), 7.41–7.33 (4H, m), 7.31–7.27 (2H, m), 7.22–7.16 (3H, m), 7.04 (1H, s), 6.83 (1H, d, *J* 8.5), 6.76 (1H, s), 5.77 (1H, s), 5.60–5.38 (2H, m), 5.32–5.17 (2H, m), 4.61 (1H, dd, *J* 13.7, 7.6), 4.34 (2H, dd, *J* 21.2, 7.9), 4.13 (1H, t, *J* 6.7), 3.15–3.00 (2H, m). δ_C ($CDCl_3$, 75 MHz) 171.4, 162.0, 161.5, 160.8, 156.1, 155.8, 155.6, 148.3, 148.0, 143.6, 143.1, 141.3, 135.5, 129.3, 128.7, 127.8, 127.2, 120.0, 114.0, 111.5, 109.8, 103.9, 67.2, 58.2, 55.0, 47.1, 38.0, 29.8. *m*/*z* (HRMS ESI) 665.2021; $[M + Na]^+$ requires 665.2007.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-leucinate* (**8e**) (0.235 g, 84 %), white solid, mp 146–148°C. δ_H ([D6]DMSO, 400 MHz) 8.30 (1H, s), 7.90 (2H, d, *J* 7.4), 7.85 (1H, t, *J* 8.7), 7.74–7.67 (2H, m), 7.43 (2H, t, *J* 7.2), 7.38–7.30 (2H, m), 6.84 (1H, d, *J* 8.5), 6.77 (1H, s), 6.29 (1H, s), 5.92 (2H, d, *J* 10.5), 5.53–5.51 (1H, m), 5.28–5.18 (2H, m), 4.36–4.19 (2H, m), 4.12–4.01 (1H, m), 3.39 (1H, q, *J* 6.8), 1.66–1.38 (2H, m), 1.10 (1H, t, *J* 6.9), 0.85 (6H, 2d, *J* 5.5 each). δ_C ([D6]DMSO, 101 MHz) 172.6, 162.1, 159.9, 156.1, 155.1, 150.4, 143.8, 142.5, 142.2, 139.6, 126.0, 125.2, 121.3, 113.3, 109.7, 109.1, 108.9, 102.5, 65.6, 64.9, 57.5, 52.2, 49.1, 46.6, 24.1, 22.7, 21.1. *m*/*z* (HRMS ESI) 631.2189; $[M + Na]^+$ requires 631.2163.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-isoleucinate* (**8f**) (0.235 g, 85 %), white solid, mp 143–146°C. δ_H ([D6]DMSO + $CDCl_3$, 400 MHz) 10.58 (1H, s), 8.26 (1H, s), 7.84 (2H, d, *J* 7.5), 7.76–7.69 (3H, m), 7.66 (1H, d, *J* 8.8), 7.40 (2H, t, *J* 7.4), 7.31 (2H, t, *J* 6.6), 6.81 (1H, dd, *J* 8.7, 2.3), 6.74 (1H, d, *J* 2.3), 5.87 (2H, s), 5.55 (1H, s), 5.24 (2H, dd, *J* 27.9, 12.7), 4.28 (2H, d, *J* 7.2), 4.24–4.18 (1H, m), 4.04–3.99 (1H, m), 1.84–1.75 (1H, m), 1.42–1.33 (1H, m), 1.24 (1H, s), 0.83–0.73 (6H, m). δ_C ([D6]DMSO + $CDCl_3$, 101 MHz) 171.5, 161.6, 159.8, 156.2, 155.1, 150.1, 143.7, 143.6, 142.2, 140.7, 127.5, 126.9, 125.8, 125.7, 125.2, 119.9, 113.1, 109.2, 109.2, 102.5, 65.7, 58.5, 57.2, 49.1, 46.6, 36.1, 24.8, 15.3, 10.9. *m*/*z* (HRMS ESI) 631.2164; $[M + Na]^+$ requires 631.2164.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl((benzyloxy)carbonyl)-*L*-phenylalanyl-*L*-tryptophanate* (**9a**) (0.240 g, 72 %), yellow solid, mp 155–158°C. δ_H ([D6]DMSO, 300 MHz) 10.88 (1H, s), 8.51 (1H, d, *J* 7.0), 8.14 (1H, s), 7.69 (1H, d, *J* 8.6), 7.46 (2H, dd, *J* 18.6, 8.3), 7.34–7.17 (14H, m), 7.10–6.95 (2H, m), 6.84 (1H, d, *J* 8.6), 6.77 (1H, s), 5.88 (2H, s), 5.54 (1H, s), 5.21–5.11 (2H, m), 4.98–4.86 (2H, m), 4.57 (1H, dd, *J* 12.4, 6.0), 4.32 (1H, t, *J* 7.4), 3.22–3.09 (2H, m), 2.94 (1H, d, *J* 14.1), 2.67 (1H, t, *J* 11.7). δ_C ([D6]DMSO, 75 MHz) 172.4, 171.9, 162.3, 160.5, 156.3, 155.9, 155.6, 155.3, 151.3, 150.9, 142.7, 138.6, 137.5, 136.6, 129.7, 128.8, 128.5, 128.2, 128.0, 127.6, 126.7, 126.6, 126.3, 124.3, 121.5, 119.0, 118.5, 113.8, 112.0, 109.7, 103.10, 65.8, 58.3, 56.4, 53.7, 49.7, 38.0, 27.4. *m*/*z* (HRMS ESI) 763.2480; $[M + Na]^+$ requires 763.2487.

