Organic & Biomolecular Chemistry

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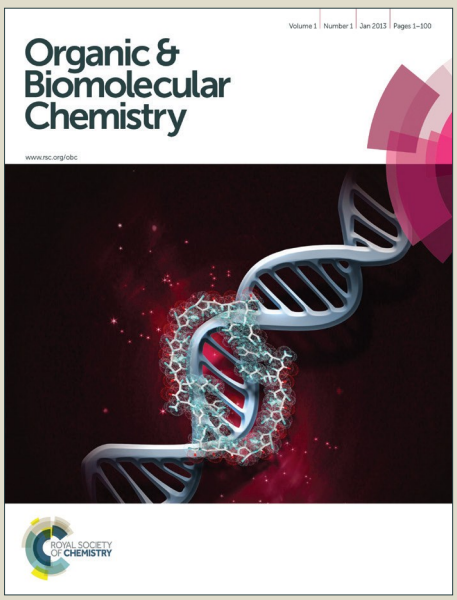

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DOI: 10.1039/C6OB01080F

Journal Name

ARTICLE

Readily functionalizable phosphonium-tagged fluorescent coumarins for enhanced detection of conjugates by mass spectrometry

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Fluorescent coumarins are an important class of small-molecule organic fluorophores ubiquitous in different well-established and emerging fields of research including, among other, biochemistry and chemical biology. The present work aims at covering the poor detectability of coumarin-based conjugates by mass spectrometry while keeping important photophysical properties of the coumarin core. In this context, the synthesis of readily functionalizable phosphonium-tagged coumarin derivatives enabling a dual mass-tag & fluorescence labelling of analytes or (bio)molecules of interest through a single-step protocol, is reported. The utility of these coumarins is illustrated through the preparation of a fluorogenic substrates that facilitated the identification of the peptide fragment released by specific proteolytic cleavages.

Introduction

Interest in the development and the use of small-molecule organic fluorophores has dramatically increased in recent years in particular due to the growing number of imaging applications in life, environmental, and material sciences. Benzo-α-pyrones, or coumarins, are well represented in the arena of organic fluorescent dyes with applications in various areas of research as evidenced by the following selected examples.[1] For instance, coumarin-based fluorescence resonance energy transfer (FRET) probes are routinely used in biochemistry for fluorometric assays of kinase, lipase, protease, peptidase, or glucosidase activity.[2, 3] Besides, in analytical sciences, different coumarin-containing chemosensors have been developed for the selective recognition of environmentally and biologically important organic, inorganic, or metallic analytes.[4] Recently, coumarins have also been involved in the design of innovative probes for theranostics, an appealing biomedical approach that advantageously enables both the detection of the disease and the delivery of the therapeutic agent.[5] In the emerging field of chemical biology, coumarin scaffolds are also used in association with bioorthogonal reactions in order to specifically trigger their fluorescence unveiling thus important biological processes or biomolecules.[6, 5c]

This widespread use of coumarin scaffolds for fluorescence purposes is due to serious advantageous features such as their stability, their straightforward access from inexpensive starting materials, if not already commercially available. Moreover, these bicyclic dyes exhibit outstanding photophysical properties, *i.e.* high fluorescent quantum yields, and large Stokes'shifts, associated with good photochemical stabilities. Although they are usually emitting fluorescence in the range of ~400-500 nm, their emission could be readily red-shifted above 600 nm due to their great versatility in terms of synthetic modularity.[7]

The aforementioned fields of research often require the preparation, characterization and use of small amount of precious and sometimes sophisticated fluorescently labelled compounds. However, several fluorescent members of the coumarin family such as 7-methoxycoumarin derivatives, suffer from low solubility in aqueous media, and a poor intrinsic electrospray ionization ability making their use sometimes difficult for biological applications.

A few fluorescent or fluorogenic mass tag derivatives have been reported with specific applications in proteomic research or sensing of amines or thiols,[8] however, there is no report concerning the general access to a readily and diversely functionalizable family of fluorescent mass tags. To cover this issue, herein is reported the synthesis of bioconjugatable or clickable coumarin-containing fluorescent mass tags. The synthetic strategy relies on the successive introduction of the bioconjugatable handle and the mass tag on each side of the fluorescent scaffold, the mass tag being introduced at the late stage of the synthesis so as to facilitate the purification steps. An extension of this strategy to the 4-amino-1,8-naphthalimide framework was also investigated. The labelling approach would advantageously combine both the fluorescence and the mass-tag labelling through a single and chemoselective tagging step.

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†Electronic Supplementary Information (ESI) available: ^{1}H, ^{13}C NMR, RP-HPLC elution profiles, and spectroscopic data. See DOI: 10.1039/x0xx00000x

Results and discussion

Since (7-methoxycoumarin-4-yl)acetic acid derivatives are extensively used as fluorescent dyes in a wide range of studies, the preparation of model analogues containing a fixed positive charge, has first been undertaken in order to evaluate: a) the influence of the introduction of a mass-tag on their photoluminescence properties; b) their relative ability to be readily detected by mass spectrometry in comparison to parent reference coumarins.

Accordingly, coumarins **5** and **6** bearing either a phosphonium or an ammonium moiety were prepared from resorcinol in 3 and 4 steps, respectively (Fig. 1a).[9] In fact, methyl (7-hydroxycoumarin-4-yl)acetate **3**, obtained in 75% from resorcinol, underwent Mitsunobu reaction with 3-bromopropanol to provide the key intermediate **4** in 54% yield. Next, nucleophilic displacement of the bromide in compound **4** by triphenylphosphine afforded the desired coumarin **5** in 93% yield. The same strategy was used for the synthesis of the ammonium analogue **6**, which however, required an additional transesterification step to recover the methyl ester transesterified into the corresponding ethyl ester during the S_N2 displacement step.

Compounds **1**, **2**, **5** and **6** in hand, their photoluminescence properties were determined and summarized in Table 1. Satisfyingly, both analogues exhibited almost the same maximum absorption (*i.e.* λ_{abs} ~ 325 nm) and emission (*i.e.* λ_{em} ~ 395 nm, entries 1, 3, 4) wavelengths as the parent coumarin **1** upon an excitation at 315 nm in 0.1 M PBS pH 7.4. Furthermore, coumarins **5**, and **6** displayed useful fluorescence quantum yields (0.32 and 0.33 respectively), which is also in accordance with that of the parent compound **1** (Φ_F = 0.40, entry 1).

Next, their relative ability to be readily detected by electrospray ionization mass spectrometry (ESI-MS) in positive ionization mode was investigated by analysing a stoichiometric solution of compounds **1**, **5** and **6**. The phosphonium **5** proved to be the most readily detectable by ESI-MS, exhibiting a ~4-fold higher sensitivity than the quaternary ammonium analogue **6**, as shown in Fig. 1b. Moreover, the signal corresponding to the parent coumarin **1** (m/z 249.2 $[M+H]^+$) was not detected. It is worth noting that the presence of the phosphonium salt moiety in compound **5** improved not only the ability of the coumarin scaffold to be detected by mass spectrometry, but also its solubility in water, which is a key feature in the aim of future biological applications (Fig. 1c). At this stage, the synthesis of ammonium-based coumarins was not pursued to focus on more promising phosphonium salt derivatives.

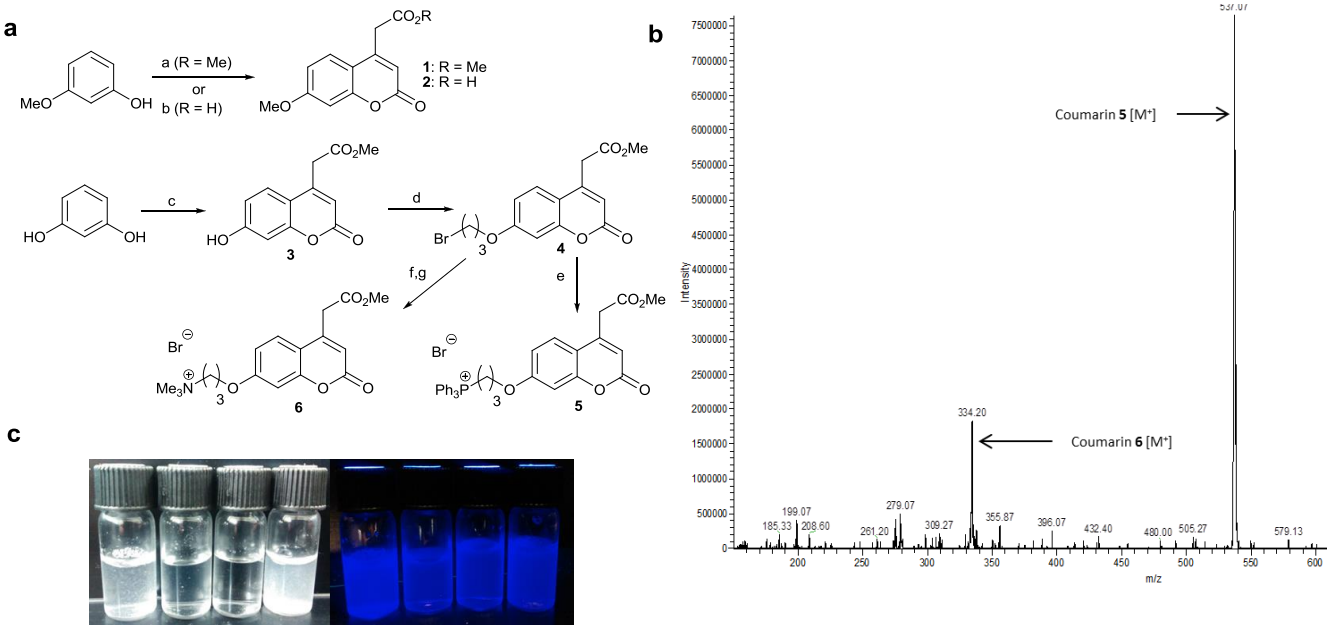

Fig. 1 Synthesis of coumarin derivatives **1**, **2**, **5**, **6**. **a.** Reagents and conditions: (a) Dimethyl-1,3-acetonedicarboxylate, MeOH, conc. H_2SO_4, rt, 16 h, 83%; (b) Citric acid, conc. H_2SO_4, 0 °C, 16 h, 33%; (c) Dimethyl-1,3-acetonedicarboxylate , MeOH, conc. H_2SO_4, rt, 16 h, 75%; (d) 3-bromo-1-propanol, PPh_3, DEAD, rt, 16 h, 54%; (e) PPh_3, ACN, reflux, 24 h, 93%; (f) Me_3N, EtOH, 85 °C, 12 h; (g) MeOH, DIPEA, reflux, 48 h, 87% (over 2 steps). **b**. ESI-MS analysis of a 2.17×10^2 µM solution of coumarins **1, 5, 6** in a 1:1:1 molar ratio. **c**. Photographs of compounds **1**, **6**, **5**, **2** (from left to right) under visible (left) and UV light (right, λ_{ex} = 365 nm)

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Table 1 Photophysical properties of coumarin derivatives[a]

Entry	Compound	λ_{abs} [nm][b]	λ_{em} [nm][b]	Stokes'shift [nm] (cm^{-1})	Φ_F[c]
1	**1**	326	396	70 (5423)	0.40
2	**2**	322	387	65 (5216)	0.40
3	**5**	326	395	69 (5358)	0.32
4	**6**	325	396	71 (5517)	0.33
5	**7**	323	390	67 (5319)	0.30
6	**10**	326	393	67 (5229)	0.31
7	**13**	326	395	69 (5358)	0.32
8	**14**	325	393	68 (5324)	0.16
9	**15**	326	395	69 (5358)	0.14

[a] All measurements were performed at 25 °C. [b] Maxima of the main absorption (λ_{abs}) and emission (λ_{em}) bands. [c] Determined in PBS pH 7.4 at λ_{ex} 315 nm by using 7-hydroxycoumarin (Φ_F = 0.76 in 0.1 M PBS pH 7.4, λ_{ex} = 315 nm) as standard.[10]

Accordingly, we next turned our attention towards the preparation of phosphonium-tagged coumarins bearing readily bioconjugatable (*i.e.* carboxylic acid, amine, and iodoacetamide) or clickable functional groups (*i.e.* 5-ethoxyoxazole,[11] and terminal alkyne).[12] These functions were specifically attached on the carboxylic moiety positioned at the 4-position of the coumarin ring system to assure both the preservation of the crucial photophysical properties of the parent dye **1**,[13] and a rapid access to the desired conjugates from simple starting materials. In fact, the bromocoumarin **4** described in Fig. 1a, was used as key intermediate to provide the small collection of functionalizable coumarins **7**, **10**, **13-15** (Scheme 1). The water solubilizing triphenylphosphonium moiety was strategically introduced on the coumarin ring as late as possible in the synthesis in order to avoid one or more RP-HPLC purifications. First, the carboxylic acid-containing coumarin **7** was obtained in 89% yield by saponification of the methyl ester precursor **5**. Furthermore, the clickable coumarin **10** was formed upon amidation of the carboxylic acid functionality of bromocoumarin **8**, followed by a S_N2 displacement of the alkylbromide **9** by triphenylphosphine, providing **10** in 71% yield. Besides, the amine derivative **13** was also prepared by the successive amidation/S_N2 sequence, followed by the removal of the Boc protecting group of **12** under standard acidic conditions. This compound was used as a key intermediate for the synthesis of iodoacetamide- and 5-alkoxyoxazole-containing coumarins **14** and **15**. It is worth noting that these syntheses required few column chromatographic purifications, the products being purified either by crystallization or washing with organic solvents. Gratifyingly, most of the photophysical properties of these derivatives were found to be comparable to those of the model dye **5**, with the exception of quantum yields of compounds **14** and **15**, which were found to be slightly lower (Table 1, entries 5-9).

