DOI: 10.1002/ejoc.201000042

# Click Chemistry to Fluorescent Amino Esters: Synthesis and Spectroscopic Studies

**Chun Li,[a,b] Etienne Henry,[c] Naresh Kumar Mani,[c] Jie Tang,[b] Jean-Claude Brochon,[c] Eric Deprez,*[c] and Juan Xie*[a]**

**Keywords:** Amino acids / Click chemistry / Fluorescent probes / Fluorescence / Solvent effects

A new series of fluorescent amino esters have successfully been prepared by click chemistry by introducing different fluorophores (fluorescein, dansyl, 4-nitro-2,1,3-benzoxadiazole, coumarin and benzothiadiazole) into the side-chain of either serine, lysine or phenylalanine. The newly synthesized fluorescent amino esters displayed spectroscopic properties similar to their native fluorophores, with high quantum yields and fluorescence in the visible region (420–520 nm). Steady-state as well as time-resolved studies indicated that benzothiadiazole derivatives **22**–**25** are promising probes for studying protein dynamics or molecular interactions, especially in the cell, due to their emissions in the visible region, their high quantum yields and their relatively long lifetimes (14.7–18.3 ns). Moreover, the fluorescence of 7-hydroxycoumarin-substituted amino ester **27** was very sensitive to the solvent proticity and pH value. This compound appears to be a suitable pH-sensitive amino acid for probing the local pH in cell compartments.

## Introduction

The fluorescent labelling of biomolecules is a powerful method for investigating various biological events at a molecular level.[1] With fluorescence spectroscopy, different aspects of fluorescence output, for example, steady-state intensity, Stokes shifts, lifetimes, steady-state and time-resolved anisotropies and resonance energy transfer, can be used for studying biological structures, dynamics and functions and for visualizing intracellular processes or molecular interactions.[2] Considerable progress has been realized since the discovery of green fluorescent protein (GFP) and its derivatives, which allowed the labelling of a protein of interest by a fluorescent protein.[3] However, such fusion proteins, obtained by molecular biology techniques, can lead to steric hindrance problems and, consequently, to perturbation of the natural function of the protein of interest. Proteins can also be chemically labelled in vitro by using extrinsic small organic fluorophores. Depending on the number of reactive groups available on the protein, chemical labelling may lead to heterogeneous populations of fluorescently labelled proteins. Several strategies have recently been proposed to circumvent the problem of non-specific labelling, mainly based on the use of specific and small peptidyl tags fused to the protein of interest. The first one allows a specific and enzyme-mediated labelling of a target protein at a single and predefined position. This enzymatic labelling, by transglutaminase, occurs specifically on a Gln residue of the PKPQQFM peptide substrate by using various commercially available fluorophore–cadaverine compounds such as fluorescein–cadaverine or TAMRA–cadaverine.[4] Specific covalent labelling can also occur in an enzymatic-independent process by using a specific peptide tag containing four Cys residues and a bis-(arsenic) fluorescein derivative. The latter strategy is compatible with labelling in a cellular environment.[5] Alternatively, synthetic fluorescent amino acids are useful tools for protein labelling because they can be introduced into peptide sequences by chemical synthesis[6] or into proteins by translational machinery.[7] Increasing effort has been devoted to the chemical synthesis of fluorescent amino acids[8] as a result of their various biological applications.[9] However, there are no general methods for introducing a fluorophore into the amino acid sequence: different strategies have to be employed to obtain each fluorescent amino acid.[8]

$Cu^{I}$-catalysed Huisgen 1,3-dipolar cycloaddition (CuAAC) between terminal alkynes and organic azides (also known as "click chemistry") has recently been extensively used and has found wide applications in organic synthesis, medicinal chemistry, molecular biology and polymer and materials science because of its high efficiency, versatility, regioselectivity and excellent functional-group compatibility.[10] Furthermore, the 1,2,3-triazole ring formed is re-

[a] PPSM, Institut d'Alembert, ENS de Cachan, CNRS UMR 8531,
61 Avenue du Pt Wilson, 94235 Cachan, France
Fax: +33-1-47402454
E-mail: joanne.xie@ens-cachan.fr
[b] Institute of Medicinal Chemistry, Department of Chemistry, East China Normal University,
Shanghai 200062, China
[c] LBPA, Institut d'Alembert, ENS de Cachan, CNRS UMR 8113,
61 Avenue du Pt Wilson, 94235 Cachan,France
Fax: +33-147407671
E-mail: deprez@lbpa.ens-cachan.fr

sistant to hydrolysis, oxidation, reduction, and other modes of cleavage. Owing to their electronic properties, 1,2,3-triazoles have been employed as rigid linking units to mimic amide and ester bonds.[10d,11] Click chemistry has been used for the fluorescent labelling of alkyne- or azide-functionalized proteins[12] or for profiling enzyme activities.[13] Triazole amino acids and peptides have also been reported.[14] However, to the best of our knowledge, the synthesis of fluorescent amino acids by click chemistry has never been reported. We have successfully used natural amino acids as the starting materials to obtain their fluorescent derivatives, and we report herein the preparation and basic photophysical characterization of these molecules in the amino ester forms. Several of these molecules were found to be suitable for biological applications, especially compounds **22**–**25** and **27**. It is important to note that the amino ester derivatives are characterized by poor water solubility. However, the $CO_2Me$ group can be easily deprotected by saponification. The saponification procedure was tested on compound **22**, and its soluble version **23** was successfully obtained and characterised.

## Results and Discussion

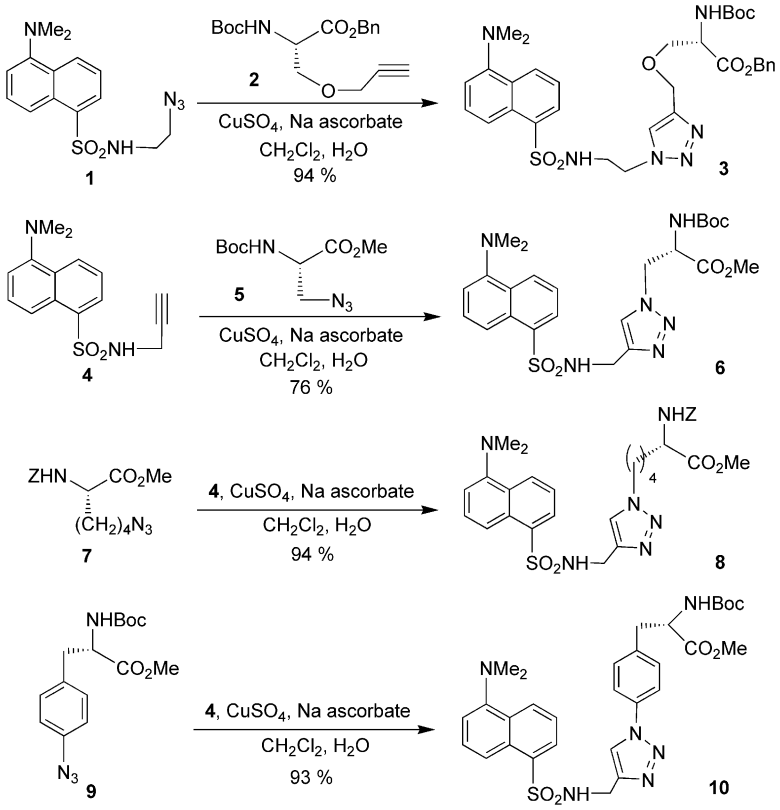
### Synthesis

Fluorophores like dansyl, fluorescein, 4-nitro-2,1,3-benzoxadiazole, benzothiadiazole and coumarin with emission at around 500 nm were chosen to prepare the fluorescent amino acids. As amino acids, we used Ser, Lys and Phe as the starting materials. To obtain dansyl amino acids, *O*-propargyl-Boc-Ser-OBn (**2**)[15] was treated with the dansyl azide **1**[16] in the presence of $CuSO_4$/Na ascorbate in a mixture of $CH_2Cl_2/H_2O$ at room temperature (Scheme 1). The dansyl serine derivative **3** was obtained in 94% yield. Similarly, the click reaction of the dansyl alkyne **4**[17] with azido amino esters **5**,[18] **7**[15] and **9**[19] afforded the corresponding compounds **6** (76%), **8** (94%) and **10** (93%).

Fluorescein-substituted amino esters **12**, **13** and **14** were prepared in excellent yields by CuAAC between the alkyne **11**[20] and the azido amino esters **5**, **7** and **9**, respectively (Scheme 2).

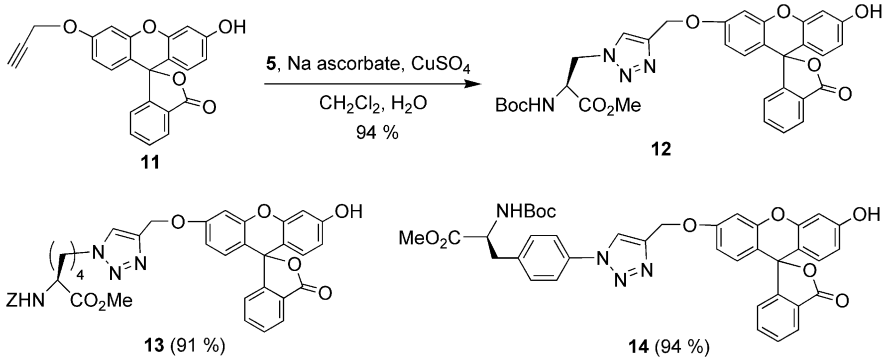
To synthesize NBD derivatives [7-(alkylamino)-substituted 4-nitro-2,1,3-benzoxadiazole], we first prepared azido- or alkyne-substituted NBDs **15** and **17** by treating NBD-Cl with 2-azidoethylamine or propargylamine under basic conditions (Scheme 3). The click reaction of **2** with **15** was realized with CuI as catalyst at 90 °C to afford compound **16**. No reaction was observed with $CuSO_4$/Na ascorbate at room temperature. Propargyl-NBD **17** reacted with azides **5** and **7** at room temperature to give the corresponding alanine and lysine derivatives **18** and **19**, respectively. The phenylalanine derivative **20** was obtained after reaction under reflux.

We have also prepared benzothiadiazole-substituted amino acid derivatives (Scheme 4). The reaction of TMS-alkyne **21**[21] with azide **5** in the presence of $CuSO_4$/Na ascorbate/TBAF at room temperature led to **22** in 81% yield.