*(1-((7-Hydroxy-2-oxo-2*H*-chromen-4-yl)methyl)-1*H*-1,2,3-triazol-4-yl)methyl((benzyloxy)carbonyl)-*L*-phenylalanyl-*L*-alaninate* (**9b**) (0.220 g, 74 %), yellow solid, mp 153–155°C. δ_H ([D6]DMSO, 400 MHz) 10.79 (1H, br s), 8.57 (1H, s), 8.32 (1H, s), 7.70 (1H, d, *J* 8.3), 7.48 (1H, d, *J* 7.9), 7.38–7.17 (10H, m), 6.85 (1H, d, *J* 8.2), 6.78 (1H, s), 5.91 (2H, s), 5.55 (1H, s), 5.28–5.17 (2H, m), 4.94 (2H, s), 4.31 (2H, d, *J* 7.5), 2.99 (1H, d, *J* 13.6), 2.69 (1H, t, *J* 11.8), 1.31 (3H, br s). δ_C ([D6]DMSO, 101 MHz) 172.2, 171.7, 161.8, 159.9, 155.8, 155.1, 150.4, 142.3, 138.1, 137.0, 129.2, 128.2, 128.0, 127.6, 127.4, 127.0, 127.0, 126.2, 126.0, 125.9, 113.2, 109.3, 109.1, 102.5, 65.1, 57.6, 55.7, 49.1, 47.6, 37.3, 16.7. *m*/*z* (HRMS ESI) 648.2085; $[M + Na]^+$ requires 648.2065.

*(1-(2-Oxo-2*H*-chromen-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-*L*-phenylalaninate* (**10a**) (0.220 g, 85 %), white solid, mp 138–140°C. δ_H ($CDCl_3$, 300 MHz) 8.05 (1H, s), 7.93 (1H, d, *J* 7.6), 7.77 (1H, t, *J* 7.7), 7.56 (1H, d, *J* 8.3), 7.45 (1H, t, *J* 7.5), 7.36–7.27 (3H, m), 7.19 (2H, d, *J* 6.8), 6.62 (1H, s), 5.47 (2H, s), 5.08 (1H, d, *J* 6.5), 4.68 (1H, d, *J* 6.0), 3.18 (2H, d, *J* 6.8), 1.47 (9H, s). δ_C ($CDCl_3$, 75 MHz) 172.0, 159.6, 155.3, 154.4, 146.7, 135.8, 133.8, 129.4, 128.7, 127.3, 125.6, 125.2, 117.7, 114.3, 110.2, 80.4, 58.1, 54.7, 38.1, 28.4. *m*/*z* (HRMS ESI) 513.1751; $[M + Na]^+$ requires 513.1745.