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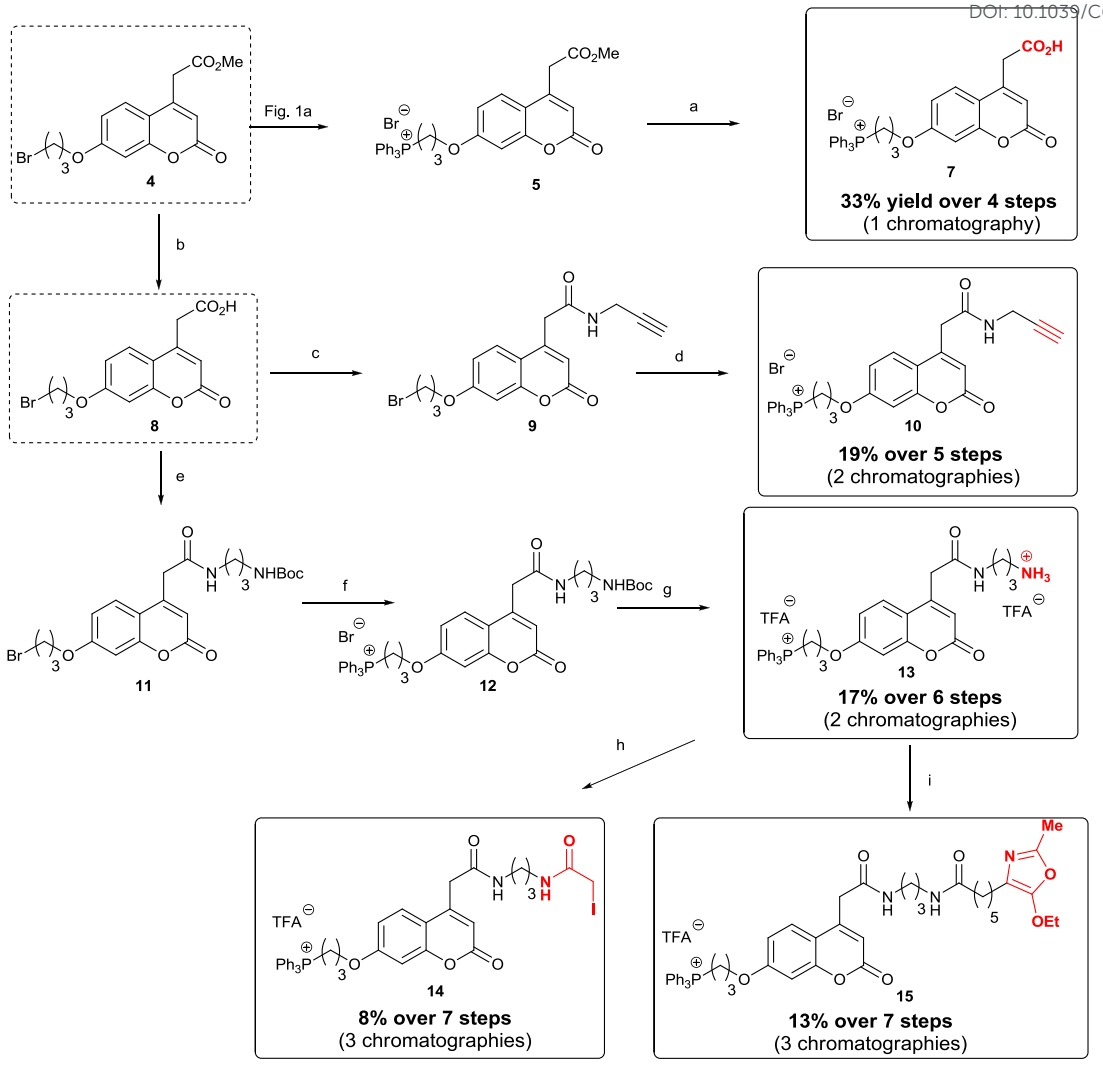

Scheme 1 Derivatizable phosphonium-tagged coumarins. Reagents and conditions: (a) K_2CO_3, MeOH/H_2O (5/1), rt, 4 h, 89%; (b) LiOH, THF, H_2O, rt, 4 h, 83%; (c) propargylamine, PyBOP, ACN, DMF, DIPEA, rt, 2 h, 78%; (d) PPh_3, ACN, reflux, 12 h, 71%; (e) N-Boc-1,3-propanediamine hydrochloride, PyBOP, ACN, DMF, DIPEA, rt, 3 h, 57%; (f) PPh_3, ACN, reflux, 24 h, 97%; (g) TFA, DCM, rt, 2 h, 90%; (h) 2,5-dioxopyrrolidin-1-yl 2-iodoacetate, DIPEA, THF, rt, 1 h, 47%; (i) 6-(5-ethoxy-2-methyloxazol-4-yl)hexanoic acid, DCC, DMAP, DIPEA, DCM, rt, 24 h, 75%.

The usefulness of these phosphonium-labelled coumarin derivatives in the field of bioorganic chemistry was illustrated first by two examples involving the bioconjugation of **10** and **7** with peptides through Huisgen click chemistry and standard amide bond formation strategy, respectively. Both peptides **16** and **18** were prepared according to standard solid-phase peptide synthesis protocols (See Experimental Section). In the first example, the copper-mediated azide-alkyne cycloaddition (CuAAC) of **10** with the tetrapeptide **16** afforded **17** in 80% yield (Fig. 2a). A 1:1 molar ratio solution of the unlabelled and labelled peptide **16** and **17** analysed by ESI-MS, showed only the signal corresponding to the phosphonium salt derivative (Fig 2b). This result confirms the beneficial effect of the fixed charge tag on the detection by mass spectrometry. In the second application, coumarin **7** was involved in the design of a fluorescence resonance energy transfer (FRET)-based protease-sensitive probe, which relied on a fluorophore-quencher pair covalently attached to each end of a peptide substrate. Upon enzymatic digestion, the fluorogenic probe will be cleaved separating the fluorophore and the quencher from one another, thus generating a fluorescent signal. The incorporation of a fixed positive charge tag into the fluorophore moiety should enable the easy identification of the phosphonium-containing peptide fragment formed by the enzymatic digestion, and accordingly, to conclude unambiguously on the specificity of the proteolysis. The proof-of-concept was illustrated using a peptide substrate of β-secretase. This aspartic protease, also called β-amyloid-converting enzyme 1 (BACE-1), has been recognized as an important target for the treatment of Alzheimer's disease.[14] Indeed, BACE-1 is responsible for the aggregation of β-amyloid plaques through the proteolytic process of the amyloid precursor protein (APP). In this context, different BACE-1 FRET substrates have been reported, such as **19** involving a FRET pair that consists of a 7-methoxy-coumarin dye and a 2,4-dinitrophenylamino quencher connected at each extremity of the decapeptide substrate Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys.[15] This peptide was later on modified by incorporation

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of two Arg residues to increase its hydrosolubility, as recently reported by Rademann et al. Accordingly, both fluorogenic probes **19** and **20** were prepared from unlabelled and phosphonium-tagged coumarin **2** and **7** respectively, using the same reaction sequence. The structure and purity of the probes were confirmed by ESI-MS and RP-HPLC analysis (see Supplementary Information).

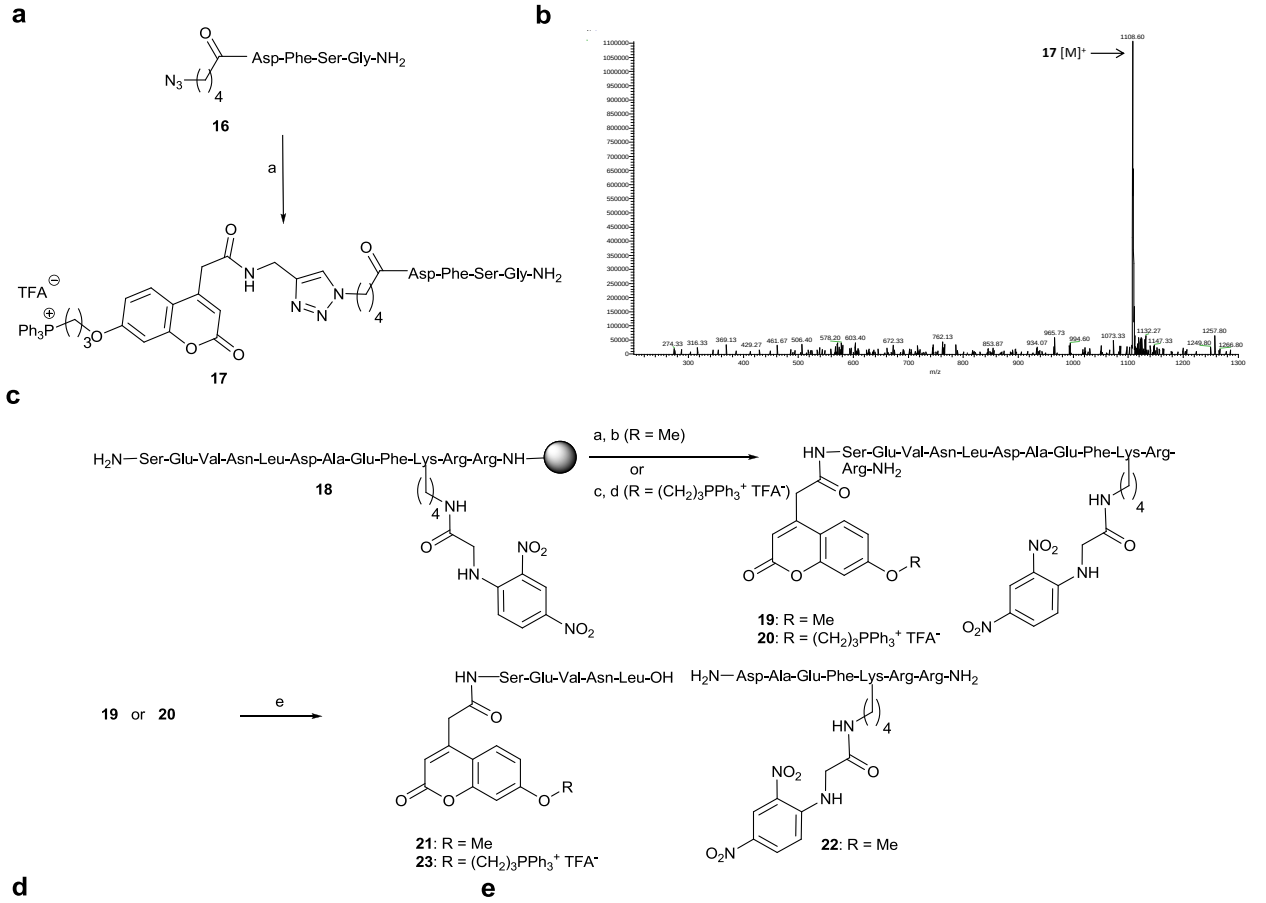

Fig. 2 Bioconjugation applications of coumarins **7** and **10. a.** Reagents and conditions: (a) **10**, $CuSO_4.5H_2O$, sodium ascorbate, TBTA, ACN/H_2O, rt, 18 h, 80%. **b.** ESI-MS analysis of a 5.0 × 10 µM solution of **16** and **17** in a 1:1 molar ratio. **c.** Reagents and conditions: (a) **2**, HOBt, DIC, DMF, rt, 12 h; (b) TFA/TIS/H_2O/EDT, rt, 3 h, 11% (over the total synthetic sequence); (c) **7**, HOBt, DIC, NMP, rt, 72 h; (d) TFA/TIS/H_2O/EDT, rt, 3 h, 1.5% (over the total synthetic sequence); (e) BACE-1, acetate buffer 0.1 M pH = 4.5, 25 °C, 48 h. **d.** Fluorescence emission spectrum of probe **19** (in black) ad **20** (in red) before (dotted line) and after (continuous line) incubation with BACE-1. **e.** LC/ESI-MS analysis in TIC mode of the crude BACE-1 mediated hydrolysis of a 5.0 × 10 µM solution of **19** (in black) and **20** (in red).