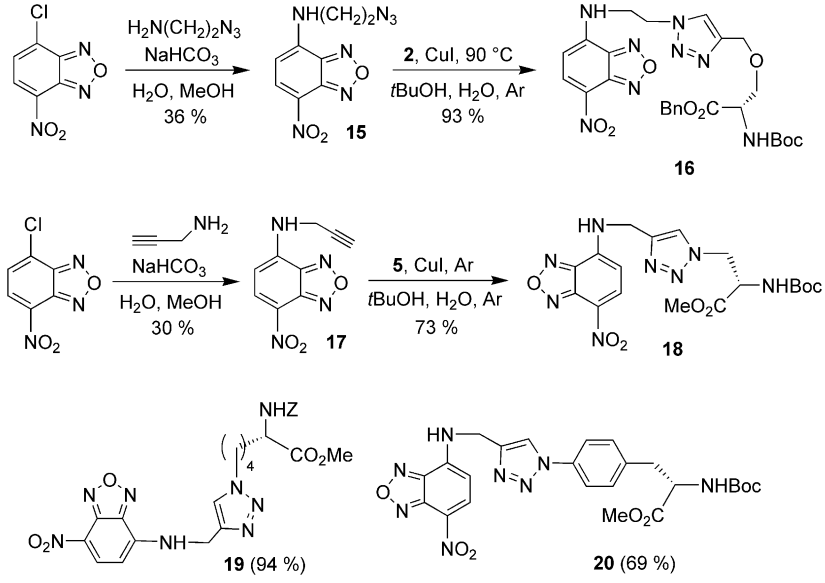



Scheme 1. Synthesis of dansyl-substituted amino esters **3**, **6**, **8** and **10**.



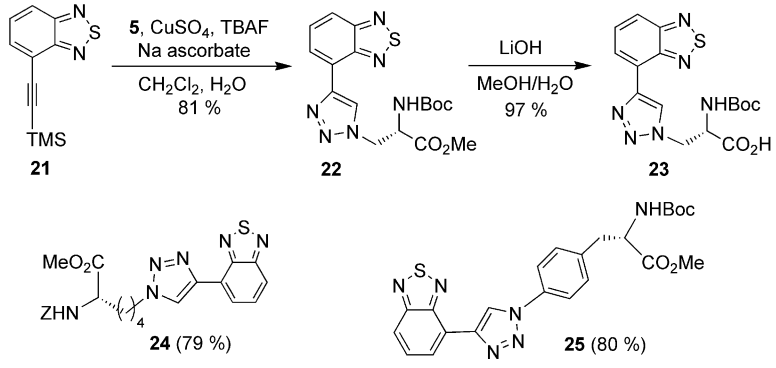

Scheme 2. Synthesis of fluorescein-substituted amino esters **12**–**14**.




Scheme 3. Synthesis of NBD-substituted amino esters **16**, **18**–**20**.

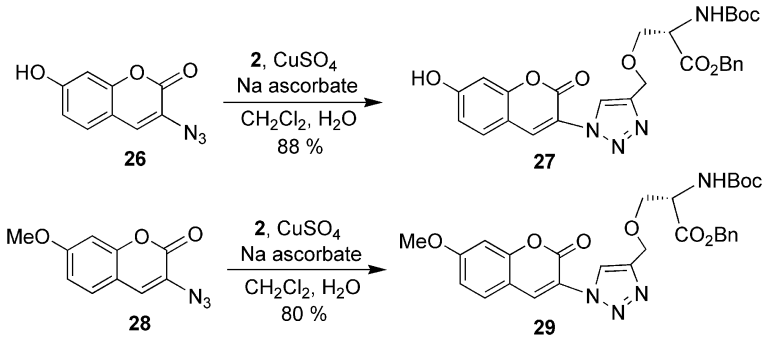
Saponification of **22** led to the free acid **23** in 97% yield. The corresponding Lys and Phe derivatives **24** and **25** were synthesized in a similar way.

It was also convenient to prepare coumarin-substituted serine derivatives **27** and **29** by CuAAC by treating **2** with the azidocoumarins **26**[22] and **28**[22] (Scheme 5).




Scheme 4. Synthesis of benzothiadiazole-substituted amino acid derivatives **22**–**25**.




Scheme 5. Synthesis of coumarin-substituted amino esters **27** and **29**.

## Spectroscopic Properties

### *General Spectroscopic Properties*

First, the steady-state fluorescence parameters of several fluorescent amino acids were determined in two solvents, DMSO and ethanol, characterized by different polarities (Table 1). The fluorescence quantum yields were determined by using fluorescein or quinine sulfate as standards for compounds **12** and **18** and for compounds **6** and **22**–**29**, respectively (see the Exp. Sect.).

With the exception of the fluorescein derivative (compound **12**), the spectroscopic properties of the newly synthesized fluorophores in EtOH and DMSO were similar to the native molecules: they exhibited large Stokes shifts and high quantum yields. Compound **12** (lactone form of fluorescein) displayed excitation and emission characteristics qualitatively similar to the standard anionic fluorescein, albeit with a dramatic decrease in the quantum yield, as shown previously.[23] Identical results were obtained with compounds **13** and **14**. Thus, compounds **12**–**14** were studied under conditions that favour the anionic forms,[23] that is, in DMSO/buffer solutions of increasing pH (see Table 1). In the pH range of 7–9, their quantum yields were significantly higher ($\Phi_F$ = 0.31–0.36) than those obtained in ethanol or DMSO, which shows that the low quantum yields in ethanol and DMSO are due to the prevalence of the non-fluorescent lactone form.

The fluorescence emissions of the different compounds presented in Table 1 were affected by solvent polarity to different extents, with the most marked redshifts in emission being observed for compounds **6** and **18**. Note that compound **23**, the water-soluble version of compound **22**, was characterized by a significant redshift of its fluorescence emission in water compared with DMSO ($\lambda_{max}^F$ =

Table 1. Spectroscopic steady-state data of the fluorescent amino acid derivatives.[a,b]

| Compound | Solvent | $\lambda_{max}^A$ [nm] | $\lambda_{max}^F$ [nm] | $\Delta\nu_{A-F}$ [cm$^{-1}$] | $\varepsilon_{00}$ [L mol$^{-1}$ cm$^{-1}$] | $\Phi_F$ |
|---|---|---|---|---|---|---|
| **6** | EtOH | 336 | 517 | 10420 | 3600 | 0.40 |
| | DMSO | 342 | 532 | 10443 | 3000 | 0.64 |
| **12**[c] | EtOH | 276/480 | 512 | 16701/1302 | 6400/720 | 0.02 |
| | DMSO | 277 | 538 | 17514 | 6300 | 0.005 |
| | T7D[d] | 455/480 | 516 | 2598/1454 | 27500/25500 | 0.31 |
| | T8D[d] | 455/480 | 516 | 2598/1454 | 30000/27000 | 0.35 |
| | T9D[d] | 455/480 | 516 | 2598/1454 | 30000/27000 | 0.36 |
| **18** | EtOH | 326/456 | 527 | 11700/2954 | 20000/7600 | 0.57 |
| | DMSO | 341/471 | 537 | 10704/2609 | 21000/6600 | 0.83 |
| **22** | EtOH | 316/360 | 476 | 10637/6769 | 14000/5200 | 0.86 |
| | DMSO | 318/366 | 473 | 10305/6181 | 10000/3600 | 0.73 |
| **23** | EtOH | 316/359 | 473 | 10504/6714 | 14800/5400 | 0.39 |
| | DMSO | 316/360 | 475 | 10593/6725 | 10400/3600 | 0.35 |
| | Water | 316/350 | 500 | 11646/8571 | 10200/3200 | 0.22 |
| **24** | EtOH | 316/360 | 481 | 10856/6988 | 14000/6100 | 0.82 |
| | DMSO | 317/359 | 479 | 10669/6978 | 11500/4100 | 0.75 |
| **25** | EtOH | 316/360 | 478 | 10725/6857 | 15000/6300 | 0.49 |
| | DMSO | 319/362 | 480 | 10515/6791 | 14800/7800 | 0.46 |
| **27** | EtOH | 346 | 421 | 4875 | 20000 | 0.65 |
| | DMSO | 346 | 426 | 5289 | 19000 | 0.59 |
| **29** | EtOH | 344 | 416 | 5031 | 20000 | 0.80 |
| | DMSO | 344 | 419 | 5203 | 18000 | 0.53 |

[a] $\lambda_{max}^A$: absorbance wavelength, $\lambda_{max}^F$: emission wavelength, $\Delta\nu_{A-F}$: Stokes shift, $\varepsilon_{00}$: molar absorbance coefficient, $\Phi_F$: fluorescence quantum yield. [b] When molecules are characterized by two absorption modes (**12** in EtOH and **18**–**25** in both EtOH and DMSO), both of the absorption modes are responsible for fluorescence emission and the same $\lambda_{max}^F$ was obtained regardless of the excitation wavelength. For such compounds, $\Phi_F$ was measured by using the highest absorption wavelength. [c] Compounds **13** and **14** have spectroscopic and solubility properties identical to **12** (data not shown). [d] T7D, T8D and T9D correspond to DMSO/Tris buffer mixtures (2:8, v/v) at pH = 7, 8 and 9, respectively.

500 and 475 nm, respectively). Importantly, all the amino acid derivatives emitted fluorescence in the visible region of the spectrum, from 420 nm for compounds **27** and **29** to 520 nm for compounds **6**, **12**–**14** and **18**. Thus, the different amino acid derivatives tested appear to be suitable fluorescent probes for protein/peptide labelling and cellular imaging applications, in contrast to the natural tryptophan amino acid which emits at 330–350 nm.

### *Time-Resolved Fluorescence*

To compare the fluorescence lifetimes of the non-natural amino esters and tryptophan, we next performed time-resolved fluorescence measurements. The influence of solvent polarity on the lifetimes was also assessed, because fluorescence lifetimes are known to be highly sensitive to environment, with particularly high polar solvents responsible for a substantial decrease in the lifetimes because of solvent relaxation. Most of the compounds are not fully soluble in water due to the presence of Boc and methyl groups (except for compound **23** which is in the free acid form). The fluorescence lifetimes were thus measured in pure DMSO and in a DMSO/water mixture (2:8, v/v) (Table 2).