*(1-(2-Oxo-2*H*-chromen-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl ((benzyloxy)carbonyl)-*L*-alaninate* (**10b**) (0.215 g, 91 %), white solid, mp 139–142°C. δ_H ($CDCl_3$, 300 MHz) 7.97

(1H, s), 7.78–7.63 (1H, m), 7.59–7.46 (1H, m), 7.38–7.27 (1H, m), 7.23–7.03 (6H, m), 6.43 (1H, s), 5.39–5.02 (3H, m), 4.94 (2H, s), 4.24 (1H, d, *J* 3.5), 1.29 (3H, br s). δ_C ($CDCl_3$, 75 MHz) 173.1, 159.6, 156.4, 155.8, 154.4, 146.6, 136.5, 136.2, 133.7, 128.6, 128.2, 128.0, 125.6, 125.1, 117.7, 114.3, 110.2, 67.1, 58.2, 49.9, 18.2. *m/z* (HRMS ESI) 471.1282; $[M + Na]^+$ requires 471.1275.

*(1-(2-Oxo-2*H*-chromen-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl ((benzyloxy)carbonyl)-L-phenylalaninate* (**10c**) (0.260 g, 93 %), white solid, mp 143–145°C. δ_H ($CDCl_3$, 300 MHz) 7.84 (1H, s), 7.76 (1H, d, *J* 7.9), 7.61 (1H, t, *J* 7.7), 7.40 (1H, d, *J* 8.4), 7.30 (4H, d, *J* 7.7), 7.19–7.16 (2H, m), 7.17–7.10 (3H, m), 7.01 (2H, d, *J* 6.7), 6.46 (1H, s), 5.34 (2H, t, *J* 8.2), 5.20 (1H, d, *J* 7.9), 5.01 (2H, s), 4.61 (1H, dd, *J* 12.8, 6.2), 3.06 (2H, d, *J* 5.4). δ_C ($CDCl_3$, 75 MHz) 171.6, 159.7, 159.4, 155.8, 154.4, 146.6, 143.4, 136.1, 135.4, 133.8, 129.4, 128.7, 128.3, 128.1, 127.4, 125.6, 125.2, 121.5, 117.7, 114.2, 110.2, 67.3, 58.2, 55.0, 38.1. *m/z* (HRMS ESI) 547.1599; $[M + Na]^+$ requires 547.1588.

*(1-(2-Oxo-2*H*-chromen-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)-L-phenylalaninate* (**10d**) (0.290 g, 89 %), white solid, mp 134–136°C. δ_H ($CDCl_3$, 300 MHz) 7.90 (1H, s), 7.76 (4H, m), 7.63 (1H, t, *J* 7.5), 7.53 (2H, d, *J* 7.0), 7.41 (4H, dd, *J* 18.5, 7.8), 7.33–7.27 (3H, m), 7.24–7.17 (2H, m), 7.14–7.04 (1H, m), 6.50 (1H, s), 5.50–5.35 (2H, m), 5.34–5.25 (1H, m), 4.71 (1H, t, *J* 6.1), 4.39 (2H, dd, *J* 17.8, 10.5), 4.18 (1H, d, *J* 6.1), 3.15 (2H, d, *J* 6.2). δ_C ($CDCl_3$, 75 MHz) 171.5, 159.5, 155.6, 154.2, 146.4, 143.6, 141.3, 135.4, 134.7, 133.8, 133.6, 129.3, 128.8, 128.6, 127.7, 127.3, 127.1, 125.4, 124.9, 120.0, 117.6, 114.1, 110.1, 67.0, 58.2, 54.9, 47.1, 37.9. *m/z* (HRMS ESI) 635.1919; $[M + Na]^+$ requires 635.1901.