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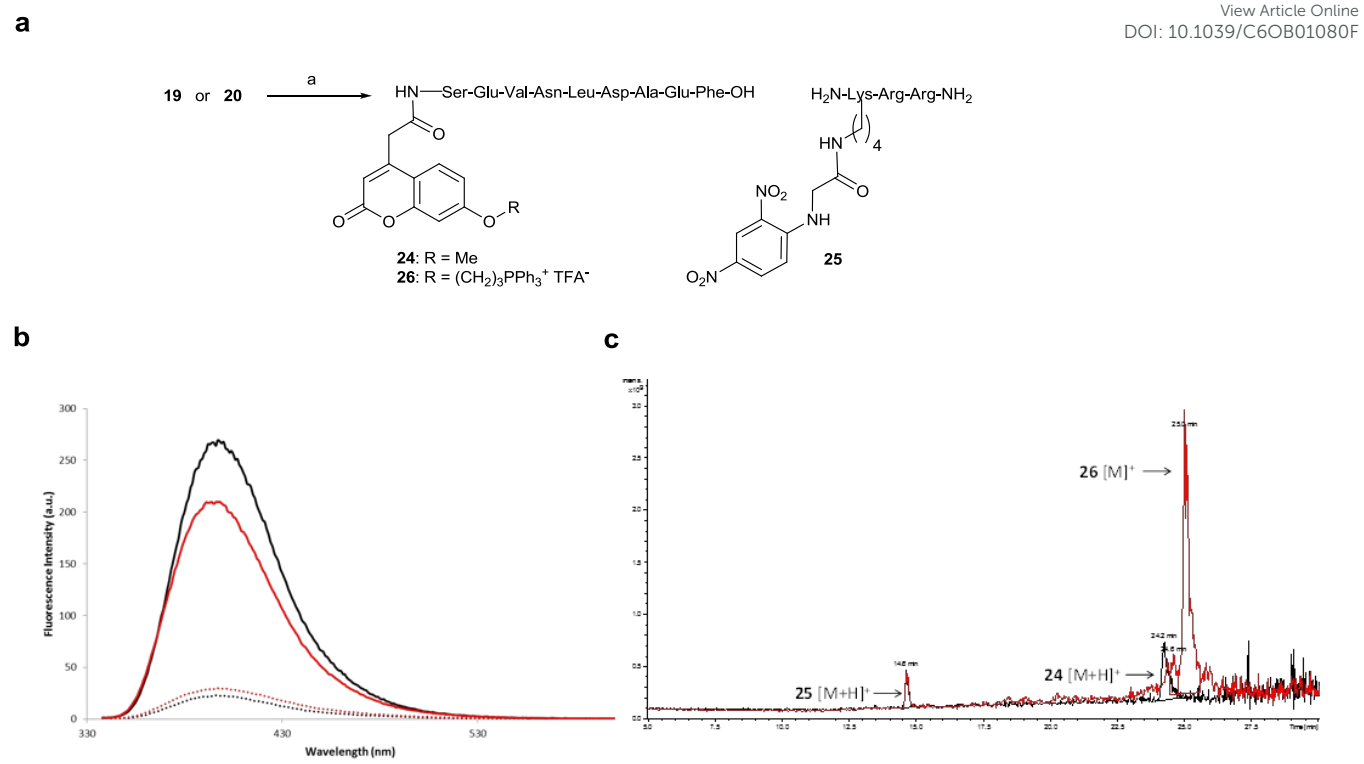

Fig. 3 Bioconjugation applications of coumarins **7** and **10**. **a.** Reagents and conditions: (a) α-chymotrypsin, tris buffer 0.1 M + 10 mM $CaCl_2$ pH = 7.8, 25 °C, 48 h. **b.** Fluorescence emission spectrum of probe **19** (in black) and **20** (in red) before (dotted line) and after (continuous line) incubation with α-chymotrypsin. **c.** LC/ESI-MS analysis in TIC mode of the crude for α-chymotrypsin-mediated hydrolysis of a 5 × 10 µM solution of **19** (in black) and **20** (in red).

Next, each probe was incubated in the presence of BACE-1, in sodium acetate buffer pH 4.5, at 25 °C for 48 hours. Their fluorescence quenching efficiency was measured and found to be 91% and 84% for **19** and **20**, respectively. This difference may be attributed to the slight red-shift in fluorescence emission observed between **2** and **7**, decreasing the spectral overlap between the donor emission and quencher absorption (c.f. Table 1, and Supplementary Information). Their crude enzymatic mixture was subsequently analysed by RP-HPLC coupled with ESI-MS in the full scan (FS) and total ion current (TIC) chromatography mode (Fig. 2e). As the result, the signal corresponding to the fragment **23** (m/z 1065.5 $[M]^+$, t_R = 24.4 min) was significantly higher than that of the unlabelled analogue **21** (m/z 777.3 $[M+H]^+$, t_R = 22.7 min). Besides, the cleavage site positioned between the Leu and Asp residues was consistent with the mode of action of BACE-1. Probes **19** and **20** were next incubated with α-chymotrypsin, a serine protease of pancreatic juice. After 48 h of reaction in Tris buffer pH 7.8, the fluorescence emission spectra of crude enzymatic mixtures produced similar profiles to those obtained with BACE-1, with a wavelength of maximum emission at ~ 395 nm (Fig. 3). Moreover, the fluorescence quenching efficiency in this buffer was also found to be 91% and 84% for **19** and **20**, respectively. In contrast, the TIC chromatogram showed analytes with different retention times and mass-to-charge ratios, which was due to a proteolytic cleavage of the amide bond occurring this time specifically between the Phe and Lys residues. Again, a marked difference in peak intensity was observed between the unlabelled fragment **24** (m/z 1240.5 $[M+H]^+$, t_R = 24.2 min) and the tagged fragment **26** (m/z 1528.7 $[M]^+$, t_R = 25.0 min). Together, outcomes obtained with functionalizable phosphonium-based coumarins **7** and **10** are consistent with preliminary results obtained with model coumarins **1** and **5**, and illustrate the importance of attaching a fixed positive charge to increase the ionizability of conjugates. Then, the bioconjugation ability of the phosphonium-tagged coumarin **14** was investigated through the labelling of a model protein, bovine serum albumin (BSA). First, a solution of BSA dissolved in PBS buffer pH 8.3 was treated with a five-fold molar excess of compound **14**, and incubated for 2.5 h at 37 °C. The excess of iodoacetamide reagent was then removed by repeated ultrafiltration until no detectable absorbance at 325 nm corresponding to the coumarin core was observed in the filtrate. Characterization of the resulting coumarin-BSA conjugate was achieved by means of UV-Vis and fluorescence spectroscopy, as well as mass spectroscopy (Fig. 4). The absorbance spectrum of the conjugate is a linear combination of the absorption of the fluorophore **14** (λ_{max} = 326 nm) and BSA (λ_{max} = 278 nm), which confirms the labeling of the BSA protein. Besides, the quantum yield was determined to be 0.20 in milliQ water at an excitation wavelength of 315 nm, and the fluorophore/protein molar ratio (F/P) was found to be 1.2. The BSA protein having a single reactive cysteine residue (Cys34), this over-labelling may be explained by the conjugation of a small fraction of the 30-35 lysine residues which are accessible

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for bioconjugation. A comparative matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass analysis of native BSA and labelled-BSA showed a noticeable mass difference on the order of several hundred daltons, which is consistent with the attachment of about one phosphonium-tagged coumarin derivative to the protein. Besides, the labelling was further confirmed by MALDI-TOF/TOF MS analysis of chymotrypsin and trypsin digests of modified BSA (c.f. Electronic Supplementary Information).

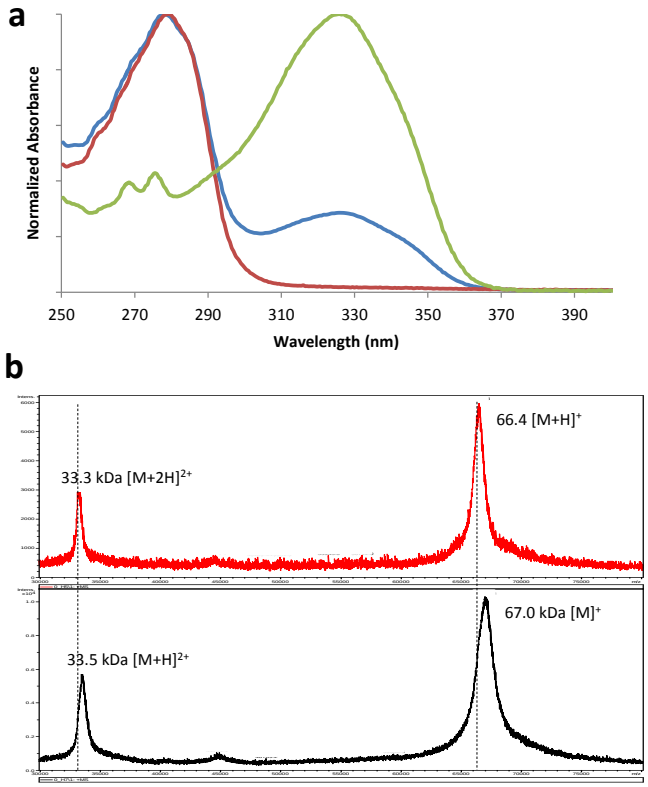


Fig. 4 Bioconjugation of BSA. **a.** Normalized absorption of compound **14** (green), native BSA (red), and modified BSA (blue). **b.** MALDI-TOF MS spectra of native BSA (top), and modified BSA (bottom).

Finally, the dual fluorescence and mass-tag labelling strategy was investigated with a yellow-green fluorescent dye based on the 4-amino-1,8-naphthalimide framework. This was illustrated through the expeditious preparation of terminal alkyne derivatives **28** and **29** which were obtained in two steps from the commercially available 4-chloro-1,8-naphthalic anhydride. As observed with the coumarin family, the presence of the phosphonium moiety improved both the water solubility and the ionization ability of the naphthalimide core. Satisfyingly, the quantum yield of fluorescence of **29** also proved to be better than that of the unlabelled analogue **28**.

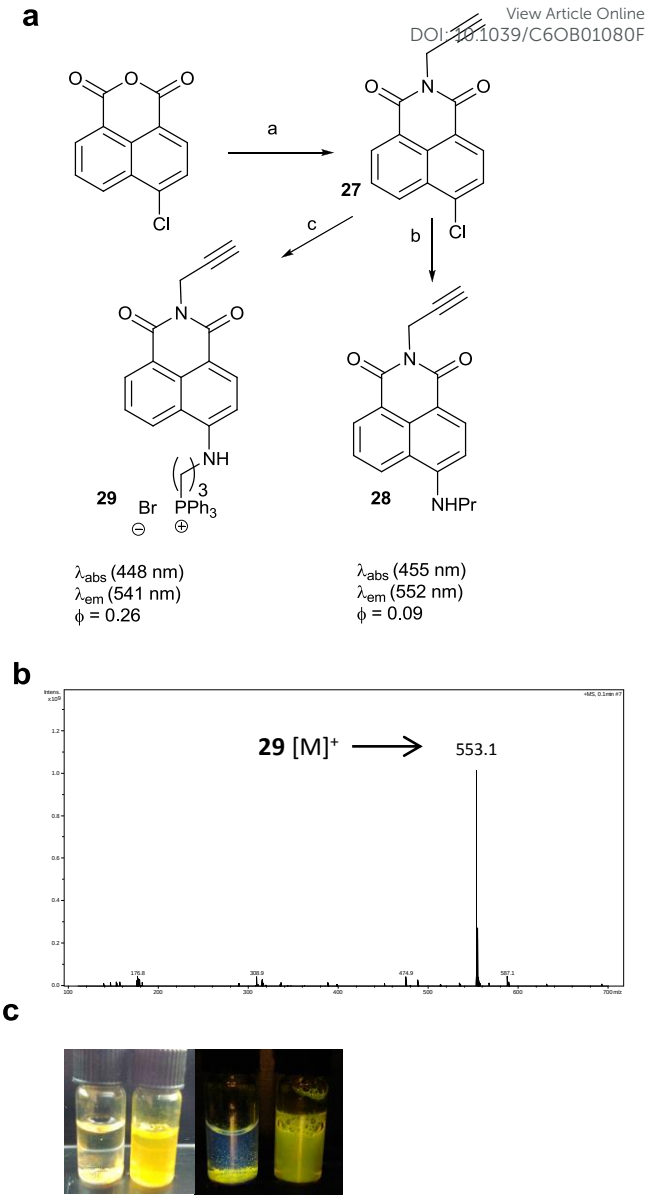


Fig. 5 Derivatizable yellow-green fluorescent dye. **a.** Reagents and conditions: (a) 3-bromopropargylamine, just below reflux for 12 h then 50 °C for 5 h, 88%; (b) n-propylamine, DMSO, 90 °C, 4h, 87%; (c) (3-aminopropyl)triphenylphosphonium bromide hydrobromide, DMSO, 90 °C, 2 d, 48%. **b.** ESI-MS analysis of a 2.17 × 10^2 µM solution of naphthalimide **28** and **29** in a 1:1 molar ratio. **c.** Photographs of compounds **28** and **29** (from left to right) under visible (left) and UV light (right, λ_{ex} = 365 nm)

Conclusions

In conclusion, we have developed a series of conjugatable phosphonium-tagged fluorescent coumarin derivatives obtained from a readily prepared shared-intermediate bromocoumarin **4**. Importantly, this strategy allows covalent attachment of both a fluorophore and a mass-tag to the (bio)molecule of interest in a single step reaction procedure. From this study, it comes out that the detection ability of the phosphonium derivative by ESI-MS was superior to that of

their corresponding quaternary ammonium salt analogue. Furthermore, the carboxylic acid derivative **7** proved to be a suitable phosphonium-tagged surrogate of the traditionally used 7-methoxy-coumarin-4-acetic acid.