Table 2. Analysis of fluorescence decay.

| Compound | $\tau$ [nm] | |
|---|---|---|
| | DMSO | DMSO/$H_2O$[a] |
| **6** | 17 | 7.1 |
| **12** | 0.75 | 3.4 |
| **18** | 7.77 | 2.0 |
| **22** | 14.0 | 18.3 |
| **23** | 13.6 | 15.6[b] |
| **24** | 15.4 | 18.6 |
| **25** | 9.47 | 14.7 |
| **27** | 2.4 | 4.2 |
| **29** | 2.17 | 3.1 |

[a] DMSO/$H_2O$ in a ratio of 2:8 (v/v). [b] $\tau$ measured in Tris buffer at pH = 7.

All tested compounds exhibited a monoexponential $I(t)$ decay (Figure 1). Moreover, compounds **6**, **18** and **22**–**25** were characterized by long fluorescence lifetimes. However, compounds **6** and **18** were highly sensitive to solvent polarity, as judged by the significant decrease in their lifetimes upon the addition of water (from 17 to 7.1 ns and from 7.77 to 2.0 ns, respectively). In contrast, the lifetimes of the benzothiadiazole-substituted amino acid derivatives **22**–**25** were long, above 14 ns in DMSO/water mixtures, much higher than the fluorescence lifetime of tryptophan, which is about 3 ns.

In conclusion, the steady-state and time-resolved studies indicate that compounds **22**–**25** are promising probes for studying protein dynamics or molecular interactions, especially in the cell environment due to their emissions in the visible region of the spectrum and their high quantum yields. More specifically, owing to their relative long lifetimes, they could be suitable as donor molecules in time-resolved fluorescence resonance energy transfer (FRET) experiments (i.e., when FRET efficiency is determined by measuring the donor lifetime) and, more generally, in fluorescence lifetime imaging (FLIM) by enhancing image contrasts and accuracy in the determination of FRET efficiency when measuring the decrease in the donor lifetime upon interaction between donor- and acceptor-labelled partners. In addition, another application of such long lifetime probes is related to the enhancement of image contrast by time-gated fluorescence imaging, which reduces cellular auto-fluorescence characterized by short fluorescence lifetimes.

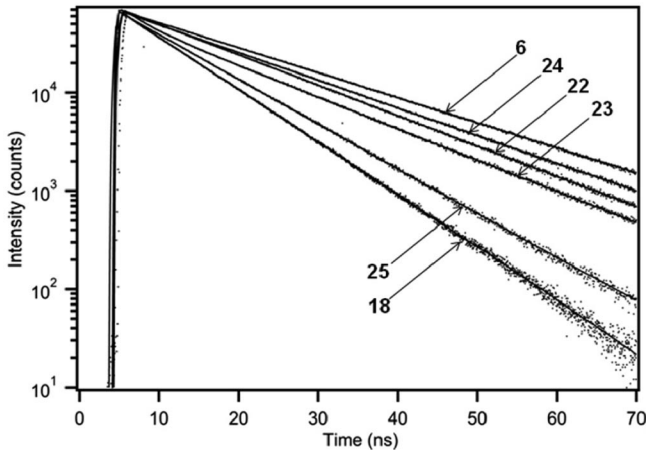



Figure 1. Fluorescence intensity decays of compounds **6**, **18** and **22**–**25** in DMSO (5 μM). Acquisition and analysis of the intensity decays were performed as described in the Exp. Sect.

Furthermore, long lifetimes are also of particular interest for time-resolved fluorescence anisotropy experiments. This approach allows rotational correlation times to be determined. A long correlation time is related in a first approximation to the hydrated volume of an entire particle by the Stokes–Einstein relationship and, for example, can be used to discriminate between different higher-order oligomeric states of a protein. However, by using the intrinsic tryptophan fluorescence, this approach suffers from the short lifetime of tryptophan, which is severely limited for estimating precisely the long rotational correlation times characterizing large molecules (typically above 80–100 ns) due to a limited time window for measuring the fluorescence anisotropy decay.[24] Therefore, extrinsic fluorescent probes with long lifetimes could be suitable for improving the accurate recovery of long rotational correlation times in protein studies. In this context, the benzothiadiazole-substituted amino acid derivatives **22**–**25** represent promising fluorophores for both in vitro and in vivo biological applications based on fluorescence anisotropy or FLIM methodologies.

### *Insight into the Properties of Coumarin Derivatives 27 and 29*

7-Hydroxycoumarin is a well-known pH-sensitive blue-fluorescent fluorophore with a p$K_a$ of 7.8.[25] Indeed, protonation of the 7-hydroxy group induces significant changes in both the emission and absorption bands depending on the proton-donating property of the solvent or pH.[26] Thus, we assessed whether compound **27** behaves as a 7-hydroxycoumarin. The spectroscopic properties of **27** were then fur-

Table 3. Spectral fluorescence characteristics of coumarin derivatives **27** and **29**.[a]

| Entry | Solvent | $\lambda_{max}^{A1}$ [nm] | $\lambda_{max}^{A2}$ [nm] | $\lambda_{max}^{F1}$ [nm] | $\lambda_{max}^{F2}$ [nm] | $I_{max}^{F1}/I_{max}^{F2}$ |
|---|---|---|---|---|---|---|
| Compound **27** | | | | | | |
| 1 | $CH_3CN$ | 341 | – | 420 | – | |
| 2 | EtOH | 346 | – | 421 | – | |
| 3 | DMSO | 346 | – | 426 | – | |
| 4 | DMSO/$H_2O$ (8:2, v/v) | 346 | – | 426 | 483 | 0.567 |
| 5 | DMSO/$H_2O$ (6:4, v/v) | 346 | – | 426 | 481 | 0.164 |
| 6 | DMSO/$H_2O$ (4:6, v/v) | 346 | – | 426 | 479 | 0.08 |
| 7 | DMSO/$H_2O$ (2:8, v/v) | 346 | – | 426 | 478 | 0.06 |
| 8 | DMSO/Tris buffer pH = 7 (2:8, v/v)[b] | 346 | 400 | 423 | 478 | |
| 9 | DMSO/Tris buffer pH = 9 (2:8, v/v) | – | 400 | – | 476 | |
| 10 | DMSO + 10 μM NaOH[c] | 345 | – | 424 | – | |
| 11 | DMSO + 50 μM NaOH[c,d] | 345 | 436 | 424 | 485 | |
| 12 | DMSO + 100 μM NaOH[c] | – | 436 | – | 485 | |
| Compound **29** | | | | | | |
| 13 | DMSO | 344 | – | 419 | – | |
| 14 | DMSO/$H_2O$ (2:8, v/v) | 346 | – | 423 | – | |
| 15 | DMSO/Tris buffer pH = 7 (2:8, v/v) | 346 | – | 423 | – | |
| 16 | DMSO/Tris buffer pH = 9 (2:8, v/v) | 346 | – | 423 | – | |
| 17 | DMSO + 100 μM NaOH[c] | 344 | – | 419 | – | |

[a] $I_{max}^{F1}/I_{max}^{F2}$ corresponds to the intensity ratio of the emission maxima. [b] Excitation at 346 nm is responsible for the two fluorescence emissions at 423 and 478 nm, whereas excitation at 400 nm is responsible for fluorescence emission at 478 nm only. [c] 10, 50 and 100 μM NaOH corresponds to an NaOH/compound molar ratio of 2.8, 13.9 and 28, respectively. [d] Excitation at 345 nm is responsible for the fluorescence emission at 424 nm only, and excitation at 436 nm is responsible for the fluorescence emission at 485 nm only.

ther studied by varying (1) the proticity of solvents by using aprotic solvents (acetonitrile, DMSO) and solvents with either low (ethanol) or high (DMSO/water mixtures) proticity or (2) the pH by adding NaOH or by using buffered solutions (Table 3). Compound **29** was used as a control, because the 7-OH group in **27** is substituted by a methyl group in **29**.

In aprotic solvents or solvents with low proticity, only slight differences appeared in the absorption and fluorescence spectra of compound **27** (Table 3, Entries 1–3). In sharp contrast, the emission spectra of compound **27** changed dramatically in solvents with higher proticity (DMSO/water mixtures): by increasing the solvent proticity (achieved by decreasing the DMSO/water ratio), the fluorescence emission band intensity decreased at 426 nm, and a second emission band appeared at 478 nm (Figure 2; Table 3, Entries 3–7). This ratiometric behaviour is due to the presence of the 7-OH group, because compound **29** was unaffected by modulation of the DMSO/water ratio (Table 3, Entries 13 and 14). According to the prototype compound 7-hydroxycoumarin,[25] this dual emission of compound **27** is likely due to a protonation equilibrium in the excited state, [7-OH]* ⇌ [7-O$^-$]*, with the 7-OH* form responsible for the emission band at 426 nm and the 7-O$^-$* form responsible for the emission band at 478 nm upon excitation of the 7-OH form at 346 nm (see Figure 3). Moreover, by increasing the pH, a dual absorption is observed that originates in a protonation equilibrium in the ground state, 7-OH ⇌ 7-O$^-$: increasing the pH of the Tris buffer (Table 3, Entries 8 and 9) or directly adding NaOH in DMSO (Table 3, Entries 10–12) favours the 7-O$^-$ ground-state form. Consequently, the absorbance at 346 nm (7-OH) decreased, and a second absorption band appeared at above 400 nm (7-O$^-$). At pH = 9 or upon addition of 100 μM NaOH, only the excitation mode above 400 nm was observed (Entries 9 and 12, respectively). Under equilibrium conditions and high proticity (Entry 8), excitation at 346 nm (selective excitation of the 7-OH form) leads to dual emission, that is, at 423 (7-OH*) and 478 nm (7-O$^-$*) (as found in Entries 4–7), whereas excitation at 400 nm (selective excitation of the 7-O$^-$ form) only leads to emission at 478 nm (7-O$^-$*). In contrast, different results were obtained under aprotic conditions (Entry 11): excitation at 346 nm yields a single emission (at 424 nm, as found in Entries 1–3). The emission at 485 nm can be observed only with excitation above 400 nm. Again, the dual absorption was not observed with compound **29** (Entries 16 and 17). The 7-

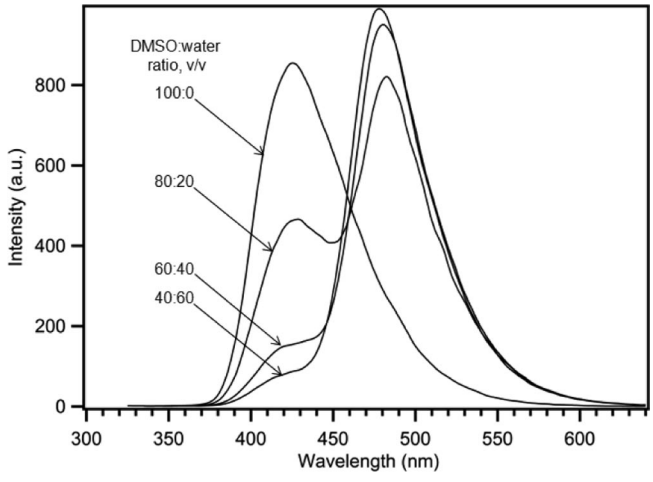



Figure 2. Emission spectra of compound **27** (2.6 μM) as a function of the DMSO/water ratio. $\lambda_{exc}$ = 346 nm.

hydroxycoumarin derivative thus appears to be a suitable pH-sensitive amino acid to probe local pH in cell compartments.