*(1-(2-Oxo-2*H*-chromen-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl (tert-butoxycarbonyl)-L-alaninate* (**10g**) (0.180 g, 81 %), white solid, mp 133–135°C. δ_H ($CDCl_3$, 300 MHz) 8.16 (1H, s), 7.87 (1H, d, *J* 7.8), 7.67 (1H, t, *J* 7.8), 7.46 (1H, d, *J* 8.1), 7.36 (1H, t, *J* 7.8), 6.58 (1H, s), 5.43 (2H, q, *J* 13.0), 5.01 (1H, d, *J* 4.8), 4.30 (1H, t, *J* 6.6), 1.42 (3H, br s), 1.39 (9H, s). δ_C ($CDCl_3$, 75 MHz) 173.5, 159.6, 155.3, 154.4, 146.7, 143.9, 133.7, 125.6, 125.2, 125.0, 117.7, 114.3, 110.2, 80.3, 58.1, 49.5, 28.4, 18.2. *m/z* (HRMS ESI) 437.1441; $[M + Na]^+$ requires 437.1432.

*(1-(2-Oxo-2*H*-chromen-4-yl)-1*H*-1,2,3-triazol-4-yl)methyl ((benzyloxy)carbonyl)-L-phenylalanyl-L-tryptophanate* (**11a**) (0.295 g, 78 %), yellow solid, mp 150–152°C. δ_H ([D6]DMSO, 300 MHz) 11.1 (1H, s), 8.79 (2H, br s), 7.94 (2H, br s), 7.80–7.62 (4H, m), 7.49–7.33 (12H, m), 7.25–7.12 (2H, m), 7.04 (1H, s), 5.46 (2H, s), 5.09 (2H, s), 4.87–4.76 (1H, m), 4.53–4.44 (1H, m), 3.42–3.27 (2H, m), 3.13–3.04 (1H, m), 2.86–2.75 (1H, m). δ_C ([D6]DMSO, 101 MHz) 171.9, 171.3, 159.4, 155.8, 153.6, 145.7, 142.6, 138.0, 136.9, 136.0, 133.5, 129.1, 128.2, 127.9, 127.6, 127.4, 127.0, 126.3, 125.4, 125.0, 123.8, 123.7, 121.0, 118.4, 118.0, 117.1, 114.20, 111.4, 110.6, 109.1, 65.2, 57.3, 55.8, 53.1, 37.3, 26.8. *m/z* (HRMS ESI) 733.2369; $[M + Na]^+$ requires 733.2381.

*Typical Procedure for the Synthesis of Triazolyl-Linked Amino Acids **13a–f***

To a solution of 4-methyl-7-(prop-2-ynyloxy)-coumarin (**2a**) or 4-(prop-2-ynyloxy)-coumarin (**2b**) (0.100 g, 1equiv.) was added α-azido esters **12a–c** (1 equiv.) in ethanol/H_2O (1 : 1), followed by addition of $CuSO_4$ (0.01 equiv.) and sodium ascorbate (0.1 equiv.). The reaction mixture was stirred at room temperature for 6–10 h and monitored via TLC. After completion of the reaction, the reaction mixture was evaporated and the residue was diluted with water and ammonium hydroxide (2–3 mL) and extracted into dichloromethane. Organic layer was separated dried over anhydrous sodium sulfate to evaporate the volatiles. The crude compound was recrystallized from ethanol to yield pure **13a–f**.

*Ethyl 2-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetate* (**13a**) (0.140 g, 88 %), white solid, mp 129–132°C. δ_H ($CDCl_3$, 300 MHz) 7.82 (1H, s), 7.50 (1H, d, *J* 8.6), 6.98–6.89 (2H, m), 6.14 (1H, s), 5.28 (2H, s), 5.18 (2H, s), 4.27 (2H. q, *J* 7.1), 2.39 (3H, s), 1.30 (3H, t, *J* 7.1). δ_C ($CDCl_3$, 75 MHz) 161.2, 155.2, 152.6, 143.6, 126.0, 125.8, 124.5, 114.2, 112.5, 112.4, 102.2, 62.7, 62.3, 51.0, 18.8, 14.2. *m/z* (HRMS ESI) 366.1056; $[M + Na]^+$ requires 366.1060.