Experimental

General

Flash column chromatography purifications were performed on Kieselgel 60 Silica gel (40-63 µm) from Macherey-Nagel. TLC analyses were carried out on Merck DC Kieselgel 60 F-254 coated aluminium sheets. Compounds were visualised by: illumination with a short wavelength UV lamp (*i.e.*, λ = 254 or 365 nm) or staining with potassium permanganate ($KMnO_4$) solution. All reactions were carried out under a nitrogen (Solid Phase Peptide Synthesis) or argon atmosphere with dry solvents under anhydrous conditions. N,N-Diisopropylethylamine (DIPEA) was distilled from CaH_2 and tetrahydrofuran (THF) was distilled over sodium / benzophenone. Acetonitrile (ACN), Methylene chloride (DCM), toluene were obtained from MB SPS-800 apparatus from MBRAUN. N,N'-dimethylformamide (DMF), dimethylsulfoxide (DMSO) and methanol (MeOH) were purchased in anhydrous form and used without further purification, unless otherwise stated. Cyclohexane and ethyl acetate (EtOAc) were purchased at ACS grade quality and used without further purification, unless otherwise stated. Commercially available reagents were used without further purification, unless otherwise stated. BACE-1 full protein (His*Tag®, human, recombinant, NSO Cells) was purchased from Calbiochem (PF 125). α-Chymotrypsin (from bovine pancreas) and BSA were purchased from Sigma. The HPLC-grade ACN was obtained from VWR. Aqueous buffers (0.1 M PBS, aqueous 0.1 M sodium acetate buffers, aqueous 0.1 M Tris buffer + 10 mM $CaCl_2$, used for UV-Fluo analysis, BACE-1 assays and α-chymotrypsin assays) and aqueous mobile phases for HPLC was prepared using water purified with a MilliQ system (purified to 18.2 MΩ.cm).

Instruments and methods

^1H and ^{13}C NMR spectra were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France). Chemical shifts are expressed in parts per million (ppm) using residual solvent peak for calibration, *i.e.* $CDCl_3$ (δ_H = 7.26, δ_C = 77.16), $(CD_3)_2CO$ (δ_H = 2.05, δ_C = 29.84), $(CD_3)_2SO$ (δ_H = 2.50, δ_C = 39.52), CD_3CN (δ_H = 1.94, δ_C = 118.26) and CD_3OD (δ_H = 3.31, δ_C = 49.00).[16] *J* values are expressed in Hz. Infrared (IR) spectra were recorded with a universal ATR accessory on a Perkin-Elmer Spectrum One FT-IR spectrum 100 spectrometer. Bond vibration frequencies are expressed in reciprocal centimetre (cm^{-1}). Analytical HPLC was performed on a Thermo Scientific Surveyor Plus instrument equipped with PDA detector. Semi-preparative HPLC was performed on a Thermo Scientific Spectra System liquid chromatography system (P400) equipped with a UV-Vis 2000 detector. UV/Vis absorption spectra were obtained on a Varian Cary 50 scan spectrometer by using a rectangular quartz cell (Varian, standard cell, Open Top, light path 10 × 10 mm, chamber volume: 3.5 mL). Fluorescence spectroscopic studies (emission/excitation spectra) were performed with a Varian Cary Eclipse spectrometer with a semi-micro quartz fluorescence cell (Hellma, 104F-QS, light path: 10 × 4 mm, chamber volume: 1400 µL). All absorption spectra of the "coumarin derivatives" were recorded (250-600 nm) at 25 °C in PBS 0.1 M, pH 7.4. Excitation/emission spectra were recorded at 390/315 nm respectively (excitation filter: auto and emission filter: open, excitation and emission slit: 5 nm). Fluorescence quantum yields were measured at 25 °C by a relative method using 7-hydroxycoumarin (Φ_F = 0.76 in PBS, pH 7.4),[17] or Lucifer Yellow (Φ_F = 0.21 in water λ_{ex} = 410 nm) as a standard.[18] The following equation was used to determine the relative fluorescence quantum yield:

$$\Phi_F(x) = (A_S/A_X)(F_X/F_S)(n_X/n_S)^2 \Phi_F(s)$$

where A is the absorbance (in the range of 0.01-0.1 A.U.), F is the area under the emission curve, n is the refractive index of the solvents (at 25 °C) used in measurements, and the subscripts s and x represent standard and unknown, respectively. The following refractive index value was used: 1.337 for PBS, and 1.333 for milliQ water. The syntheses of peptides were carried out on an Applied Biosystems 433A synthesizer using the standard Fmoc/tBu chemistry.[19] Low-resolution mass spectra (LRMS) from Fig. 2b and Fig. 2b were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray ionisation (ESI) source. LC-MS data from Fig 2e and Fig 3c were recorded on a Agilent workstation: Agilent quaternary 1200 series equipped with a UV detector, a Bruker HCT (ion trap) apparatus equipped with an electrospray ionisation (ESI) source and a Thermo Hypersil Gold C_{18} column, 5 µm, 2.1 x 100 mm. ACN and 0.1% formic acid (FA) as eluents [0% ACN (5min) then linear gradient from 0% to 100% (40 min) of ACN] at a flow rate of 0.25 mL/min. High-resolution mass spectra (HRMS) were recorded on a Thermo LTQ Orbitrap XL apparatus with an ESI source. MALDI-TOF data were performed on a Autoflex III time-of-light mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a frequency-tripled Nd:YAG laser emitting at 355 nm. The microwave reaction was performed with a Monowave 300 from Anton Paar apparatus using a G30 vial as reaction vessel (linear gradient from 20 to 120 °C, then 15 min at 120 °C, and finally cooled to rt).

HPLC systems used for purity control or purification

System QC: was used for analytical experiments (Thermo Hypersyl GOLD C_{18} column, 5 µm, 2.1 x 100 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min) followed by linear gradient from 0% to 100% (40 min) of ACN] at a flow rate of 0.25 mL/min. Several chromatographic systems were used for the purification steps (by semi-preparative HPLC): System A: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 10.0 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min),

followed by linear gradient from 0% to 20% (20 min) of ACN, followed by linear gradient from 20% to 60% (60 min) of ACN and followed by linear gradient from 60% to 100% (20 min) of ACN] at a flow rate of 13 mL/min. UV-vis wavelength used for the detection was performed at 235 and 335 nm. System B: RP-HPLC (Varian Kromasil C_{18} column, 10 µm, 21.2 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min), followed by linear gradient from 0% to 60% (100 min) of ACN and followed by linear gradient from 60% to 100% (10 min) of ACN] at a flow rate of 20 mL/min. UV-vis wavelength used for the detection was performed at 235 and 338 nm. System C: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 10.0 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min), followed by linear gradient from 0% to 30% (60 min) of ACN and followed by linear gradient from 30% to 100% (35 min) of ACN] at a flow rate of 13 mL/min. UV-vis wavelength used for the detection was performed at 225 and 235 nm. System D: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 10.0 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (10 min), followed by linear gradient from 0% to 100% (100 min) of ACN] at a flow rate of 13 mL/min. UV-vis wavelength used for the detection was performed at 225 and 235 nm. System E: RP-HPLC (Varian Kromasil C_{18} column, 10 µm, 21.2 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min), followed by linear gradient from 0% to 60% (80 min) of ACN and followed by linear gradient from 60% to 100% (30 min) of ACN] at a flow rate of 20 mL/min. UV-vis wavelength used for the detection was performed at 235 and 330 nm. System F: RP-HPLC (Varian Kromasil C_{18} column, 10 µm, 10 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min), followed by linear gradient from 0% to 20% (15 min) of ACN, followed by linear gradient from 20% to 60% (80 min) of ACN and followed by linear gradient from 60% to 100% (20 min) of ACN] at a flow rate of 8 mL/min. UV-vis wavelength used for the detection was performed at 230 and 320 nm. System G: RP-HPLC (Varian Kromasil C_{18} column, 10 µm, 21.2 x 250 mm) with ACN and 0.1% aq. TFA as eluents [0% ACN (5 min), followed by linear gradient from 0% to 60% (120 min) of ACN, followed by linear gradient from 60% to 100% (10 min) of ACN] at a flow rate of 20 mL/min. UV-vis wavelength used for the detection was performed at 325 and 350 nm.

Synthesis

Methyl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate 1

To a solution of 3-methoxyphenol (10 mL, 91.1 mmol) and dimethyl-1,3-acetonedicarboxylate (13.2 mL, 91.1 mmol) in MeOH (135 mL) cooled to 0 °C was slowly added sulphuric acid (90 mL). The resulting solution was allowed to warm up to rt and stirred overnight. Then the reaction mixture was poured into ice water with vigorous stirring for 15 min. The precipitate was filtered off and washed with cold water to neutrality and recrystallised from MeOH to give methyl 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetate **1** as a white solid (18.7 g, 83% yield). m.p. 120-122 °C; IR : $\tilde{\nu}$ = 1695, 1604, 1553, 1396, 1339, 1209, 1198, 1179, 1140, 1065 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) : δ = 7.48 (d, *J* = 8.6 Hz, 1H), 6.94-6.80 (m, 2H), 6.23 (s, 1H), 3.87 (s, 3H), 3.73 (s, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm *:* 169.3, 163.0; 160.9, 155.7, 148.1, 125.6, 114.0, 112.7, 112.6, 101.3, 55.9, 52.8, 38.1; HRMS (ESI^+): calcd. for $C_{13}H_{13}O_5$ $[M+H]^+$: 249.0757; found 249.0761. HPLC (system QC): t_R = 23.4 min, purity 95.9%.

2-(7-Methoxy-2-oxo-2H-chromen-4-yl)acetic acid 2[20]

A mixture of citric acid (21.2 g, 109.3 mmol, 1.4 equiv.) and sulphuric acid (28 mL) was stirred at rt for 1 h, then the solution was slowly heated to 70 °C. After 35 min at this temperature, no effervescence was any longer, then the clear solution was rapidly cooled to 0 °C. To the stirred solution was added 3-methoxyphenol (10.2 g, 80 mmol) and sulphuric acid (11 mL), each in three equal portions, at such a rate that the internal temperature did not exceed 10 °C. The resulting mixture was stirred at 0 °C for 16 h, poured into ice-water (200 mL), and the resulting precipitate was filtered off and washed with cold water. The solid was stirred with aq Na_2CO_3 1 N (100 mL) for 1 h at 65 °C, filtered and washed with water. Acidification with aq HCl 1 N of the combined filtrate and washing gave the 2-(7-methoxy-2-oxo-2H-chromen-4-yl)acetic acid **2** as a white solid (6.2 g, 33% yield). ^1H NMR (300 MHz, $(CD_3)_2CO$) : δ = 7.68 (d, *J* = 8.8 Hz, 1H), 6.94 (dd, *J* = 8.8 Hz and 1.9 Hz, 1H), 6.91 (d, *J* = 1.9 Hz, 1H), 6.29 (s, 1H), 3.93 (s, 3H), 3.91 (s, 2H). HPLC (system QC): t_R = 21.5 min, purity 97.0%.

Methyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate 3[21]

To a solution of resorcinol (10 g, 91 mmol) and dimethyl-1,3-acetonedicarboxylate (13.2 mL, 91 mmol) in MeOH (135 mL) cooled to 0 °C was slowly added sulphuric acid (90 mL). The resulting solution was allowed to warm up to rt and stirred overnight. Then the reaction mixture was poured into ice water with vigorous stirring for 15 min. The precipitate was filtered off and washed with cold water to neutrality and recrystallised from MeOH to give methyl 2-(7-hydroxy-2-oxo-2H-chromen-4-yl)acetate **3** as a yellow solid (16 g, 75% yield). ^1H NMR (300 MHz, $(CD_3)_2SO$) : δ = 10.59 (br, 1H), 7.51 (d, *J* = 8.7 Hz, 1H), 6.91-6.66 (m, 2H), 6.24 (s, 1H), 3.94 (s, 2H), 3.64 (s, 3H).

Methyl 2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)-acetate 4

To a solution of **3** (8.7 g, 37.1 mmol, 1.2 equiv.), 3-bromo-1-propanol (2.8 mL, 30.9 mmol) and PPh_3 (12.1 g, 46.4 mmol, 1.5 equiv.) in THF (150 mL) cooled to 0°C was slowly added DEAD (5.8 mL, 37.1 mmol, 1.2 equiv.). The resulting solution was allowed to warm up to rt and stirred overnight. After completion of the reaction, followed by TLC, the mixture was concentrated under reduced pressure and purified by chromatography (DCM/EtOAc, 98:2, R_f : 0.5) followed by recrystallization from MeOH to give methyl 2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)acetate **4** as a white solid (6 g, 54% yield). m.p. 88-90 °C; IR : $\tilde{\nu}$ = 1723, 1712, 1609, 1550, 1429, 1393, 1299, 1207, 1147, 1026 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) : δ = 7.48 (d, *J* = 9.1 Hz, 1H), 6.89-6.84 (m, 2H), 6.23 (s, 1H), 4.18 (t, *J* = 5.8 Hz, 2H), 3.73 (s, 5H), 3.60 (t, *J* = 6.4 Hz, 2H), 2.35 (p, *J* = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 169.3, 162.0, 160.8, 155.6, 148.0, 125.7, 114.0, 112.8, 112.7, 101.9, 66.0, 52.8, 38.0, 32.0, 29.6; HRMS (ESI^+): calcd. for $C_{15}H_{16}Br_{79}O_5$ $[M+H]^+$: 355.0181; found 355.0184.