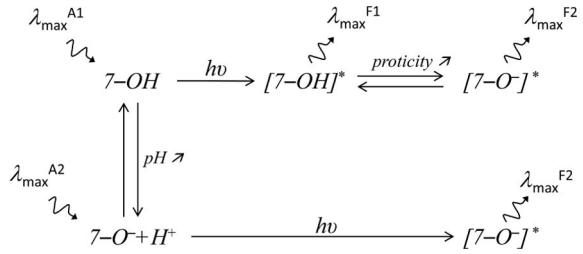



Figure 3. Model for the influence of proticity and pH on the absorption and emission bands of compound **27**.

## Conclusions

We have reported an efficient synthesis of new fluorescent amino ester derivatives by introducing fluorophores into the side-chain of natural amino acids like serine, lysine and phenylalanine by click chemistry. Dansyl, NBD, fluorescein, coumarin and benzothiadiazole derivatives have been prepared by this approach. Spectroscopic studies showed that all the newly synthesized fluorophores exhibit similar fluorescence properties compared with the native molecules, with large Stokes shifts and high quantum yields. Time-resolved fluorescence measurements demonstrated that all compounds exhibited a monoexponential $I(t)$ decay. In particular, the benzothiadiazole-substituted amino ester derivatives **22**–**25** showed long fluorescence lifetimes (above 14 ns in DMSO/water mixtures), much longer than that of tryptophan (about 3 ns). Consequently, compounds **22**–**25** represent promising fluorophores for both in vitro and in vivo biological applications based on fluorescence anisotropy or FLIM methodologies. Moreover, the fluorescence properties of 7-hydroxycoumarin-substituted amino ester **27** appeared to be very sensitive to the proticity of the solvent and the pH, which makes this molecule a suitable pH-sensitive amino acid for probing the local pH in cell compartments. Finally, the different amino esters can be easily deprotected to yield hydrosoluble amino acids, as testified by the successful conversion of ester **22** into carboxylic acid **23** by saponification.

## Experimental Section

**General:** $^1$H and $^{13}$C NMR spectra were recorded with a Jeol ECS400 spectrometer. Column chromatography was performed with silica gel 60 (230–400 mesh). Analytical thin-layer chromatography was performed with aluminium precoated plates of silica gel 60F-254 with detection carried out by using UV light and ninhydrin. ESI-HRMS were recorded with a Thermo LTQ-Orbitrap spectrometer at the Service of Mass Spectrometry of the University Pierre and Marie Curie. UV/Vis absorption spectra were recorded with a double-beam Uvikon 933 spectrophotometer. Corrected excitation and emission spectra were obtained with a Cary Eclipse spectrofluorimeter (Varian). For the emission measurements, the absorbance at the excitation wavelengths was kept below 0.05 to avoid inner filter effects. DMSO, ethanol, acetonitrile as well as Tris buffer (20 mM), prepared with Millipore-filtered water (resistivity $> 18$ MΩ cm$^{-1}$ at 20 °C), were from Sigma–Aldrich (spectrometric grade). Quantum yields were estimated by using fluorescein in 0.1 M NaOH ($\Phi_F = 0.95$) and quinine sulfate in 0.1 M $H_2SO_4$ ($\Phi_F = 0.58$ for $\lambda_{ex} = 350$ nm) as standards, taking into account the refractive index of the standard and the sample solvent according to ref.[2] Time-resolved fluorescence experiments were performed to determine the fluorescence lifetimes, which were obtained from the fluorescence intensity decays, $I(t)$, by using the time-correlated single photon counting technique. The instrumentation setup was essentially the same as those previously described[27] with modifications: the excitation light pulse source was a Ti:sapphire laser (Maitai femtosecond laser, Spectra Physics) associated with either a second or a third harmonic generator tuned at 420 or 300 nm, respectively. The polarizer was set at the magic angle (54.7°), and the emission monochromator (ARC SpectraPro-150) was set at the maximum emission pick ($\Delta\lambda = 15$ nm). To determine the lifetime ($\tau$) distribution, $I(t)$ was analysed by the maximum entropy method.[24,28]

**General Procedure A for the Click Reaction:** $CuSO_4 \cdot 5H_2O$ (0.008 to 0.02 mmol) and Na ascorbate (0.0017 to 0.04 mmol) were added successively to a solution of alkyne (0.1 mmol) and azide (0.1 mmol) in $CH_2Cl_2$ (2 mL) and water (2 mL). The reaction mixture was vigorously stirred at room temp. for 12 h. $CH_2Cl_2$ (5 mL) was then added. After separation of the organic layer, the aqueous layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried with $MgSO_4$ and the solvents evaporated.

**General Procedure B for the Click Reaction:** CuI (0.03 mmol) was added to a solution of alkyne (0.1 mmol) and azide (0.1 mmol) in *t*BuOH (2 mL) and water (2 mL). The reaction mixture was vigorously stirred under argon at 90 °C for 48 h. Then water (15 mL) was added. After separation of the organic layer, the aqueous layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried with $MgSO_4$ and the solvents evaporated.

**Benzyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-[(1-{2-[5-(dimethylamino)naphthalen-1-ylsulfonamido]ethyl}-1*H*-1,2,3-triazol-4-yl)methoxy]propanoate (3):** From alkyne **2** (23 mg, 0.07 mmol) and azide **1** (21.8 mg, 0.07 mmol), the click reaction according to the General Procedure A led to **3** as a light-green solid (42 mg, 94%). $[\alpha]_D = -1.0$ ($c = 0.5$, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta = 8.57$ (d, $^3J_{H,H} = 8.7$ Hz, 1 H, Ar-H), 8.23 (d, $^3J_{H,H} = 7.3$ Hz, 1 H, Ar-H), 8.17 (d, $^3J_{H,H} = 8.7$ Hz, 1 H, Ar-H), 7.53–7.48 (m, 2 H, Ar-H), 7.30–7.27 (m, 5 H, Ph), 7.23 (s, 1 H, Ar-H), 7.17 (d, $^3J_{H,H} = 7.4$ Hz, 1 H, Ar-H), 5.69 (t, $^3J_{H,H} = 6.2$ Hz, 1 H, NH), 5.44 (d, $^3J_{H,H} = 8.7$ Hz, 1 H, NH), 5.23 (d, $^2J_{H,H} = 12.4$ Hz, 1 H, CHPh), 5.13 (d, $^2J_{H,H} = 12.4$ Hz, 1 H, CHPh), 4.55–4.44 (m, 3 H, 2-H, $CH_2$), 4.35 (t, $^3J_{H,H} = 5.3$ Hz, 2 H, $CH_2$), 3.90 (dd, $^3J_{H,H} = 2.8$, $^2J_{H,H} = 9.2$ Hz, 1 H, 3-H), 3.71 (dd, $^3J_{H,H} = 3.2$, $^2J_{H,H} = 9.6$ Hz, 1 H, 3′-H), 3.39–3.35 (m, 2 H, N-$CH_2$), 2.86 (s, 6 H, 2 N-Me), 1.41 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta = 170.7$, 155.6, 152.2, 135.6, 134.4, 130.9, 130.0, 129.6, 128.8, 128.6, 128.4, 128.2, 123.2, 118.6, 115.5, 80.2, 70.1, 67.3, 64.6, 54.2, 50.4, 45.5, 42.9, 28.4 ppm. HRMS (ESI): calcd. for $C_{32}H_{41}N_6NaO_7S$ 675.2577; found 675.2571.

**Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-(4-{[5-(dimethylamino)naphthalen-1-ylsulfonamido]methyl}-1*H*-1,2,3-triazol-1-yl)propanoate (6):** From alkyne **4** (36 mg, 0.13 mmol) and azide **5** (30 mg,

0.13 mmol), the click reaction according to the General Procedure A followed by purification by column chromatography (petroleum ether/EtOAc, 1:2) afforded **6** as a colourless liquid (134 mg, 76%). $R_f$ = 0.1 (petroleum ether/EtOAc, 1:2). $[\alpha]_D$ = +26.4 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.57 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 8.27–8.22 (m, 2 H, Ar-H), 7.59–7.52 (m, 2 H, Ar-H), 7.28 (s, 1 H, CH=C), 7.20 (d, $^3J_{H,H}$ = 6.8 Hz, 1 H, Ar-H), 5.29 (d, $^3J_{H,H}$ = 6.4 Hz, 1 H, NH), 5.15 (t, $^3J_{H,H}$ = 6.0 Hz, 1 H, NH), 4.73–4.63 (m, 3 H, 2-H, N-$CH_2$), 4.19 (d, $^3J_{H,H}$ = 6.4 Hz, 2 H, $CH_2$), 3.76 (s, 3 H, OMe), 2.90 (s, 6 H, 2 N-$CH_3$), 1.45 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 169.4, 155.1, 152.1, 143.8, 134.4, 130.8, 129.8, 128.8, 123.5, 123.2, 118.6, 115.4, 80.9, 53.7, 53.3, 50.9, 45.5, 38.9, 28.3 ppm. HRMS (ESI): calcd. for $C_{24}H_{32}N_6NaO_6S$ 555.2002; found 555.2003.