*Methyl-(S)-2-(4-(((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) methyl)-1*H*-1,2,3-triazol-1-yl)-3-phenylpropanoate* (**13b**) (0.165 g, 85 %), white solid, mp 133–135°C. δ_H ($CDCl_3$, 300 MHz) 7.69 (1H, s), 7.48 (1H, d, *J* 6.6), 7.21–7.09 (3H, m), 7.02–6.81 (4H, m), 6.12 (1H, s), 5.62–5.52 (1H, m), 5.20 (2H, s), 3.74 (3H, s), 3.52–3.38 (2H, m), 2.37 (3H, s). δ_C ($CDCl_3$, 75 MHz) 168.6, 161.3, 161.2, 155.2, 152.5, 143.4, 143.1, 134.5, 128.9, 127.7, 125.8, 123.3, 114.1, 112.6, 112.4, 102.2, 64.2, 62.4, 53.3, 39.1, 18.9. *m/z* (HRMS ESI) 442.1385; $[M + Na]^+$ requires 442.1373.

*Methyl-(S)-3-(1*H*-indol-3-yl)-2-(4-(((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)propanoate* (**13c**) (0.175 g, 82 %), white solid, mp 138–140°C. δ_H ([D6]DMSO, 300 MHz) 10.85 (1H, s), 8.44 (1H, s), 7.70 (1H, d, *J* 8.8), 7.51 (1H, d, *J* 7.8), 7.32 (1H, d, *J* 8.0), 7.14 (1H, s), 7.10–6.94 (4H, m), 6.24 (1H, s), 5.87 (1H, t, *J* 7.6), 5.24 (2H, s), 3.73 (3H, s), 3.71–3.67 (2H, m), 2.42 (3H, s). δ_C ([D6]DMSO, 75 MHz) 169.6, 161.6, 160.7, 155.2, 154.0, 142.3, 136.5, 127.2, 127.0, 125.7, 124.3, 121.7, 119.1, 118.5, 114.0, 113.1, 112.0, 108.6, 102.1, 63.3, 62.2, 53.5, 27.8, 18.7. *m/z* (HRMS ESI) 481.1496; $[M + Na]^+$ requires 481.1482.

*Ethyl-2-(4-(((2-oxo-2*H*-chromen-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)acetate* (**13d**) (0.145 g, 90 %), white solid, mp 128–130°C. δ_H ($CDCl_3$, 300 MHz) 7.87 (1H, s), 7.72 (1H, d, *J* 7.8), 7.48 (1H, t, *J* 7.7), 7.24–7.14 (2H, m), 5.81 (1H, s), 5.32 (2H, s), 5.18 (2H, s), 4.24 (2H, q, *J* 7.0), 1.27 (3H, t, *J* 7.1). δ_C ($CDCl_3$, 75 MHz) 166.1, 165.0, 162.7, 153.4, 142.0, 132.6, 125.0, 124.0, 123.2, 116.8, 115.5, 91.3, 62.7, 51.1, 14.2. *m/z* (HRMS ESI) 352.0911; $[M + Na]^+$ requires 352.0904.

*Methyl-(S)-2-(4-(((2-oxo-2*H*-chromen-4-yl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)-3-phenylpropanoate* (**13e**) (0.175 g, 87 %), white solid, mp 130–132°C. δ_H ($CDCl_3$, 300 MHz) 7.80 (1H, s), 7.69 (1H, d, *J* 7.3), 7.48 (1H, t, *J* 7.7), 7.27–7.09 (5H, m), 6.93 (2H, d, *J* 3.2), 5.75 (1H, s), 5.65–5.56 (1H, m), 5.23 (2H, s), 3.71 (3H, d, *J* 6.9), 3.51–3.38 (2H, m). δ_C ($CDCl_3$, 75 MHz) 168.5, 164.9, 162.6, 153.4, 134.4, 132.6, 128.9, 127.8, 124.0, 123.2, 116.8, 115.5, 91.2, 64.4, 62.7, 53.3, 39.1. *m/z* (HRMS ESI) 428.1226; $[M + Na]^+$ requires 428.1217.