(3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7yl)oxy)-propyl)triphenylphosphonium bromide 5

Compound **4** (500 mg, 1.4 mmol) and PPh_3 (4.05 g, 15.5 mmol, 11 equiv.) was suspended in dry ACN (20 mL) and the corresponding medium refluxed for 24 h. The mixture was cooled, concentrated in *vacuo* and washed several times with EtOAc to give (3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-triphenylphosphonium bromide **5** as a white solid (863 mg, 93% yield). m.p. 175-177 °C; IR : $\tilde{\nu}$ = 1715, 1610, 1433, 1390, 1261, 1135, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) : δ = 7.84-7.63 (m, 15H), 7.43 (t, *J* = 8.9 Hz, 1H), 6.84 (dd, *J* = 8.9 and 2.5 Hz, 1H), 6.70 (d, *J* = 2.4 Hz, 1H), 6.17 (s, 1H), 4.25 (t, *J* = 5.4 Hz, 2H), 3.77-3.59 (m, 7H), 2.28-2.09 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 169.4, 161.2, 160.9, 155.3, 148.2, 135.4 (d, $J_{P\text{-}C}$ = 3.0 Hz, 3C), 133.6 (d, $J_{P\text{-}C}$ = 10.0 Hz, 6C), 130.7 (d, $J_{P\text{-}C}$ = 12.6 Hz, 6C), 125.9, 118.5, 117.4, 113.5 (d, $J_{P\text{-}C}$ = 80.1 Hz, 3C), 112.1, 102.5, 67.2 (d, $J_{P\text{-}C}$ = 17.5 Hz, 1C), 52.8, 37.9, 22.7 (d, $J_{P\text{-}C}$ = 3.3 Hz, 1C), 19.4 (d, $J_{P\text{-}C}$ = 54.1 Hz, 1C); HRMS (ESI^+): calcd. for $C_{33}H_{30}O_5P^+$ $[M]^+$: 537.1831; found 537.1831. HPLC (system QC): t_R = 28.4 min, purity 97.9%.

3-((4-(2-methoxy-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)-N,N,N-trimethylpropan-1-ammonium bromide 6

To a solution of **4** (300 mg, 0.84 mmol) in dry EtOH (4 mL) was added a 4.2 M solution of trimethylamine in EtOH (2.21 mL, 9.29 mmol, 11 equiv.). The resulting solution was stirred at 85 °C for 12 h. After completion of the reaction monitored by TLC, the mixture was cooled to rt and concentrated under reduced pressure to give the crude 3-((4-(2-ethoxy-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)-N,N,N-trimethylpropan-1-aminium bromide. The crude product was dissolved in dry MeOH (12 mL) and DIPEA (150 µL) was added. The resulting mixture was refluxed for 48 h, cooled to rt, concentrated under reduced pressure and washed several times with EtOAc to give the desired salt **6** as a yellow solid (304 mg, 87% yield). m.p. 131-133 °C; IR : $\tilde{\nu}$ = 3478, 1717, 1611, 1392, 1263, 1200, 1141, 1064 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) : δ = 7.21 (d, *J* = 8.7 Hz, 1H), 7.01-6.92 (m, 2H), 6.28 (s, 1H), 4.23 (t, *J* = 5.7 Hz, 2H), 3.91 (s, 2H), 3.72 (s, 3H), 3.68-3.60 (m, 2H), 3.23 (s, 9H), 2.42-2.30 (m, 2H); ^{13}C NMR (75 MHz, CD_3CO) : δ = 171.3, 163.2, 162.8, 156.6, 151.1, 127.5, 114.5, 114.3, 113.8, 102.7, 66.4, 65.3, 53.8, 53.76, 53.70, 53.0, 38.1, 24.2; HRMS (ESI^+): calcd. for $C_{18}H_{24}NO_5$ $[M]^+$: 334.1654; found 334.1641. HPLC (system QC): t_R = 19.7 min, purity > 99%.

(3-((4-(carboxymethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)-triphenylphosphonium bromide 7

To a solution of **5** (600 mg, 0.97 mmol) in a solution of MeOH (10 ml) and H_2O (2 ml) was slowly added K_2CO_3 (403 mg, 2.92 mmol, 3 equiv.). The resulting mixture was stirred at rt for 4 h, then aq HCl 1 N (20 mL) and DCM (20 mL) was added. The organic layer that formed was extracted, dried over Na_2SO_4, concentrated in *vacuo* and washed with Et_2O and acetone to give **7** as a white solid (586 mg, 89% yield). m.p. 145-147 °C; IR : $\tilde{\nu}$ = 2311, 1715, 1693, 1609, 1438, 1376, 1267, 1243, 1137, 1114, 1053, 1016 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) : δ = 7.92-7.72 (m, 15H), 7.59 (d, *J* = 8.9 Hz, 1H), 6.93 (dd, *J* = 8.9 and 2.4 Hz, 1H), 6.81 (d, *J* = 2.4 Hz, 1H), 6.21 (s, 1H), 4.26 (t, *J* = 5.3 Hz, 2H), 3.81 (s, 2H), 3.75-3.62 (m, 2H), 2.27-2.13 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) : δ = 172.48, 162.9, 162.8, 156.4, 151.61, 151.58, 136.3 (d, $J_{P\text{-}C}$ = 3.0 Hz, 3C), 134.8 (d, $J_{P\text{-}C}$ = 10.1 Hz, 6C), 131.6 (d, $J_{P\text{-}C}$ = 12.6 Hz, 6C), 127.5, 119.6 (d, $J_{P\text{-}C}$ = 86.8 Hz, 3C), 114.3, 114.2, 113.8, 102.7, 68.5 (d, $J_{P\text{-}C}$ = 16.9 Hz, 1C), 23.5 (d, $J_{P\text{-}C}$ = 3.3 Hz, 1C), 20.0 (d, $J_{P\text{-}C}$ = 54.0 Hz, 1C); HRMS (ESI^+): calcd. for $C_{32}H_{28}O_5P^+$ $[M]^+$: 523.1674; found 532.1677. HPLC (system QC): t_R = 26.3 min, purity 96.6%.

2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)acetic acid 8

To a cooled solution of **4** (547 mg, 1.5 mmol) in THF (7 ml) was slowly added an aqueous solution (2 N) of lithium hydroxide (74 mg, 3.0 mmol, 2 equiv.). The resulting mixture was stirred at rt for 4 h, then diluted with EtOAc (10 mL) and aq HCl 1 N (20 mL). The organic layer that formed was extracted, dried over Na_2SO_4, concentrated in *vacuo* and washed with Et_2O to give **8** as a white solid (434 mg, 83% yield). m.p. 128-130 °C; IR : $\tilde{\nu}$ = 2957, 1709, 1609, 1388, 1296, 1195, 1146, 1067, 1024 cm^{-1}; ^1H NMR (300 MHz, $(CD)_3CO$) : δ = 7.69 (d, *J* = 8.8 Hz, 1H), 6.98 (dd, *J* = 8.8 and 2.4 Hz, 1H), 6.94 (d, *J* = 2.4 Hz, 1H), 6.30 (s, 1H), 4.29 (t, *J* = 5.9 Hz, 2H), 3.91 (s, 2H), 3.70 (t, *J* = 6.6 Hz, 2H), 2.38 (p, *J* = 6.4 Hz, 2H); ^{13}C NMR (75 MHz, $(CD)_3CO$) : δ = 170.6, 162.9, 160.8, 156.5, 150.1, 127.3, 114.4, 113.8, 113.2, 102.3, 67.0, 37.9, 33.0, 30.7; HRMS (ESI^-): calcd. for $C_{13}H_{12}Br_{79}O_3$ $[M\text{-}CO_2\text{-}H]^-$: 294.9970; found 294.9968.

2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)-N-(prop-2-yn-1-yl)acetamide 9

To a cooled solution of **8** (50 mg, 146 µmol), propargylamine (14 µl, 219 µmol, 1.5 equiv.) and PyBOP (83 mg, 160 µmol, 1.1 equiv.) in a mixture of ACN (1 mL) and DMF (100 µL) was added dropwise DIPEA (36 µL, 219 µmol, 1.5 equiv.). The resulting mixture was stirred at rt for 2 h and then diluted with EtOAc (5 mL) and aq HCl 1 N (5 mL). The organic layer that formed was extracted, washed with $NaHCO_3$ (5 mL), brine (5 mL), dried over Na_2SO_4 and concentrated in *vacuo*. Flash column chromatography (cyclohexane/EtOAc, 40:60) R_f : 0.3 gave the desired product **9** as a white solid (43 mg, 78% yield). m.p. 141-143 °C; IR : $\tilde{\nu}$ = 3369, 3288, 2949, 1703, 1662, 1614, 1583, 1532, 1391, 1296, 1254, 1146, 1017 cm^{-1}; ^1H NMR (300 MHz, $(CD_3)_2CO$) : δ = 7.81 (s, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 6.95 (dd, *J* = 8.8 and 2.5 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.26 (s, 1H), 4.28 (t, *J* = 5.9 Hz, 2H), 4.00 (dd, *J* = 5.5 and 2.5 Hz, 2H), 3.79 (s, 2H), 3.70 (t, *J* = 6.6 Hz, 2H), 2.66 (t, *J* = 2.6 Hz, 1H), 2.37 (p, *J* = 6.4 Hz, 2H); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) : δ = 168.2, 162.8, 160.8, 156.4, 150.9, 127.5, 114.2, 113.9, 113.1, 102.2, 81.0, 72.2, 67.0, 39.9, 32.9, 30.7, 29.0; HRMS (ESI^+): calcd. for $C_{17}H_{17}Br_{79}NO_4$ $[M+H]^+$: 378.0341; found 378.0339.

(3-((2-oxo-4-(2-oxo-2-(prop-2-yn-1-ylamino)ethyl)-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium bromide 10

Compound **9** (30 mg, 79 µmol) and PPh_3 (62 mg, 238 µmol, 3 equiv.) was suspended in dry ACN (1 mL). The mixture was stirred and refluxed under Ar atmosphere. Refluxing was continued for 12 h. The mixture was cooled, concentrated in *vacuo* and washed several times with EtOAc to give **10** as a white solid (36 mg, 71% yield). m.p. 88-90 °C; IR : $\tilde{\nu}$ = 3207, 3044, 1709, 1669, 1609, 1437, 1383, 1268, 1139, 1112 cm^{-1}; ^1H NMR (300 MHz, CD_3CN) : δ = 8.14 (br, 1H), 7.90-7.60 (m, 16H), 6.84 (dd, *J* = 8.9 and 2.5 Hz, 1H), 6.77 (d, *J* = 2.4 Hz, 1H), 6.23 (s, 1H), 4.20 (t, *J* = 5.4 Hz, 2H), 3.90 (dd, *J* = 5.7 and 2.5 Hz, 2H), 3.76 (s, 2H), 3.60-3.46 (m, 2H), 2.42 (t, *J* = 2.5 Hz, 1H), 2.16-2.02 (m, 2H); ^{13}C NMR (75 MHz, CD_3CN) : δ = 168.9, 162.2, 161.5, 156.2, 151.5, 136.1 (d, $J_{P\text{-}C}$ = 3.0 Hz, 3C), 134.7 (d, $J_{P\text{-}C}$ = 10.1 Hz, 6C), 131.3 (d, $J_{P\text{-}C}$ = 12.6 Hz, 6C), 127.8, 119.7, 118.5, 114.1, 113.7 (d, $J_{P\text{-}C}$ = 84.7

Hz, 3C), 102.6, 81.3, 71.8, 68.1 (d, $J_{P\text{-}C}$ = 16.8 Hz, 1C), 39.9, 29.2, 23.0 (d, $J_{P\text{-}C}$ = 3.3 Hz, 1C), 19.7 (d, $J_{P\text{-}C}$ = 54.1 Hz, 1C); HRMS (ESI^+): calcd. for $C_{35}H_{31}NO_4P$ $[M+H]^+$: 560.1991; found 560.1972. HPLC (system QC): t_R = 27 min, purity >99%.

Tert-butyl (3-(2-(7-(3-bromopropoxy)-2-oxo-2H-chromen-4-yl)acetamido)propyl)carbamate 11

To a cooled solution of **8** (100 mg, 293 µmol), N-Boc-1,3-propanediamine hydrochloride (92 mg, 439 µmol, 1.5 equiv.) and PyBOP (167 mg, 322 µmol, 1.1 equiv.) in a mixture of ACN (2 mL) and DMF (200 µL) was added dropwise DIPEA (145 µL, 879 µmol, 3 equiv.). The resulting mixture was stirred at rt for 3 h, then diluted with EtOAc (5 mL) and sat. NH_4Cl (5 mL). The organic layer that formed was extracted, washed with aq $NaHCO_3$ (5 mL), brine (5 mL), dried over Na_2SO_4 and concentrated in *vacuo*. Flash column chromatography (cyclohexane/EtOAc 15:85) gave **11** as a white solid (83 mg, 57% yield). m.p. 128-130 °C; IR : $\tilde{\nu}$ = 3328, 2928, 1715, 1679, 1939, 1614, 1525, 1389, 1251, 1146, 1058 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) : δ = 7.59 (d, *J* = 8.9 Hz, 1H), 6.96 (br, 1H), 6.88-6.82 (m, 2H), 6.27 (s, 1H), 4.69- 4.60 (m, 1H), 4.17 (t, *J* = 5.8 Hz, 2H), 3.67 (s, 2H), 3.61 (t, *J* = 6.4 Hz, 2H), 3.24 (dd, *J* = 12.0 and 6.1 Hz, 2H), 3.00 (dd, *J* = 12.2 and 6.6 Hz, 2H), 2.40-2.30 (m, 2H), 1.5 (2H under water) 1.38 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 168.0, 162.0, 161.1, 157.0, 155.7, 149.5, 126.1, 114.0, 112.9, 112.7, 101.8, 79.7, 66.0, 40.7, 36.8, 35.9, 32.0, 30.0, 29.6, 28.3 (3C); HRMS (ESI^+): calcd. for $C_{22}H_{30}Br_{79}N_2O_6$ $[M+H]^+$: 497.1287; found 497.1263.