**Methyl (*S*)-2-(Benzyloxycarbonylamino)-6-(4-{[5-(dimethylamino)-naphthalen-1-ylsulfonamido]methyl}-1*H*-1,2,3-triazol-1-yl)hexanoate (8):** From alkyne **4** (23 mg, 0.08 mmol) and azide **7** (25 mg, 0.08 mmol), the click reaction according to the General Procedure A led to **8** as a colourless liquid (45 mg, 94%). $[\alpha]_D$ = +6.2 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.54 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 8.25 (dd, $^4J_{H,H}$ = 1.4, $^3J_{H,H}$ = 7.8 Hz, 2 H, Ar-H), 7.53–7.47 (m, 2 H, Ar-H), 7.35–7.27 (m, 5 H, Ph), 7.21 (s, 1 H, CH=C), 7.16 (d, $^3J_{H,H}$ = 7.4 Hz, 1 H, Ar-H), 5.58 (t, $^3J_{H,H}$ = 6.2 Hz, 1 H, NH), 5.42 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, NH), 5.07 (s, 2 H, $PhCH_2$), 4.34–4.30 (m, 1 H, 2-H), 4.18 (d, $^3J_{H,H}$ = 6.4 Hz, 2 H, N-$CH_2$), 4.14 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, N-$CH_2$), 3.71 (s, 3 H, OMe), 2.86 (s, 6 H, 2 N-$CH_3$), 1.82–1.19 (m, 6 H, 3 $CH_2$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.7, 155.9, 152.1, 143.9, 136.2, 134.7, 130.7, 129.9, 129.7, 128.6, 128.3, 128.2, 122.2, 118.8, 115.4, 67.2, 53.5, 52.6, 49.9, 45.5, 38.9, 31.9, 29.5, 22.1 ppm. HRMS (ESI): calcd. for $C_{30}H_{36}N_6NaO_6S$ 631.2315; found 631.2292.

**Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-[4-(4-{[5-(dimethylamino)naphthalen-1-ylsulfonamido]methyl}-1*H*-1,2,3-triazol-1-yl)-phenyl]propanoate (10):** From alkyne **4** (30 mg, 0.1 mmol) and azide **9** (33.8 mg, 0.1 mmol), the click reaction according to the General Procedure A led to **10** as a colourless liquid (60 mg, 93%). $[\alpha]_D$ = +30.6 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.49 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 8.27–8.23 (m, 2 H, Ar-H), 7.53 (s, 1 H, Ar-H), 7.51–7.43 (m, 4 H, Ar-H), 7.22 (d, $^3J_{H,H}$ = 7.8 Hz, 2 H, Ar-H), 7.13 (dd, $^4J_{H,H}$ = 1.4, $^3J_{H,H}$ = 7.8 Hz, 2 H, Ar-H), 5.74 (s, 1 H, NH), 5.07 (d, $^3J_{H,H}$ = 7.4 Hz, 1 H, NH), 4.62–4.57 (m, 1 H, 2-H), 4.30 (d, $^3J_{H,H}$ = 6.0 Hz, 2 H, $CH_2$), 3.71 (s, 3 H, OMe), 3.20 (dd, $^3J_{H,H}$ = 5.5, $^2J_{H,H}$ = 13.3 Hz, 1 H, 3-H), 3.09 (dd, $^3J_{H,H}$ = 6.4, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3′-H), 2.81 (s, 6 H, 2 N-$CH_3$), 1.40 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.1, 155.1, 152.1, 144.6, 137.3, 135.7, 134.7, 130.8, 130.6, 129.9, 129.8, 129.6, 128.7, 123.2, 120.5, 120.3, 118.7, 115.4, 80.3, 54.4, 52.5, 45.4, 38.8, 38.0, 28.4 ppm. HRMS (ESI): calcd. for $C_{30}H_{37}N_6O_6$ 609.2495; found 609.2490.

**Methyl (2*S*)-2-(*tert*-Butoxycarbonylamino)-3-{4-[(3′-hydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthen]-6′-yloxy)methyl]-1*H*-1,2,3-triazol-1-yl}propanoate (12):** From alkyne **11** (37 mg, 0.1 mmol) and azide **5** (24 mg, 0.1 mmol), the click reaction according to the General Procedure A led to **12** as a brown solid (55 mg, 94%). $[\alpha]_D$ = –16.5 ($c$ = 0.3, MeOH). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.01 (d, $^3J_{H,H}$ = 7.4 Hz, 1 H, Ar-H), 7.91 (s, 1 H, OH), 7.67–7.60 (m, 3 H, Ar-H), 7.15 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, Ar-H), 6.82 (m, 1 H, Ar-H), 6.74 (s, 1 H, Ar-H), 6.67 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 6.63–6.60 (m, 1 H, Ar-H), 6.58–6.55 (m, 2 H, Ar-H), 5.45 (d, $^3J_{H,H}$ = 6.4 Hz, 1 H, NH), 5.19 (s, 2 H, O-$CH_2$), 4.83–4.81 (m, 2 H, N-$CH_2$), 4.74–4.70 (m, 1 H, 2-H), 3.76 (s, 3 H, OMe), 1.42 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 169.9, 169.4, 159.7, 158.6, 155.2, 153.1, 152.5, 152.4, 143.5, 135.2, 129.8, 129.2, 126.7, 125.0, 124.4, 124.1, 112.7, 111.9, 111.8, 110.5, 103.1, 102.1, 77.4, 61.8, 53.7, 53.3, 51.2, 28.3 ppm. HRMS (ESI): calcd. for $C_{32}H_{30}N_4NaO_9$ 637.1911; found 637.1915.

**Methyl (2*S*)-2-(Benzyloxycarbonylamino)-6-{4-[(3′-hydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthen]-6′-yloxy)methyl]-1*H*-1,2,3-triazol-1-yl}hexanoate (13):** From alkyne **11** (30 mg, 0.08 mmol) and azide **7** (25 mg, 0.08 mmol), the click reaction according to the General Procedure A led to **13** as a brown solid (50 mg, 91%). $[\alpha]_D$ = +3.0 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.13 (s, 1 H, OH), 7.98 (dd, $^4J_{H,H}$ = 0.9, $^3J_{H,H}$ = 7.3 Hz, 1 H, Ar-H), 7.66–7.56 (m, 2 H, Ar-H), 7.57 (dd, $^3J_{H,H}$ = 0.9, $^3J_{H,H}$ = 7.4 Hz, 1 H, Ar-H), 7.32–7.25 (m, 5 H, Ph), 7.12 (d, $^3J_{H,H}$ = 7.3 Hz, 1 H, Ar-H), 6.82 (d, $^4J_{H,H}$ = 2.3 Hz, 1 H, Ar-H), 6.74 (d, $^4J_{H,H}$ = 1.8 Hz, 1 H, Ar-H), 6.64–6.58 (m, 2 H, Ar-H), 6.56–6.53 (m, 2 H, Ar-H), 5.43 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH), 5.16 (s, 2 H, $CH_2$), 5.07 (s, 2 H, $CH_2$), 4.36–4.28 (m, 3 H, 2-H, $CH_2$), 3.69 (s, 3 H, OMe), 1.93–1.30 (m, 6 H, 3 $CH_2$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.6, 169.9, 159.7, 158.7, 156.1, 153.1, 152.5, 152.4, 143.3, 136.1, 135.2, 12.7, 129.2, 128.6, 128.3, 128.1, 123.1, 112.8, 111.9, 111.8, 110.5, 103.1, 102.2, 67.2, 61.8, 53.5, 52.6, 50.2, 32.0, 29.5, 22.2 ppm. HRMS (ESI): calcd. for $C_{38}H_{34}N_4NaO_9$ 713.2224; found 713.2222.

**Methyl (2*S*)-2-(*tert*-Butoxycarbonylamino)-3-(4-{4-[(3′-hydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthen]-6′-yloxy)methyl]-1*H*-1,2,3-triazol-1-yl}phenyl)propanoate (14):** From alkyne **11** (25 mg, 0.07 mmol) and azide **9** (22 mg, 0.07 mmol), the click reaction according to the General Procedure A followed by purification by column chromatography (petroleum ether/EtOAc, 1:2) afforded **14** as a yellow solid (45 mg, 94%). $[\alpha]_D$ = +24.8 ($c$ = 0.3, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.06 (s, 1 H, Ar-H), 8.01 (d, $^3J_{H,H}$ = 7.3 Hz, 1 H, Ar-H), 7.66–7.60 (m, 5 H, Ar-H), 7.29 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, Ar-H), 7.16 (d, $^3J_{H,H}$ = 7.3 Hz, 1 H, Ar-H), 6.86 (s, 1 H, Ar-H), 6.76–6.74 (m 1 H, Ar-H), 6.67–6.66 (m, 2 H, Ar-H), 6.59 (s, 1 H, Ar-H), 6.58 (dd, $^4J_{H,H}$ = 2.3, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 5.28 (s, 2 H, $CH_2$), 5.11 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH), 4.64–4.60 (m, 1 H, 2-H), 3.70 (s, 3 H, OMe), 3.23 (dd, $^3J_{H,H}$ = 5.5, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3-H), 3.11 (dd, $^3J_{H,H}$ = 6.4, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3′-H), 1.41 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.1, 169.9, 159.8, 158.6, 155.2, 153.2, 152.6, 152.5, 144.2, 137.5, 135.8, 135.2, 130.8, 129.8, 129.3, 126.8, 125.1, 124.1, 121.3, 120.8, 112.7, 111.9, 110.7, 103.2, 102.1, 80.4, 61.7, 54.4, 52.5, 38.1, 28.4 ppm. HRMS (ESI): calcd. for $C_{38}H_{34}N_4NaO_9$ 713.2224; found 713.2218.

***N*-(2-Azidoethyl)-7-nitrobenzo[*c*][1,2,5]oxadiazol-4-amine (15):** A solution of 4-chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole (200 mg, 1 mmol) in methanol (3.9 mL) was added to a solution of (2-azidoethyl)amine (90 mg, 1 mmol) in 0.3 M $NaHCO_3$ (3.9 mL), and the reaction mixture was stirred at 50 °C for 3 h. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography to afford **15** as a black solid (90 mg, 36%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.52 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, 6-H), 6.30 (s, 1 H, NH), 6.27 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, 5-H), 3.78–3.76 (m, 2 H, $CH_2$), 3.73–3.69 (m, 2 H, $CH_2$) ppm. $^{13}$C NMR (100 MHz, [$D_6$]DMSO): $\delta$ = 145.7, 145.0, 138.4, 121.9, 100.1, 49.4, 43.4 ppm. HRMS (ESI): calcd. for $C_8H_7N_7NaO_3$ 272.0508; found 272.0503.

**Benzyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-({1-[2-(7-nitrobenzo-[*c*][1,2,5]oxadiazol-4-ylamino)ethyl]-1*H*-1,2,3-triazol-4-yl}methoxy)-propanoate (16):** From alkyne **2** (40 mg, 0.12 mmol) and azide **15** (30 mg, 0.12 mmol), the click reaction according to the General Procedure B followed by purification by column chromatography

(petroleum ether/EtOAc, 1:2) afforded **16** as a yellow solid (66 mg, 93%). $[\alpha]_D$ = –2.0 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.40 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, Ar-H), 7.41–7.29 (m, 6 H, Ar-H), 6.17 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, Ar-H), 5.43 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, NH), 5.21 (d, $^2J_{H,H}$ = 12.4 Hz, 1 H, CHPh), 5.09 (d, $^2J_{H,H}$ = 12.4 Hz, 1 H, CHPh), 4.68 (s, 2 H, $CH_2$), 4.62–4.52 (m, 2 H, $CH_2$), 4.46 (m, 1 H, 2-H), 4.09 (s, 2 H, $CH_2$), 3.92 (dd, $^3J_{H,H}$ = 2.8, $^2J_{H,H}$ = 9.2 Hz, 1 H, 3-H), 3.75 (dd, $^3J_{H,H}$ = 2.8, $^2J_{H,H}$ = 9.2 Hz, 1 H, 3′-H), 1.40 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 170.7, 155.6, 145.1, 144.3, 143.9, 143.5, 136.2, 135.5, 128.6, 128.4, 128.0, 124.6, 123.8, 80.3, 76.8, 70.4, 64.7, 54.2, 48.6, 43.6, 28.4 ppm. HRMS (ESI): calcd. for $C_{26}H_{30}N_8NaO_8$ 605.2084; found 605.2079.

**7-Nitro-*N*-(prop-2-ynyl)benzo[*c*][1,2,5]oxadiazol-4-amine (17):** A solution of 4-chloro-7-nitrobenzo[*c*][1,2,5]oxadiazole (124.3 mg, 0.623 mmol) in methanol (10 mL) was added to a solution of propargylamine (44 μL, 0.64 mmol) in 0.3 M $NaHCO_3$ (2.5 mL), and the reaction mixture was stirred at 50 °C for 3 h. After solvent evaporation, the residue was purified by column chromatography (petroleum ether/EtOAc, 7:3) to afford 35 mg (30%) of **17** as a black solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.55 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, 6-H), 6.36 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, 5-H), 6.32 (s, 1 H, NH), 4.3 (dd, $^4J_{H,H}$ = 2.3, $^3J_{H,H}$ = 5.5 Hz, 2 H, $CH_2$), 2.44 (t, $^4J_{H,H}$ = 2.5 Hz, 1 H, CH≡C) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 144.6, 144.0, 136.6, 123.5, 99.7, 77.4, 72.8, 32.2 ppm. HRMS (ESI): calcd. for $C_9H_6N_4NaO_3$ 241.0338; found 241.0332.

**Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-{4-[(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylamino)methyl]-1*H*-1,2,3-triazol-1-yl}propanoate (18):** From alkyne **17** (35 mg, 0.16 mmol) and azide **5** (39 mg, 0.16 mmol), the click reaction according to the General Procedure B followed by purified by column chromatography (petroleum ether/EtOAc, 1:2) afforded **18** as a yellow solid (54 mg, 73%). $[\alpha]_D$ = –23.1 ($c$ = 0.3, MeOH). $^1$H NMR (400 MHz, [D$_6$]-DMSO): $\delta$ = 9.86 (s, 1 H, NH), 8.49 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 8.03 (s, 1 H, Ar-H), 7.38 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH), 6.48 (d, $^3J_{H,H}$ = 9.2 Hz, 1 H, Ar-H), 4.71–4.65 (m, 3 H, 3-H, $CH_2$), 4.54–4.42 (m, 2 H, 2,3-H), 3.60 (s, 3 H, OMe), 1.20 (s, 9 H, Boc) ppm. $^{13}$C NMR (400 MHz, [D$_6$]DMSO): $\delta$ = 170.5, 155.6, 145.0, 144.7, 143.0, 138.3, 124.7, 122.0, 100.4, 79.2, 54.1, 52.8, 49.9, 38.9, 28.4 ppm. HRMS (ESI): calcd. for $C_{18}H_{22}N_8NaO_7$ 485.1509; found 485.1511.

**Methyl (*S*)-2-(Benzyloxycarbonylamino)-6-{4-[(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylamino)methyl]-1*H*-1,2,3-triazol-1-yl}hexanoate (19):** From alkyne **17** (17.6 mg, 0.081 mmol) and azide **7** (25 mg, 0.081 mmol), the click reaction according to the General Procedure B followed by purification by column chromatography (petroleum ether/EtOAc, 1:2) afforded **19** as a yellow solid (40 mg, 94%). $[\alpha]_D$ = –2.0 ($c$ = 0.5, $CH_2Cl_2$). $R_f$ = 0.1 (petroleum ether/EtOAc, 1:2). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.42 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 7.64 (s, 1 H, Ar-H), 7.33–7.25 (m, 5 H, Ph), 6.32 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 5.44 (d, $^3J_{H,H}$ = 8.3 Hz, 1 H, NH), 5.05 (s, 2 H, $OCH_2$), 4.78 (d, $^3J_{H,H}$ = 4.1 Hz, 2 H, $CH_2$), 4.36–4.29 (m, 3 H, 2-H, N-$CH_2$), 3.70 (s, 3 H, OMe), 1.97–1.36 (m, 6 H, 3 $CH_2$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 170.0, 156.0, 144.3, 143.9, 143.4, 142.3, 136.4, 136.1, 128.6, 128.3, 128.0, 124.4, 122.3, 99.7, 67.1, 53.5, 52.6, 50.2, 39.4, 32.0, 29.5, 22.2 ppm. HRMS (ESI): calcd. for $C_{24}H_{26}N_8NaO_7$ 561.1822; found 561.1817.

**Methyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-(4-{4-[(7-nitrobenzo[*c*][1,2,5]oxadiazol-4-ylamino)methyl]-1*H*-1,2,3-triazol-1-yl}phenyl)propanoate (20):** From alkyne **17** (29 mg, 0.14 mmol) and azide **9** (43 mg, 0.14 mmol), the click reaction according to the General Procedure B followed by purification by column chromatography (petroleum ether/EtOAc, 1:2) afforded **20** as a yellow solid (50 mg, 69%). $[\alpha]_D$ = +1.2 ($c$ = 0.3, MeOH). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.46 (d, $^3J_{H,H}$ = 9.9 Hz, 1 H, Ar-H), 8.04 (s, 1 H, Ar-H), 7.62 (d, $^3J_{H,H}$ = 8.3 Hz, 2 H, Ar-H), 7.27 (d, $^3J_{H,H}$ = 8.3 Hz, 2 H, Ar-H), 7.03 (s, 1 H, NH), 6.39 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 5.04 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH), 4.90 (d, $^3J_{H,H}$ = 5.5 Hz, 2 H, N-$CH_2$), 4.58 (m, 1 H, 2-H), 3.72 (s, 3 H, OMe), 3.18 (dd, $^3J_{H,H}$ = 5.5, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3-H), 3.05 (dd, $^3J_{H,H}$ = 6.4, $^2J_{H,H}$ = 13.7 Hz, 1 H, 3′-H), 1.39 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.0, 155.2, 144.4, 143.9, 143.2, 143.1, 138.0, 136.3, 135.0, 130.9, 124.8, 120.7, 120.4, 99.8, 80.3, 54.4, 52.6, 39.5, 38.1, 28.4 ppm. HRMS (ESI): calcd. for $C_{24}H_{26}N_8NaO_7$ 561.1822; found 561.1817.

**Methyl (*S*)-3-[4-(Benzo[*c*][1,2,5]thiadiazol-4-yl)-1*H*-1,2,3-triazol-1-yl]-2-(*tert*-butoxycarbonylamino)propanoate (22):** From 4-(trimethylsilylethynyl)benzo[*c*][1,2,5]thiadiazole (**21**; 46.8 mg, 0.2 mmol) and azide **5** (49 mg, 0.2 mmol), the click reaction according to the General Procedure A in the presence of $Bu_4NF$ (253 mg, 0.8 mmol) followed by purification by column chromatography (petroleum ether/EtOAc, 1:1) afforded **22** as a yellow solid (65 mg, 81%). $[\alpha]_D$ = +38.8 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.71 (s, 1 H, Ar-H), 8.52 (dd, $^4J_{H,H}$ = 1.4, $^3J_{H,H}$ = 7.3 Hz, 1 H, Ar-H), 7.97 (dd, $^4J_{H,H}$ = 1.4, $^3J_{H,H}$ = 9.2 Hz, 1 H, Ar-H), 7.71–7.67 (m, 1 H, Ar-H), 5.48 (d, $^3J_{H,H}$ = 6.9 Hz, 1 H, NH), 4.97 (t, $^3J_{H,H}$ = 3.7 Hz, 2 H, $CH_2$), 4.80–4.76 (m, 1 H, 2-H), 3.82 (s, 3 H, OMe), 1.44 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 169.6, 155.4, 155.2, 151.9, 143.2, 130.0, 125.5, 125.4, 123.3, 120.9, 80.8, 53.9, 53.3, 51.0, 28.3 ppm. HRMS (ESI): calcd. for $C_{17}H_{20}N_6NaO_4S$ 427.1164; found 427.1166.