*Methyl-(S)-3-(1*H*-indol-3-yl)-2-(4-(((2-oxo-2H-chromen-4-yl) oxy)methyl)-1*H*-1,2,3-triazol-1-yl)propanoate* (**13f**) (0.185 g, 85 %), white solid, mp 134–136°C. δ_H ([D6]DMSO, 300 MHz) 10.88 (1H, s), 8.52 (1H, s), 7.71–7.65 (2H, m), 7.50–7.29 (5H, m), 7.06–7.00 (1H, m), 6.95 (1H, t, *J* 7.4), 6.14 (1H, s), 5.90 (1H, t, *J* 7.6), 5.39 (2H, s), 3.75 (3H, s), 3.72–3.68 (2H, m). δ_C ([D6] DMSO, 75 MHz) 169.5, 164.8, 162.1, 153.27, 141.3, 136.4, 133.4, 127.3, 126.0, 124.8, 124.4, 123.4, 121.6, 119.1, 118.4, 117.0, 115.6, 112.0, 108.6, 91.9, 63.5, 63.3, 53.7, 27.8. *m/z* (HRMS ESI) 462.1776; $[M + NH_4]^+$ requires 462.1772.

*Procedure for the Synthesis of (1-(2-oxo-2H-chromen-4-yl)-1H-1,2,3-triazol-4-yl)methyl-L-alaninate Hydrochloride (**14**)*

A solution of dioxane/HCl was added dropwise to **10g** (0.150 g, 1 equiv.) that was initially dissolved in dioxane at 0°C. The reaction was stirred at room temperature for 2 h and monitored via TLC. After reaction completion, the reaction mixture was completely concentrated, washed with diethyl ether, and filtered off to give **14** (0.085 g, 68 %), white solid, mp 160–162°C. δ_H ([D6]DMSO + $CDCl_3$, 300 MHz) 8.99 (1H, s), 7.87 (1H, d, *J* 8.1), 7.78 (1H, t, *J* 7.6), 7.57 (1H, s), 7.45 (1H, t, *J* 7.4), 6.95 (1H, s), 5.47 (2H, s), 4.15 (1H, dd, *J* 13.4, 6.5), 3.39 (2H, br s (merged with moisture of DMSO)), 1.50 (3H, d, *J* 6.8). δ_C ([D6]DMSO + $CDCl_3$, 101 MHz) 169.6, 159.3, 153.6, 145.7, 142.2, 133.4, 126.6, 125.5, 124.9, 117.1, 114.1, 110.4, 58.2, 47.9, 15.6.

*(1-(2-Oxo-2H-chromen-4-yl)-1H-1,2,3-triazol-4-yl) methyl(tert-butoxycarbonyl)-L-phenylalanyl-L-alaninate (**15**)*

To the stirred solution of **14** (0.050, 1 equiv.) in DMF, triethyl amine (2.5 equiv.) was added at 0°C and subsequently EDC·HCl (1.5 equiv.) and HOBt (1.2 equiv.) were added, and the reaction mixture was stirred for 15 min at 0°C. Then, Boc-L-phenylalanine (0.038 g, 1 equiv.) was added and the reaction was stirred at room temperature for 8 h. The completion of the reaction was monitored by TLC. After the completion of the reaction, crushed ice was added that resulted in the precipitation of the product which was filtrated, washed with cold water, and recrystallized with ethanol to afford **15** (0.075 g, 86 %), white solid, mp 145–147°C. δ_H ([D6]DMSO + $CDCl_3$, 400 MHz) 8.81 (1H, s), 8.42 (1H, s), 7.83 (1H, d, *J* 8.4), 7.75 (1H, s), 7.54 (1H, d, *J* 8.1), 7.40 (1H, d, *J* 8.0), 7.27–7.10 (5H, m), 6.90–6.83 (1H, m), 6.71 (1H, d, *J* 7.7), 5.34 (2H, s), 4.45–4.34 (1H, m), 4.25–4.18 (1H, m), 2.95 (1H, d, *J* 13.8), 2.68 (1H, d, *J* 12.0), 1.37 (3H, br s), 1.29 (9H, s). δ_C ([D6]DMSO + $CDCl_3$, 101 MHz) 172.1, 171.8, 159.2, 155.1, 153.6, 145.7, 142.7, 137.9, 133.3, 129.1, 127.8, 126.2, 126.0, 125.5, 124.8, 117.0, 114.1, 110.3, 77.9, 57.3, 55.2, 47.6, 37.4, 28.1, 16.8.