tert-butyl (3-(2-(2-oxo-7-(3-(triphenyl-l4-phosphanyl)propoxy)-2H-chromen-4-yl)acetamido)propyl)carbamate 12

A solution of **11** (350 mg, 0.70 mmol) and PPh_3 (555 mg, 2.11 µmol, 3 equiv.) in ACN (12 mL) was refluxed and stirred for 24 h. The mixture was cooled to rt, concentrated in *vacuo* and washed several times with EtOAc to give **12** as a white solid (517 mg, 97% yield). m.p. 86-88 °C; IR : $\tilde{\nu}$ = 3226, 2933, 1704, 1610, 1512, 1437, 1390, 1265, 1140, 1112 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) : δ = 8.25 (br, 1H), 7.95-7.65 (m, 15H), 7.53 (d, *J* = 8.8 Hz, 1H), 6.68-6.58 (m, 2H), 6.26 (s, 1H), 5.22 (br, 1H), 5.45-5.35 (m, 2H), 4.15-4.01 (m, 2H), 3.76 (s, 2H), 3.30 (dd, *J* = 11.7 and 5.7 Hz, 2H), 3.09 (dd, *J* = 12.5 and 6.4 Hz, 2H), 2.24-2.12 (m, 2H), 1.71-1.62 (m, 2H), 1.39 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 168.7, 161.2, 161.0, 156.3, 154.9, 151.0, 135.3 (d, $J_{P\text{-}C}$ = 2.8 Hz, 3C), 133.7 (d, $J_{P\text{-}C}$ = 10.0 Hz, 6C), 130.6 (d, $J_{P\text{-}C}$ = 12.6 Hz, 6C), 126.5, 118.7, 117.5, 113.1, 112.7 (d, $J_{P\text{-}C}$ = 83 Hz, 3C), 101.5, 78.7, 67.4 (d, $J_{P\text{-}C}$ = 17.8 Hz, 1C), 39.6, 37.2, 36.2, 29.5, 28.5 (3C), 22.7 (d, $J_{P\text{-}C}$ = 2.6 Hz, 1C), 19.6 (d, $J_{P\text{-}C}$ = 19.6 Hz, 1C); HRMS (ESI^+): calcd. for $C_{40}H_{44}N_2O_6P$ $[M]^+$: 679.2937; found 679.2951.

(3-((4-(2-((3-aminopropyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyltriphenyl-phosphonium bromide 13

To a pre-cooled solution of **12** (534 mg, 0.70 mmol) in DCM (18 ml) was slowly added TFA (2.5 mL). The resulting mixture was stirred at rt for 2 h and concentrated in *vacuo* to give **13** as a colorless oil (440 mg, 90% yield). IR : $\tilde{\nu}$ = 1668, 1610, 1553, 1442, 1389, 1133, 1113 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) : δ = 7.94-7.72 (m, 15H), 7.70 (d, *J* = 8.9 Hz, 1H), 6.98 (dd, *J* = 8.9 and 2.4 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 6.27 (s, 1H), 4.26 (t, *J* = 5.6 Hz, 2H), 3.79 (s, 2H), 3.70-3.58 (m, 2H), 3.36-3.31 (m, 2H), 2.94 (d, *J* = 7.4 Hz, 2H), 2.27-2.12 (m, 2H), 1.94-1.81 (m, 2H); ^{13}C NMR (75 MHz, CD_3OD) : δ = 171.5, 163.1, 162.9, 156.6, 152.2, 136.4 (d, $J_{P\text{-}C}$ = 3.0 Hz, 3C), 134.8 (d, $J_{P\text{-}C}$ = 10.1 Hz, 6C), 131.6 (d, $J_{P\text{-}C}$ = 12.7 Hz, 6C), 127.5, 119.6 (d, $J_{P\text{-}C}$ = 86.8 Hz, 3C), 114.4, 114.2, 113.9, 102.8, 68.5 (d, $J_{P\text{-}C}$ = 16.9 Hz, 1C), 39.9, 38.3, 37.4, 28.7, 23.5 (d, $J_{P\text{-}C}$ = 3.5 Hz, 1C), 20.0 (d, $J_{P\text{-}C}$ = 54.0 Hz, 1C); HRMS (ESI^+): calcd. for $C_{35}H_{36}N_2O_4P$ $[M]^+$: 579.2413; found 579.2407. HPLC (system QC): t_R = 24.1 min, purity 98.7%.

Synthesis of (3-((4-(2-((3-(2-iodoacetamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 14

2,5-dioxopyrrolidin-1-yl 2-iodoacetate[22]

To a solution of 2-iodoacetic acid (500 mg, 2.69 mmol) and NHS (309 mg, 2.69 mmol) in dry EtOAc (15 mL), was slowly added DIC (533 µL, 2.69 mmol). The reaction mixture was stirred for 6 h at rt. The precipitating di*iso*propylurea by-product was removed by filtration and the resulting solution was concentrated and recrystallized from anhydrous isopropyl alcohol to give the desired product as a white crystal (654 mg, 86% yield). ^1H NMR (300 MHz, $(CD_3)_2SO$) : δ = 4.38 (s, 2H), 2.81 (s, 4H).

(3-((4-(2-((3-(2-iodoacetamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 14

To a solution of **13** (30 mg, 37.1 µmol) and DIPEA (7 µL, 40.9 µmol, 1.1 equiv.) in dry THF (5 ml) was slowly added 2,5-dioxopyrrolidin-1-yl 2-iodoacetate (12.6 mg, 44.6 µmol, 1.2 equiv.). The reaction mixture was stirred for 1 h at rt. After completion, followed by HPLC, the reaction mixture was concentrated and the resulting crude product was subjected to a RP-HPLC (system A). The product-containing fractions were lyophilized to afford **14** as a colorless oil (17 mg, 47% yield). IR : $\tilde{\nu}$ = 3270, 3067, 2930, 1658, 1610, 1556, 1439, 1389, 1265, 1113 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) : δ = 7.94-7.71 (m, 15H), 7.69 (d, *J* = 8.9 Hz, 1H), 6.98 (dd, *J* = 8.9 and 2.54 Hz, 1H), 6.90 (d, *J* = 2.4 Hz, 1H), 6.27 (s, 1H), 4.25 (t, *J* = 5.5 Hz, 2H), 3.76 (s, 2H), 3.66 (s, 2H) 3.70-3.56 (m, 2H), 3.26 (t, *J* = 6.8 Hz, 2H), 3.18 (t, *J* = 6.9 Hz, 2H), 2.27-2.1 (m, 2H), 1.70 (p, *J* = 6.8 Hz, 2H); ^{13}C NMR (75 MHz, CD_3OD) : δ = 171.5, 170.8, 163.0, 162.9, 156.6, 152.2, 136.4 (d, $J_{P\text{-}C}$ = 3.0 Hz, 3C), 134.8 (d, $J_{P\text{-}C}$ = 10.1 Hz, 6C), 131.6 (d, $J_{P\text{-}C}$ = 12.7 Hz, 6C), 127.5, 119.7 (d, $J_{P\text{-}C}$ = 86.8 Hz, 3C), 114.5, 114.4, 114.0, 102.8, 68.5 (d, $J_{P\text{-}C}$ = 16.7 Hz, 1C), 40.2, 38.3, 38.0, 29.8, 23.5 (d, $J_{P\text{-}C}$ = 3.5 Hz, 1C), 20.0 (d, $J_{P\text{-}C}$ = 54.0 Hz, 1C), -2.0; HRMS (ESI^+): calcd. for $C_{37}H_{37}IN_2O_5P$ $[M]^+$: 747.1485; found 747.1509. HPLC (system QC): t_R = 26.7 min, purity > 99%.

(3-((4-(2-((3-(6-(5-ethoxy-2-methyloxazol-4-yl)hexanamido)propyl)amino)-2-oxoethyl)-2-oxo-2H-chromen-7-yl)oxy)propyl)triphenylphosphonium 2,2,2-trifluoroacetate 15

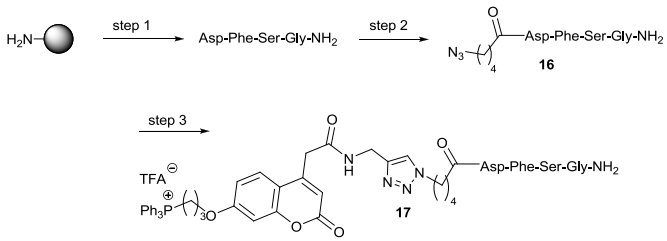
To a solution of **13** (30 mg, 37.1 µmol) in DCM (2.5 ml) was slowly added DCC (9.2 mg, 44.6 µmol, 1.2 equiv.) and DMAP (1 mg, 7.4 µmol, 0.1 equiv.). The resulting mixture was stirred at rt. for 30 min. Then a solution of 6-(5-ethoxy-2-methyloxazol-4-yl)hexanoic acid[10] (10.8 mg, 44.6 µmol, 1.2 equiv.) in DCM (2.5 mL) was added, followed by DIPEA (7.6 µl, 44.6 µmol, 1.2 equiv.) dropwise. The resulting solution was stirred at rt. for 18 h and concentrated in *vacuo.* The resulting crude product was subjected to a RP-HPLC C_{18} cartridge (system B). The product-containing fractions were lyophilized to afford **15** as a colorless oil (30 mg, 75% yield). IR : $\tilde{\nu}$ = 3276, 3061, 2934, 2865, 1713, 1661, 1612, 1554, 1438, 1388, 1201, 1136, 1113, 1020 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) : δ = 7.95-7.65

(m, 15H), 7.69 (d, *J* = 8.9 Hz, 1H), 6.97 (dd, *J* = 8.9 and 2.4 Hz, 1H), 6.91 (d, *J* = 2.4 Hz, 1H), 6.28 (s, 1H), 4.25 (t, *J* = 5.5 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.79 (s, 2H), 3.69-3.56 (m, 2H), 3.23 (t, *J* = 6.7 Hz, 2H), 3.17 (t, *J* = 6.9 Hz, 2H), 2.31 (t, *J* = 7.3 Hz, 2H), 2.29 (s, 3H), 2.25-2.12 (m, 4H), 1.72-1.5 (m, 7H), 1.35-1.27 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) : δ = 176.2, 170.8, 163.02, 162.98, 162.96, 156.6, 152.2, 136.4 (d, $J_{P\text{-}C}$ = 3.0 Hz, 3C), 134.8 (d, $J_{P\text{-}C}$ = 10.1 Hz, 6C), 131.6 (d, $J_{P\text{-}C}$ = 12.7 Hz, 6C), 127.5, 119.7 (d, $J_{P\text{-}C}$ = 86.9 Hz, 3C), 117.27, 117.25, 114.5, 114.4, 113.9, 102.8, 71.7, 68.4 (d, $J_{P\text{-}C}$ = 17.0 Hz, 1C), 40.2, 38.1, 37.6, 37.0, 30.1, 29.7, 29.2, 26.7, 24.9, 23.5 (d, $J_{P\text{-}C}$ = 3.0 Hz, 1C), 20.0 (d, $J_{P\text{-}C}$ = 54.1 Hz, 1C), 15.3, 13.8; HRMS (ESI$^+$): calcd. for $C_{47}H_{53}N_3O_7P$ $[M]^+$: 802.3621; found 802.3621. HPLC (system QC): t_R = 27.3 min, purity > 99%.

Synthesis of phosphonium-tagged tetrapeptide 17

2,5-dioxopyrrolidin-1-yl 5-azidopentanoate[23]

To a stirred solution of 5-azidopentanoic acid (200 mg, 1.4 mmol) and NHS (193 mg, 1.68 mmol, 1.2 equiv.) in DCM (1.5 mL) was added EDC.HCl (321 mg, 1.68 mmol, 1.2 equiv.). The reaction mixture was stirred at rt for 20 h. The crude reaction mixture was poured into a saturated aq NaCl solution and extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, concentrated under reduced pressure and purified by flash chromatography (100 %, DCM, R_f : 0.7) to give the product as a pale yellow oil (178 mg, 53% yield). ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 3.34 (t, *J* = 6.5 Hz, 2H), 2.84 (s, 4H), 2.66 (t, *J* = 7.1 Hz, 2H), 1.91-1.78 (m, 2 H), 1.78-1.66 (m, 2H).



Step 1: Tetrapeptide Gly-Ser-Phe-Asp

The SPPS of peptide was performed using the standard Fmoc/tBu chemistry. A 4-Methylbenzhydrylamine Hydrochloride Salt Resin (MBHA Resin) (131 mg, loading 1.9 mmol/g) on a scale of 0.25 mmol was placed in SPPS coupling reactor and elongated by sequentially coupling : rink amide linker, Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Phe-OH, Fmoc-Asp-(tBu)-OH, using HBTU (4 equiv.), HOBt (4 equiv.) and DIEA (12 equiv.) in peptide-grade DMF. The peptide was cleaved from the resin and the protective amino acid side chains were removed by addition of a pre-cooled cocktail of TFA/TIS/H_2O/EDT (94/1/2.5/2.5 6 mL) to the above resin. After 3 h of stirring at rt, the resin was filtered and washed with TFA. The filtrate was evaporated to dryness and cold Et_2O was added to the oily residue. The resulting precipitate was isolated by centrifugation, lyophilized and purified by RP-HPLC (system C). The product-containing fractions were lyophilized to give the substrate as a yellow powder. (69 mg, 65% yield). HRMS (ESI$^-$): calcd. for $C_{18}H_{24}N_5O_7$ $[M\text{-}H]^-$: 422.1676; found 422.1672.