**(*S*)-3-[4-(Benzo[*c*][1,2,5]thiadiazol-4-yl)-1*H*-1,2,3-triazol-1-yl]-2-(*tert*-butoxycarbonylamino)propanoic Acid (23):** LiOH (3.6 mg, 0.149 mmol) was added to a solution of methyl ester **22** (50 mg, 0.124 mmol) in MeOH (3 mL) and water (1 mL). The reaction mixture was stirred at room temp. for 24 h, then acidified with $H^+$ resin, filtered, and concentrated directly to give the free acid **23** (46 mg, 97%). $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ = 8.49 (s, 1 H, Ar-H), 8.36 (d, $^3J_{H,H}$ = 7.4 Hz, 1 H, Ar-H), 7.97 (dd, $^4J_{H,H}$ = 0.9, $^3J_{H,H}$ = 10.1 Hz, 1 H, Ar-H), 7.72 (dd, $^3J_{H,H}$ = 6.9, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 5.02 (dd, $^3J_{H,H}$ = 4.1, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3-H), 4.77 (dd, $^3J_{H,H}$ = 8.2, $^2J_{H,H}$ = 14.2 Hz, 1 H, 3′-H), 4.61 (m, 1 H, 2-H), 1.30 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CD_3OD$): $\delta$ = 171.9, 155.3, 151.8, 129.7, 125.6, 124.8, 123.4, 120.5, 79.5, 54.7, 51.0, 27.2 ppm.

**Methyl (*S*)-6-[4-(Benzo[*c*][1,2,5]thiadiazol-4-yl)-1*H*-1,2,3-triazol-1-yl]-2-(benzyloxycarbonylamino)hexanoate (24):** From alkyne **21** (19 mg, 0.08 mmol) and azide **7** (25 mg, 0.08 mmol), the click reaction was carried out according to the General Procedure A in the presence of $Bu_4NF$ (202 mg, 0.64 mmol). After 12 h, the reaction mixture was treated with a $CH_2Cl_2$/0.1 N EDTA solution (1:1, 16 mL). The water layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layers were combined, washed with brine, dried with $MgSO_4$ and the solvents evaporated under vacuum. Purification on silica gel with EtOAc/petroleum ether (1:1) gave **24** as a light-yellow solid (40 mg, 79%). $[\alpha]_D$ = +6.0 ($c$ = 0.3, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.72 (s, 1 H, Ar-H), 8.54 (d, $^3J_{H,H}$ = 6.9 Hz, 1 H, Ar-H), 7.97 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 7.72 (dd, $^3J_{H,H}$ = 6.9, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 7.34–7.27 (m, 5 H, Ph), 5.33 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, NH), 5.06 (s, 2 H, $CH_2$), 4.48 (t, $^3J_{H,H}$ = 7.1 Hz, 2 H, N-$CH_2$), 4.41–4.36 (m, 1 H, 2-H), 3.71 (s, 3 H, OMe), 2.09–1.38 (m, 6 H, 3 $CH_2$) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.7, 155.9, 155.4, 151.9, 143.3, 136.2, 130.1, 128.6, 128.3, 128.2,

125.4, 123.9, 123.6, 120.7, 67.1, 53.5, 52.6, 50.1, 32.2, 29.8, 22.3 ppm. HRMS (ESI): calcd. for $C_{23}H_{24}N_6NaO_4S$ 503.1477; found 503.1472.

**Methyl (*S*)-3-{4-[4-(Benzo[*c*][1,2,5]thiadiazol-4-yl)-1*H*-1,2,3-triazol-1-yl]phenyl}-2-(*tert*-butoxycarbonylamino)propanoate (25):** From alkyne **21** (23 mg, 0.1 mmol) and azide **9** (34 mg, 0.1 mmol), the click reaction was performed according to the General Procedure A in the presence of $Bu_4NF$ (252 mg, 0.8 mmol). After 12 h, the reaction mixture was treated with a $CH_2Cl_2$/0.1 N EDTA solution (1:1, 20 mL). The aqueous layer was extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layers were combined, washed with brine, dried with $MgSO_4$ and the solvents evaporated under vacuum. Purification on silica gel with EtOAc/petroleum ether (1:4) gave **25** as a light-green solid (45 mg, 80%). $[\alpha]_D$ = +42.8 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 9.17 (s, 1 H, Ar-H), 8.60 (dd, $^4J_{H,H}$ = 0.9, $^3J_{H,H}$ = 7.3 Hz, 1 H, Ar-H), 7.98 (dd, $^4J_{H,H}$ = 0.9, $^3J_{H,H}$ = 8.3 Hz, 1 H, Ar-H), 7.81 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, Ar-H), 7.72 (dd, $^3J_{H,H}$ = 6.9, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 7.32 (d, $^3J_{H,H}$ = 8.2 Hz, 2 H, Ar-H), 5.07 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH), 4.62 (m, 1 H, 2-H), 3.74 (s, 3 H, OMe), 3.21 (dd, $^3J_{H,H}$ = 6.0, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3-H), 3.10 (dd, $^3J_{H,H}$ = 2.4, $^2J_{H,H}$ = 13.8 Hz, 1 H, 3′-H), 1.40 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 172.1, 155.4, 155.1, 151.9, 143.8, 137.2, 136.1, 130.8, 130.0, 125.8, 123.2, 121.9, 121.1, 120.7, 80.3, 54.4, 52.5, 38.1, 28.4 ppm. HRMS (ESI): calcd. for $C_{23}H_{24}N_6NaO_4S$ 503.1477; found 503.1472.

**Benzyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-{[1-(7-hydroxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl]methoxy}propanoate (27):** From alkyne **2** (50 mg, 0.15 mmol) and azidocoumarin **26** (30.5 mg, 0.15 mmol), the click reaction according to the General Procedure A followed by purification by column chromatography (petroleum ether/EtOAc, 1:1) afforded **27** as a colourless liquid (70 mg, 88%). $[\alpha]_D$ = –11.4 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.46 (s, 1 H, Ar-H), 8.39 (s, 1 H, Ar-H), 7.49 (d, $^3J_{H,H}$ = 8.2 Hz, 1 H, Ar-H), 7.30–7.24 (m, 5 H, Ph), 6.93–6.90 (m, 2 H, Ar-H), 5.63 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, NH), 5.25 (d, $^2J_{H,H}$ = 12.4 Hz, 1 H, CHPh), 5.14 (d, $^2J_{H,H}$ = 12.4 Hz, 1 H, CHPh), 4.70–4.61 (m, 2 H, $CH_2$), 4.52–4.50 (m, 1 H, 2-H), 4.02 (dd, $^3J_{H,H}$ = 3.7, $^2J_{H,H}$ = 9.6 Hz, 1 H, 3-H), 3.82 (dd, $^3J_{H,H}$ = 3.2, $^2J_{H,H}$ = 9.6 Hz, 1 H, 3′-H), 1.43 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 170.9, 161.6, 156.2, 155.8, 154.7, 144.2, 135.3, 134.3, 130.4, 128.6, 128.4, 128.1, 123.9, 119.5, 114.9, 103.2, 80.5, 70.5, 67.4, 64.4, 54.2, 28.4 ppm. HRMS (ESI): calcd. for $C_{27}H_{28}N_4NaO_8$ 559.1805; found 559.1800.

**Benzyl (*S*)-2-(*tert*-Butoxycarbonylamino)-3-{[1-(7-methoxy-2-oxo-2*H*-chromen-3-yl)-1*H*-1,2,3-triazol-4-yl]methoxy}propanoate (29):** From alkyne **2** (61 mg, 0.19 mmol) and azidocoumarin **28** (40 mg, 0.19 mmol), the click reaction according to the General Procedure A followed by purification by column chromatography (petroleum ether/EtOAc, 3:2) afforded **29** as a colourless liquid (80 mg, 80%). $[\alpha]_D$ = –8.0 ($c$ = 0.5, $CH_2Cl_2$). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 8.47 (s, 1 H, Ar-H), 8.46 (s, 1 H, Ar-H), 7.54 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, Ar-H), 7.34–7.26 (m, 5 H, Ph), 6.95 (dd, $^4J_{H,H}$ = 2.3, $^3J_{H,H}$ = 8.2 Hz, 1 H, Ar-H), 6.90 (d, $^4J_{H,H}$ = 2.3 Hz, 1 H, Ar-H), 5.42 (d, $^3J_{H,H}$ = 8.7 Hz, 1 H, NH), 5.21 (d, $^2J_{H,H}$ = 12.4 Hz, 1 H, CHPh), 5.11 (d, $^2J_{H,H}$ = 12.4 Hz, 1 H, CHPh), 4.69–4.60 (m, 2 H, $CH_2$), 4.47 (m, 1 H, 2-H), 3.97 (dd, $^3J_{H,H}$ = 3.2, $^2J_{H,H}$ = 9.6 Hz, 1 H, 3-H), 3.91 (s, 3 H, OMe), 3.76 (dd, $^3J_{H,H}$ = 3.2, $^2J_{H,H}$ = 9.6 Hz, 1 H, 3′-H), 1.42 (s, 9 H, Boc) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 170.5, 163.9, 156.1, 155.6, 154.8, 144.5, 135.5, 133.9, 130.0, 128.6, 128.4, 128.1, 123.8, 120.2, 114.2, 111.6, 100.8, 80.1, 70.5, 67.2, 64.6, 56.1, 54.2, 28.4 ppm. HRMS (ESI): calcd. for $C_{28}H_{30}N_4NaO_8$ 573.1961; found 573.1956.