*2-(4-(((4-Methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)acetic Acid (**16**)*

To a solution of **13a** (0.100 g, 1 equiv.) in ethanol cooled to 0°C was added 30 % NaOH solution in water (5 mL) dropwise. The reaction was stirred at room temperature for 3 h and monitored via TLC. After the completion of the reaction, ethanol was completely concentrated under reduced pressure to give the corresponding salt of the acid, which was acidified using 4N HCl at 0°C and the aqueous layer was extracted with dichloromethane to give **16** (0.075 g, 85 %), white solid, mp 110–112°C. δ_H ([D6]DMSO + $CDCl_3$, 400 MHz) 8.23 (1H, s), 7.66 (1H, d, *J* 8.8), 7.12 (1H, s), 7.03 (1H, d, *J* 8.8), 6.17 (1H, s), 5.29 (2H, s), 5.26 (2H, s), 2.41 (3H, s). δ_C ([D6]DMSO + $CDCl_3$, 101 MHz) 168.3, 161.0, 160.0, 154.7, 152.9, 141.8, 126.2, 126.0, 113.3, 112.5, 111.3, 101.5, 61.6, 50.6, 18.1.

*Methyl-(2-(4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy) methyl)-1H-1,2,3-triazol-1-yl)acetyl)-L-phenylalaninate (**17**)*

To the stirred solution of **16** (0.050 g, 1 equiv.) in DMF, triethyl amine (2.5 equiv.) was added at 0°C and subsequently EDC·HCl (1.5 equiv.) and HOBt (1.2 equiv.) were added, and the reaction mixture was stirred for 15 min at 0°C. Later L-phenylalanine methyl ester (0.029 g, 1 equiv.) was added and the reaction was stirred at room temperature for 6 h. The completion of the reaction was monitored by TLC. After the completion of the reaction, crushed ice was added that resulted in the precipitation of the product, which was filtrated, washed with cold water, and recrystallized with ethanol to afford **17** (0.065 g, 90 %), white solid, mp 135–137°C. δ_H ([D6]DMSO + $CDCl_3$, 400 MHz) 8.89 (1H, d, *J* 7.7), 8.09 (1H, s), 7.64 (1H, d, *J* 8.8), 7.31–7.25 (2H, m), 7.24–7.18 (3H, m), 7.10 (1H, d, *J* 2.2), 7.01 (1H, dd, *J* 8.8, 2.2), 6.16 (1H, s), 5.26 (2H, s), 5.14 (2H, q, *J* 16.2), 4.57 (1H, dd, *J* 13.7, 8.1), 3.64 (3H, s), 3.08 (1H, dd, *J* 13.8, 5.5), 2.95 (1H, dd, *J* 13.8, 8.7), 2.40 (3H, s). δ_C ([D6] DMSO + $CDCl_3$, 101 MHz) 171.3, 165.3, 161.0, 160.0, 154.7, 152.9, 141.6, 136.5, 129.0, 128.2, 126.5, 126.1, 126.0, 113.3, 112.4, 111.3, 101.4, 61.5, 53.6, 51.8, 51.3, 36.8, 18.1.

Supplementary Material

Copies of ^{1}H NMR, ^{13}C NMR, and HRMS spectra of the synthesized compounds are available on Journal's website.

Acknowledgements

The authors acknowledge the Department of Science and Technology (DST), New Delhi, for providing research grant (SB/FT/CS-033/2012) for the research work. DSA also thanks DST, New Delhi for the research fellowship. SK and SMAS thank UGC, New Delhi, India for their Junior Research Fellowship and BSR Fellowship, respectively.

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