Step 2: Tetrapeptide Gly-Ser-Phe-Asp-linker-N_3 16

To tetrapeptide Gly-Ser-Phe-Asp (25 mg, 58.9 µM) in a solution of PBS/ACN (1 mL, 1:1) was added 2,5-dioxopyrrolidin-1-yl 5-azidopentanoate (15.6 mg, 64.8 µM, 1.1 equiv.) in a solution of PBS/ACN (1 mL, 1:1). The reaction mixture was stirred at rt for 18 h. After completion of the reaction monitored by HPLC, the reaction mixture was subjected to a RP-HPLC (system D). The product-containing fractions were lyophilized to give **16** as a white powder (21 mg, 65% yield). HRMS (ESI$^-$): calcd. for $C_{23}H_{31}N_8O_8$ $[M\text{-}H]^-$: 547.2265; found 547.2244.

Step 3: Phosphonium-tagged tetrapeptide 17

To a solution of **16** (10 mg, 18.2 µmol, 1.2 equiv.) in ACN/H_2O (2 mL, 1:1) were added ALJ 521 (9.5 mg, 15 µmol), copper(II) sulfate pentahydrate (4.5 mg, 18.2 µmol, 1.2 equiv.), sodium ascorbate (9 mg, 45.5 µmol, 3 equiv.) and tris((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)amine (TBTA) (2.9 mg, 5.5 µmol, 0.36 equiv.). The reaction was stirred at rt for 18 h and directly subjected to a RP-HPLC (system E).The product-containing fractions were lyophilized to give **17** as a white powder (14.6 mg, 80% yield). HRMS (ESI$^+$): calcd. for $C_{58}H_{63}N_9O_{12}P$ $[M]^+$: 1108.4334; found 1108.4331. HPLC (system QC): t_R = 25.6 min, purity > 99%.

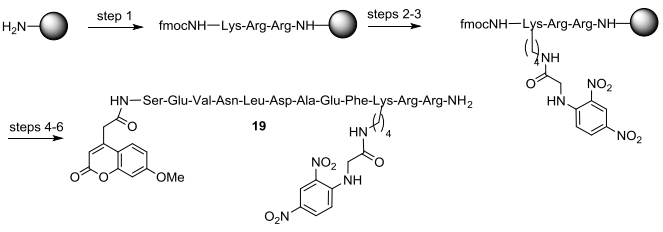
Synthesis of (MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19 and (PPh_3C)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20

(2,4-dinitrophenyl)glycine[24]

To a quartz reaction vessel was added glycine (345 mg, 4.5 mmol), a solution of bicarbonate (756 mg, 9 mmol, 2 equiv.) in H_2O (30 mL) and Sanger's reagent (837 mg, 4.5 mmol). The reaction vessel was then placed into the cavity of a microwave reactor and irradiated for 15 min at 120 °C. After completion, the desired product precipitated after addition of aq 1N HCl (50 mL) to the reaction mixture. The yellow solid was collected by filtration, washed with cold water, diluted with DCM, dried over $MgSO_4$ and concentrated under reduced pressure to give (2,4-dinitrophenyl)glycine as a yellow powder (998 mg, 94% yield).^{1}H NMR (300 MHz, CD_3OD) : δ = 9.07 (d, *J* = 2.7 Hz, 1H), 8.32 (dd, *J* = 9.5 and 2.7 Hz, 1H), 7.05 (d, *J* = 9.5 Hz, 1H), 4.30 (s, 2H).

(MOCAc)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 19

The SPPS of peptide was performed using the standard Fmoc/tBu chemistry.



Step 1 : A 4-Methylbenzhydrylamine Hydrochloride Salt Resin (MBHA Resin) (131 mg, loading 1.9 mmol/g) on a scale of 0.25 mmol was placed in SPPS coupling reactor and elongated by sequentially coupling : rink amide linker, Fmoc-Arg(Pbf)-OH (× 2) (Pdf : 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl) and Fmoc-Lys(Mtt)-OH (Mtt : 4-methyltrityl) (4 equiv.) using HBTU (4 equiv.), HOBt (4 equiv.) and DIEA (12 equiv.) in peptide-grade DMF.

Step 2: The resin was then transfer to a round bottom flask and the *N*-Mtt group on the lysine side chain was removed by using a cocktail of TFA/TIS/DCM (2:5:93; 6 mL) for 2 h at rt. The resin was washed with MeOH (3 × 5 mL), DMF (3 × 5 mL) and DCM (3 × 5 mL) and then split into four equal parts (0.062 mmol, 1 equiv.).

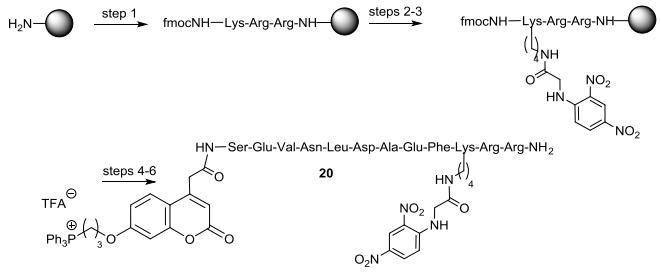
Step 3 : To a solution of (2,4-dinitrophenyl)glycine (75.3 mg, 0.31 mmol, 5 equiv.) and HOBt (41.9 mg, 0.31 mmol, 5 equiv.) in a solution of DMF/THF (1.5 mL, 2:1) was added dropwise DIC (48 µL, 0.31 mmol, 5 equiv.). The reaction mixture was stirred for 30 min at rt. Then, this solution was added to the above resin (0.062 mmol) and stirred for 12 h at rt. The resin was then washed successively with MeOH (3 × 5 mL), DMF (3 × 5 mL) and DCM (3 × 5 mL).

Step 4 : The peptide was elongated by SPPS by sequentially coupling : Fmoc-Phe-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Ala-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Leu-OH, Fmoc-Asn(trt)-OH, Fmoc-Val-OH, Fmoc-Glu(O*t*Bu)-OH and Fmoc-Ser(*t*Bu)-OH) (4 equiv.) using HBTU (4 equiv.), HOBt (4 equiv.) and DIEA (12 equiv.) in peptide-grade DMF.

Step 5 : To a solution of **2** (73.1 mg, 0.31 mmol, 5 equiv.) and HOBt (41.9 mg, 0.31 mmol, 5 equiv.) in DMF (3 mL) was added dropwise DIC (48 µL, 0.31 mmol, 5 equiv.). The reaction mixture was stirred for 30 min at rt. Then, this solution was added to the above resin (0.062 mmol) and stirred for 12 h at rt. The resin was washed successively with MeOH (3 × 5 mL), DMF (3 × 5 mL) and DCM (3 × 5 mL).

Step 6: The peptide was cleaved from the resin and the protective amino acid side chains were removed by addition of a pre-cooled cocktail of TFA/TIS/H_2O/EDT (94/1/2.5/2.5, 6 mL) to the above resin. After 3 h of stirring at rt, the resin was filtered and washed with TFA. The filtrate was evaporated to dryness and cold Et_2O was added to the oily residue. The resulting precipitate was isolated by centrifugation, lyophilized and purified by RP-HPLC (system F). The product-containing fractions were lyophilized to give the peptide **19** as a yellow powder. (13 mg, 11% yield). HRMS (ESI^+): calcd. for $C_{82}H_{118}N_{24}O_{29}/2$ $[M+2]^{2+}$: 951.4248; found 951.4250. HPLC (system QC): t_R = 26.0 min, purity 96.6%.

(PPh_3C)-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Lys-(DNPA)-Arg-Arg 20



The SPPS of peptide was performed using the standard Fmoc/tBu chemistry. The steps 1-4 are identical to those reported for the synthesis of **19**.

Step 5 : To a solution of **7** (180 mg, 0.30 mmol, 5 equiv.) and HOBt (46 mg, 0.30 mmol, 5 equiv.) in NMP (2 mL) was added dropwise DIC (46.4 µL, 0.30 mmol, 5 equiv.). The reaction mixture was stirred for 1 h at rt. Then, this solution was added to the above resin (0.06 mmol) and stirred for 72 h at rt. The resin was then successively washed with MeOH (3 × 5 mL), DMF (3 × 5 mL) and DCM (3 × 5 mL).

Step 6 : The peptide was cleaved from the resin and the protective amino acid side chains were removed by addition of a pre-cooled cocktail of TFA/TIS/H_2O/EDT (94/1/2.5/2.5, 6 mL) to the above resin. After 3 h of stirring at rt, the resin was filtered and washed with TFA. The filtrate was evaporated to dryness and cold Et_2O was added to the oily residue. The resulting precipitate was isolated by centrifugation, lyophilized and purified by RP-HPLC (system G). The product-containing fractions were lyophilized to give the peptide **20** as a yellow powder. (2 mg, 2% yield). HRMS (ESI^+): calcd for $C_{102}H_{135}N_{24}O_{29}P^+/2$ $[M+H]^{2+}$: 1095.4783; found, 1095.4785; HPLC (system QC): t_R = 27.4 min, purity > 99%.

General procedure for *in vitro* peptide cleavage by BACE-1 or α-chymotrypsin and LC-MS analyses.

Stock solutions of **19** and **20** were prepared at 433 µM in DMSO, stock solution of BACE-1 full protein (His*Tag®, human, recombinant, NSO Cells) was prepared at 741 nM in 0.1 M acetate buffer (pH = 4.5) and stock solution of α-chymotrypsin (from bovine pancreas) was prepared at 741 nM in 0.1 M tris buffer + 10 mM $CaCl_2$ (pH = 7.8). For BACE-1 reaction, BACE-1 enzyme (11.3 µL; 18.6 nM; final concentration) was added to a solution of **19** or **20** (26 µL in 424 µL of 0.1 M acetate buffer; 25 µM final concentration) to start the reaction. For α-chymotrypsin reaction, α-chymotrypsin (11.3 µL; 18.6 nM; final concentration) was added to a solution of **19** or **20** (26 µL in 424 µL of 0.1 M tris buffer; 25 µM final concentration) to start the reaction. Before and after 48 h of reaction at 25 °C, a fluorescent scan (λ_{ex} = 328 nm) with a ultra-micro quartz fluorescence cell (Hellma, 105.251-QS, light path: 3 × 3 mm, black chamber volume: 45 µL) was performed to calculate the quenching efficiency and fluorescence exaltation:

$$\textit{Quenching Efficiency}\ (x) = 1-(A_0/A_x)$$

$$\textit{Fluorescence Exaltation}\ (x) = A_x/A_0$$

where A_0 is the area under the emission curve of the probes before cleavage and A_x is the area under the emission curve of the probes after 48 h of proteolytic cleavage by BACE-1 or α-Chymotrypsin (in the range of 338-600 nm). After 48 h of reaction at 25 °C, the crude enzymatic mixture was subsequently analysed by HPLC/MS.

(3-aminopropyl)triphenylphosphonium bromide hydrobromide[25]

A solution of 3-bromopropylamine hydrobromide (2.0 g, 9.13 mmol) and PPh_3 (2.46 g, 10.04 mmol, 1.1 equiv.) in dry ACN (10 mL) was refluxed for 16 h. To the reaction mixture cooled to rt, was added cyclohexane (10 ml), and the resulting solid was separated by filtration, and washed with cyclohexane (3 x 5 mL). The solid was dissolved in isopropanol and precipitated with EtOAc to give (3-aminopropyl)triphenylphosphonium

bromide hydrobromide as a white solid (2.61 g, 60% yield). ^{1}H NMR (300 MHz, $(CD_3)_2SO$) : δ = 7.97-7.88 (m, 3H), 7.88-7.62 (m, 15H), 3.75 (m, 2H), 3.02-2.93 (m, 2H), 1.88-1.75 (m, 2H).

4-Chloro-(*N*-(2-propyn-1-yl))-1,8-naphthalimide 27[26]

To a solution of 4-chloro-1,8-naphthalic anhydride (2.0 g, 8.59 mmol) in EtOH (50 mL) was added propargylamine (1.1 mL, 17.19 mmol, 2 equiv.) and heated to just below reflux for 12 h, followed by reduced heating at 50 °C for 5 h. Upon cooling the product was collected by filtration. Washing with EtOH and subsequent drying gave 4-chloro-(*N*-(2-propyn-1-yl))-1,8-naphthalimide **27** as a yellow solid (2.31 g, 88% yield). ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 8.71 (d, *J* = 7.3 Hz, 1H), 8.63 (d, *J* = 8.5 Hz, 1H), 8.55 (d, *J* = 7.9 Hz, 1H), 7.94-7.77 (m, 2H), 4.96 (d, *J* = 2.3 Hz, 2H), 2.20 (t, J = 2.3 Hz, 1H).