## Acknowledgments

C. L. thanks the China Scholarship Council for a doctorate scholarship.

[1] M. S. T. Gonçalves, *Chem. Rev.* **2001**, *109*, 190–212.
[2] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd ed., Springer, New York, **2006**.
[3] a) N. C. Shaner, P. A. Steinbach, R. Y. Tsien, *Nat. Methods* **2005**, *2*, 905–909; b) B. N. G. Giepmans, S. R. Adams, M. H. Ellisman, R. Y. Tsien, *Science* **2006**, *312*, 217–224.
[4] a) M. Taki, M. Shiota, K. Taira, *Protein Eng. Des. Sel.* **2004**, *17*, 119–126; b) O. Delelis, K. Carayon, E. Guiot, H. Leh, P. Tauc, J.-C. Brochon, J.-F. Mouscadet, E. Deprez, *J. Biol. Chem.* **2008**, *283*, 27838–27849.
[5] a) N. Arhel, A. Genovesio, K.-A. Kim, S. Miko, E. Perret, J.-C. Olivo-Marin, S. Shorte, P. Charneau, *Nat. Methods* **2006**, *3*, 817–824; b) B. A. Griffin, S. R. Adams, R. Y. Tsien, *Science* **1998**, *281*, 269–272.
[6] a) A. R. Katritzsky, M. Yoshioka, T. Narindoshvili, A. Chung, J. V. Johnson, *Org. Biomol. Chem.* **2008**, *6*, 4582–4586; b) A. R. Katritzsky, T. Narindoshvili, *Org. Biomol. Chem.* **2009**, *7*, 627–634.
[7] a) G. Turcatti, K. Nemeth, M. D. Edgerton, U. Meseth, F. Talabot, M. Peitsch, J. Knowles, H. Vogel, A. Chollet, *J. Biol. Chem.* **1996**, *271*, 19991–19998; b) L. E. Steward, C. S. Collins, M. A. Gilmore, J. E. Carlson, J. B. A. Ross, A. R. Chamberlin, *J. Am. Chem. Soc.* **1997**, *119*, 6–11; c) T. Hohsaka, D. Kajihara, Y. Ashizuka, H. Murakami, M. Sisido, *J. Am. Chem. Soc.* **1999**, *121*, 34–40; d) B. E. Cohen, T. B. McAnaney, E. S. Park, Y. N. Jan, S. G. Boxer, L. Y. Jan, *Science* **2002**, *296*, 1700–1703; e) R. D. Anderson III, J. Zhou, S. M. Hecht, *J. Am. Chem. Soc.* **2002**, *124*, 9674–9675; f) H. Hamada, N. Kameshima, A. Szymańska, K. Wegner, L. Łankiewicz, H. Shinohara, M. Taki, M. Sisido, *Bioorg. Med. Chem.* **2005**, *13*, 3379–3384; g) D. Kajihara, R. Abe, I. Iijima, C. Komiyama, M. Sisido, T. Hohsaka, *Nature Methods* **2006**, *3*, 923–929; h) J. Wang, J. Xie, P. G. Schultz, *J. Am. Chem. Soc.* **2006**, *128*, 8738–8739; i) D. Summerer, S. Chen, N. Wu, A. Deiters, J. W. Chin, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 9785–9789; j) H. Nakata, T. Ohtsuki, R. Abe, T. Hohsaka, M. Sisido, *Anal. Biochem.* **2006**, *348*, 321–323.
[8] a) C. C. Knight, *Lett. Pept. Sci.* **1998**, *5*, 1–4; b) I. Dufau, H. Mazarquil, *Tetrahedron Lett.* **2000**, *41*, 6063–6066; c) P. Kele, G. Sui, Q. Huo, R. M. Leblanc, *Tetrahedron: Asymmetry* **2000**, *11*, 4959–4963; d) A. Rzeska, J. Malicka, K. Guzow, M. Szabelski, W. Wiczk, *J. Photochem. Photobiol. A: Chem.* **2001**, *146*, 9–18; e) K. Guzow, M. Szabelski, J. Malicka, W. Wiczk, *Helv. Chim. Acta* **2001**, *84*, 1086–1091; f) G. Sui, P. Kele, J. Orbulescu, Q. Huo, R. M. Leblanc, *Lett. Pept. Sci.* **2002**, *8*, 47–51; g) A. Szymańska, K. Wegner, L. Łankiewicz, *Helv. Chim. Acta* **2003**, *86*, 3326–3331; h) M.-P. Brun, L. Bischoff, C. Garbay, *Angew. Chem. Int. Ed.* **2004**, *43*, 3432–3436; i) A. M. Piloto, S. P. G. Costa, M. S. T. Gonçalves, *Tetrahedron Lett.* **2005**, *46*, 4757–4760; j) B. Chipon, G. Clavé, C. Bouteiller, M. Massonneau, P.-Y. Renard, A. Romieu, *Tetrahedron Lett.* **2006**, *47*, 8279–8284; k) K. Guzow, K. Kornowska, W. Wiczk, *Tetrahedron Lett.* **2009**, *50*, 2908–2910.
[9] a) C. C. Knight, *Biochem. J.* **1991**, *274*, 45–48; b) S. J. Hawthorne, P. Harriott, J. Lim, A. J. Turner, B. Walker, C. H. Williams, *Anal. Biochem.* **1997**, *253*, 13–17; c) R. R. Hudgins, F. Huang, G. Gramlich, W. M. Nau, *J. Am. Chem. Soc.* **2002**, *124*, 556–564; d) P. Venkatraman, T. T. Nguyen, M. Sainlos, O. Bilsel, S. Chitta, B. Imperiali, L. J. Stern, *Nature Chem. Biol.* **2007**, *3*, 222–228; e) K. N. Aprilakis, H. Taskent, D. P. Raleigh, *Biochemistry* **2007**, *46*, 12308–12313; f) J. C. Sauceda, R. M. Duke, M. Nitz, *ChemBioChem* **2007**, *8*, 391–394; g) L. You, G. W. Gokel, *Chem. Eur. J.* **2008**, *14*, 5861–5870; h) P. Marek, R. Gupta, D. P. Raleigh, *ChemBioChem* **2008**, *9*, 1372–1374; i)

M. Nitz, A. R. Mezo, M. H. Ali, B. Imperiali, *Chem. Commun.* **2002**, 1912–1913; j) M. J. Vázquez, M. Nitz, J. Stehn, M. B. Yaffe, B. Imperiali, *J. Am. Chem. Soc.* **2003**, *125*, 10150–10151; k) M. J. Vázquez, D. M. Rothman, B. Imperiali, *Org. Biomol. Chem.* **2004**, *2*, 1965–1966; l) M. J. Vázquez, J. B. Blanco, B. Imperiali, *J. Am. Chem. Soc.* **2005**, *127*, 1300–1306; m) G. Loving, B. Imperiali, *J. Am. Chem. Soc.* **2008**, *130*, 13630–13638; n) M. Sainlos, W. S. Iskenderian, B. Imperiali, *J. Am. Chem. Soc.* **2009**, *131*, 6680–6682.

[10] a) V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599; b) C. W. Tornoe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3069; c) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68; d) J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249–1262; e) J. F. Lutz, *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025; f) G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba, A. A. Genazzani, *Med. Res. Rev.* **2008**, *28*, 278–308; g) A. D. Moorhouse, J. E. Moses, *ChemMedChem* **2008**, *3*, 715–723.

[11] a) Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, *36*, 1674–1689; b) J. M. Beierle, W. S. Horne, J. H. van Maarseveen, B. Waser, J. C. Reubi, M. R. Ghadiri, *Angew. Chem. Int. Ed.* **2009**, *48*, 4725–4729.

[12] a) K. E. Beatty, F. Xie, Q. Wang, D. A. Tirrell, *J. Am. Chem. Soc.* **2005**, *127*, 14150–14151; b) A. Deiters, P. G. Schultz, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1521–1524; c) P. F. van Swieten, M. A. Leeuwenburgh, B. M. Kessler, H. S. Overkleeft, *Org. Biomol. Chem.* **2005**, *3*, 20–27; d) K. E. Beatty, J. C. Liu, F. Xie, D. C. Dieterich, E. M. Schuman, Q. Wang, D. A. Tirrell, *Angew. Chem. Int. Ed.* **2006**, *45*, 7364–7367; e) K. Tanaka, Y. Fujii, K. Fukase, *ChemBioChem* **2008**, *9*, 2392–2397; f) I. S. Carrico, *Chem. Soc. Rev.* **2008**, *37*, 1423–1431; g) T. Fekner, X. Li, M. M. Lee, M. K. Chan, *Angew. Chem. Int. Ed.* **2009**, *48*, 1633–1635.

[13] a) A. E. Speers, B. F. Cravatt, *Chem. Biol.* **2004**, *11*, 535–546; b) W. J. L. Wood, A. W. Patterson, H. Tsuruoka, R. K. Jain, J. A. Ellman, *J. Am. Chem. Soc.* **2005**, *127*, 15521–15527.

[14] a) M. Gajewski, B. Seaver, C. S. Esslinger, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4163–4166; b) V. S. Sudhir, C. Venkateswarlu, O. T. M. Musthafa, S. Sampath, S. Chandrasekaran, *Eur. J. Org. Chem.* **2009**, 2120–2129.

[15] C. Li, J. Tang, J. Xie, *Tetrahedron* **2009**, *65*, 7935–7941.

[16] R. F. H. Viguier, A. N. Hulme, *J. Am. Chem. Soc.* **2006**, *128*, 11370–11371.

[17] F. Bolletta, D. Fabbri, M. Lombardo, L. Prodi, C. Trombini, N. Zaccheroni, *Organometallics* **1996**, *15*, 2415–2417.

[18] M. Otsuka, A. Kittaka, T. Iimori, H. Yamashita, S. Kobayashi, M. Ohno, *Chem. Pharm. Bull.* **1985**, *33*, 509–514.

[19] Y. S. Klausner, A. M. Feigenbaum, N. De Groot, A. A. Hochberg, *Archit. Eng.* **1978**, *185*, 151–155.

[20] L. Chen, T.-S. Hu, J. Zhu, H. Wu, Z.-J. Yao, *Synlett* **2006**, 1225–1229.

[21] S. Maisonneuve, Q. Fang, J. Xie, *Tetrahedron* **2008**, *64*, 8710–8720.

[22] K. Sivakumar, F. Xie, B. M. Cash, S. Long, H. N. Barnhill, Q. Wang, *Org. Lett.* **2004**, *6*, 4603–4606.

[23] a) N. Klonis, W. H. Sawyer, *J. Fluoresc.* **1996**, *6*, 147–157; b) N. Klonis, W. H. Sawyer, *Photochem. Photobiol.* **2000**, *72*, 179–185.

[24] E. Deprez, P. Tauc, H. Leh, J.-F. Mouscadet, C. Auclair, J.-C. Brochon, *Biochemistry* **2000**, *39*, 9275–9284.

[25] O. S. Wolfbeis, E. Fürlinger, H. Kroneis, H. Marsoner, *Fresenius' J. Anal. Chem.* **1983**, *314*, 119–124.

[26] N. Nizomov, A. U. Kholov, A. A. Ishchenko, V. V. Ishchenko, V. P. Khilya, *J. Appl. Spectros.* **2007**, *74*, 626–634.

[27] a) E. Deprez, P. Tauc, H. Leh, J.-F. Mouscadet, C. Auclair, M. E. Hawkins, J.-C. Brochon, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 10090–10095; b) E. Guiot, K. Carayon, O. Delelis, F. Simon, P. Tauc, E. Zubin, M. Gottikh, J.-F. Mouscadet, J.-C. Brochon, E. Deprez, *J. Biol. Chem.* **2006**, *281*, 22707–22719.

[28] J.-C. Brochon, *Methods Enzymol.* **1994**, *240*, 262–311.

Received: January 13, 2010
Published Online: March 11, 2010