2-(prop-2-yn-1-yl)-6-(propylamino)-1H-benzo[de]isoquinoline-1,3(2H)-dione 28

A solution of 4-chloro-(*N*-(2-propyn-1-yl))-1,8-naphthalimide **27** (100 mg, 0.37 mmol) and *n*-propylamine (87.6 mg, 1.48 mmol, 4 equiv.) in DMSO (2 mL) was heated at 90 °C for 4 h. The solution was then cooled to rt, and water (8 mL) was added, which induced precipitation of a yellow solid. The reaction mixture was neutralized with a 0.1 M HCl solution and the crude product extracted into EtOAc, washed with water, dried over $MgSO_4$ and concentrated in *vacuo.* Flash column chromatography (cyclohexane/EtOAc, 70:30) R_f : 0.3 gave **28** as a yellow solid (94 mg, 87% yield). m.p. 209-211 °C; IR : ν̃ = 3384, 3316, 3232, 2936, 1677, 1639, 1573, 1544, 1408, 1375, 1334, 1245, 1147 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 8.62 (d, *J* = 6.9 Hz, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 1H), 5.29 (br, 1H), 4.96 (s, 2H), 3.40 (d, *J* = 5.0 Hz, 2H), 2.16 (s, 1H), 1.85 (dd, *J* = 14.7 and 7.8, 2H), 1.12 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 164.0, 163.3, 149.9, 135.0, 131.6, 130.0, 126.3, 124.8, 122.9, 120.3, 109.8, 104.5, 79.4, 70.2, 45.6, 29.3, 22.4, 11.8; LRMS (IE) calcd. for $C_{18}H_{16}N_2O_2$ $[M]^+$: 292.1; found 292. HPLC (system QC): t_R = 28.3 min, purity 100%.

(3-((1,3-dioxo-2-(prop-2-yn-1-yl)-2,3-dihydro-1H-benzo[de]isoquinolin-6-yl)amino)propyl)triphenylphosphonium bromide 29

A solution of 4-chloro-(*N*-(2-propyn-1-yl))-1,8-naphthalimide **27** (100 mg, 0.37 mmol), (3-aminopropyl)triphenylphosphonium bromide hydrobromide (267 mg, 0.56 mmol, 1.5 equiv.) and DIPEA (129 µL, 0.74 mmol, 2 equiv.) in DMSO (2 mL) was heated at 90 °C for 2 days. The solution was then cooled to rt, and water (8 mL) was added, which induced precipitation of a yellow solid. The reaction mixture was neutralized with a 0.1 M HCl solution and the crude product extracted into EtOAc, washed with water, dried over $MgSO_4$ and concentrated in *vacuo.* Flash column chromatography (EtOAc/MeOH, 80:20) R_f : 0.4 gave **29** as a yellow solid (112 mg, 48% yield). m.p. 146-148 °C; IR : ν̃ = 3252, 1682, 1641, 1575, 1552, 1439, 1369, 1248, 1112 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) : δ = 9.41 (d, *J* = 7.9 Hz, 1H), 9.04 (t, *J* = 5.6 Hz, 1H), 8.62 (d, *J* = 7.1 Hz, 1H), 8.32 (d, *J* = 8.6 Hz, 1H), 7.76-7.72 (m, 10H), 7.56-7.45 (m, 5H), 6.41 (d, *J* = 8.7 Hz, 1H), 4.96 (d, *J* = 1.9 Hz, 2H), 4.17-4.04 (m, 2H), 3.90-3.78 (m, 2H), 2.16 (s, 1H), 2.08-1.95 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) : δ = 163.8, 162.9, 150.3, 134.9 (d, J_{P-C} = 2.7 Hz, 3C), 134.5, 133.3 (d, J_{P-C} = 10.0 Hz, 6C), 131.3, 130.5, 130.3 (d, J_{P-C} = 12.6 Hz, 6C), 129.8, 124.6, 121.4, 120.7, 117.9 (d, J_{P-C} = 86.3 Hz, 3C), 108.2, 103.2, 79.3, 69.9, 42.0 (d, J_{P-C} = 15.7 Hz, 1C), 28.9, 20.7 (d, J_{P-C} = 52 Hz, 1C), 20.6 (d, J_{P-C} = 3.0 Hz, 1C); LRMS (ESI+): calcd. for $C_{36}H_{30}N_2O_2P$ $[M]^+$: 553.2; found 553.1. HPLC (system QC): t_R = 28.5 min, purity 96.4%.

BSA labelling

Solution A : PBS buffer (Phosphate-Buffered Saline, pH 7.47): dissolve NaCl 8 g, KCl 0.2 g, $Na_2HPO_4.H_2O$ 1.44 g and KH_2PO_4 0.24 g in 1 L milliQ water.

Solution B : 0.2 M sodium bicarbonate solution, adjusted to pH 9.0 with 2 M sodium hydroxide.

Solution C : To 10 parts of solution A add 1 part of solution B to obtain a labeling buffer of pH 8.3.

Solution D : dissolve 3.35 mg of **14** in ACN (1 mL; C = 3.89E^{-3} M).

Iodoacetamide conjugation : To BSA protein (4.406 mg, 66.7 nmol) in 1 mL of solution C was added solution D (85.7 µL, 338 nmol, 5 equiv.). The reaction mixture was incubated, protected from light, for 2.5 h at 37 °C.

Labelled BSA purification : Following the protein modification step, the excess of **14** and the buffer salts were removed by filtration through 10 kDa molecular weight cutoff Corning® Spin-X® UF 500 µL Concentrators. The protein was washed several times with 0.5 mL of milliQ water by spinning for 30 min at 6200 rpm. Subsequent washed protein was collected in milliQ water (1 mL) and stored at -25 °C until analysis.

Protocol for chymotrypsin and trypsin digestion of modified BSA:

Step 1: Redution and alkylation of disulfide bonds of modified BSA. To a solution of modified BSA (100 µl, 1 mg/mL) was successively added a solution of aq. $NaHCO_3$ (30 µL, 0.1 M) and a solution of aq. dithiothreitol (10 µL, 45 mM). The resulting solution was left for 2 h at 50 °C, then a solution of aq. iodoacetamide (10 µL, 100 mM) was added, and the resulting solution left 30 min at rt, then a solution of aq. cysteine (10 µL, 200 mM) was added. The mixture was stand for one more hour at rt before doing the enzymatic digestion.

Step 2: Enzymatic digestion of modified BSA. A solution of aq. enzyme (chymotrypsin or trypsin, 25 µL, 0.02 mg/mL) was added to 40 µL of the reaction mixture obtained in Step 1 (i.e, enzyme/modified BSA, ratio 1:50, w/w). The resulting solution was incubated for 12 h at 37 °C and stored at -25 °C, until analyzed.

Acknowledgements

We thank the Labex SynOrg (ANR-11-LABX-0029) for financial support to A. L-J. This work was also partially supported by Rouen-Normandie University, INSA de Rouen, Centre National de la Recherche Scientifique (CNRS) and Region Normandie (CRUNCh network).

Notes and references

1 For recent examples, see: (*a*) R. Baba, Y. Hori, S. Mizukami and K. Kikuchi, *J. Am. Chem. Soc.* 2012, **134**, 14310; (*b*) Y. Chen, C. M. Clouthier, K. Tsao, M. Strmiskova, H. Lachance and Jeffrey W. Keillor, *Angew Chem Int Ed*. 2014, **53**, 13785.

2 The Molecular Probes Handbook available at lifetechnologies.com/handbook

3 (*a*) S. Akita, N. Umezawa, and Tsunehiko Higuchi *Org. Lett.* 2005, **7**, 5565-5568; (*b*) M. G. Acker and Douglas S. Auld, *Persp. Sci.* 2014, **1**, 56–73; (*c*) He. M. Burke, T. Gunnlaugsson and E. M. Scanlan, *Chem. Commun.* 2015, **51**, 10576–10588, and references therein.

4 For few recent examples, see: (*a*) L. Wang, H. Li, D. Cao, Sens. *Actuator B-Chem*, 2013, 749– 755; (*b*) J.-T. Yeh, W.-C. Chen, S.-R. Liu and Shu-Pao Wu, *New J. Chem.*, 2014, **38**, 4434–4439; (*c*) Q. Wu, Z. Liu, D. Cao, R. Guan, K. Wang, Y. Shan, Y. Xu, L. Ma, Mater. Chem. Phys. 2015, 161, 43–48; (*d*) A. Romieu, *Org. Biomol. Chem*., 2015, **13**, 1294–1306; (*e*) B. Roubinet, L. Bailly, E. Petit, P.-Y. Renard, A. Romieu, *Tetrahedron Lett*., 2015, **56**,1015–1019; (*f*) B. Roubinet, A. Chevalier, P.-Y. Renard, A. Romieu*, Eur. J. Org. Chem.*, 2015, 166–182; (*g*) K. Renault, C. Sabot, P.-Y. Renard, *Eur. J. Org. Chem.* 2015, **36**, 7992–7996.

5 (*a*) S. S. Kelkar and T. M. Reineke, *Bioconjugate Chem.* 2011, **22**, 1879–1903; (*b*) Y. Cao, R. Pan, W. Xuan, Y. Wei, K. Liu, J. Zhou and W. Wang, *Org. Biomol. Chem.* 2015, **13**, 6742; (*c*) S. S. Matikonda, D. L. Orsi, V. Staudacher, I. A. Jenkins, F. Fiedler, J. Chen and A. B. Gamble, *Chem. Sci.*, 2015, **6**, 1212–1218.

6 (*a*) P. Shieha and C. R. Bertozzi, *Org. Biomol. Chem*., 2014,**12**, 9307; (*b*) L. G. Meimetis, J. C. T. Carlson, R. J. Giedt, R. H. Kohler and R. Weissleder, *Angew. Chem. Int. Ed.* 2014, **53**, 7531.

7 (*a*) O.S. Wolfbeis, E. Koller, P. Hochmuth, *Bull. Chem. Soc. Jpn.* 1985, **58**, 731-734; (*b*) J.-A. Richard, M. Massonneau, P.-Y. Renard and A. Romieu, *Org. Lett.*, 2008, **10**, 4175-4178; (*c*) E. Kisin-Finfer, D. Shabat, *Bioorg. Med. Chem.*, 2013, **21**, 3602-3608; (*d*) M.-J. Peng, Y. Guo, X.-F. Yang, L.-Y Wang, J. An, *Dyes Pigm.,* 2013, **98**, 327-332; (*e*) M.-J. Peng, X.-F. Yang, B. Yin, Y. Guo, F. Suzenet, D. En, J. Li, C.-W. Li, Y.-W. Duan, *Chem. Asian J.* 2014, **9**, 1817-1822; (*f*) M.-J. Peng, Y. Guo, X.-F. Yang, F. Suzenet, J. Li, C.-W. Li, Y.-W. Duan, *RSC Adv.* 2014, **4**, 19077-19085; (*g*) Roubinet, P.-Y Renard, A. Romieu, *Dyes Pigm.,* 2014, **110**, 270–284; (*h*) A.K. Sanap, K.K. Sanap, G.S. Shankarling, *Dyes Pigm.,* 2015, **120**, 190-199; (*i*) A.B. Tathe, V.D. Gupta, N. Sekar, *Dyes Pigm.,* 2015, **119**, 49-55.

8 (a) M. Masuda, C. Toriumi, T. Santa and K. Imai, *Anal. Chem.* 2004, *76*, 728-735; (b) A. Pashkova, E. Moskovets and B. L. Karger, *Anal. Chem.* 2004, **76**, 4550-4557; (c) E. V. Petrotchenko, D. Pasek, P. Elms, N. V. Dokholyan, G. Meissner and C. H. Borchers, *Anal. Chem.* 2006, **78**, 7959-7966; (d) J. You, H. Zhao, Z. Sun, L. Xia, T. Yan, Y. Suo and Y. Li*, J. Sep. Sci.* 2009, **32**, 1351-1362; (e) P. Zhou, J. Yao, G. Hu and J. Fang, *ACS Chem. Biol*. 2016, **11**, 1098-1105.

9 For examples of triphenylphosphonium-based coumarin developed specifically to target mitochondria, see : (*a*) A. R. Sarkar, C. H. Heo, H. W. Lee, K. H. Park, Y. H. Suh and H. M. Kim, *Anal. Chem.* 2014**, 86**, 5638−5641; (*b*) H. S. Jung, J. Han, J.-H. Lee, J. H. Lee, J.-M. Choi, H.-S. Kweon, J. H. Han, J.-H. Kim, K. M. Byun, J. H. Jung, C. Kang and J. S. Kim, *J. Am. Chem. Soc.*, 2015, **137**, 3017−3023.

10 K.-I. Setsukinai, Y. Urano, K. Kikuchi, T. Higuchi, T. Nagano, *J. Chem. Soc. Perkin Trans 2*, 2000, 2453.

11 (a) L.-A. Jouanno, A. Chevalier, N. Sekkat, N. Perzo, H. Castel, A. Romieu, N. Lange, C. Sabot and P.-Y. Renard, *J. Org. Chem.* 2014, **79**, 10353–10366 ; (b) L.-A. Jouanno, K. Renault, C. Sabot, and P.-Y. Renard, *Eur. J. Org. Chem*. 2016, in press (DOI: 10.1002/ejoc.201600127).

12 G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, 2nd edn, 2008.

13 For recent examples of bioconjugatable coumarins, see : M. D. Mertens and M. Gütschow, *Synthesis* 2014, **46**, 2191–2200.

14 A. K. Ghosh and H. L. Osswald, *Chem. Soc. Rev.*, 2014, **43**, 6765

15 M. I. Fernandez-Bachiller, A. Horatscheck, M. Lisurek and J. Rademann, *ChemMedChem* 2013, **8**, 1041–1056.

16 H.E. Gottlieb, V. Kotlyar and A. Nudelman, *J. Org. Chem.* 1997, **21**, 7512–7515.

17 K.-I. Setsukinai, Y. Urano, K. Kikuchi, T. Higuchi and T. Nagano, *J. Chem. Soc., Perkin Trans. 2* 2000, 2453-2457.

18 W. Stewart, *J. Am. Chem. Soc.* 1981, **103**, 7615-7620

19 G. B. Fields, R. L. Noble, *Int. J. Pept. Protein Res.* 1990, **35**, 161-214.

20 S.C. Laskowski and R.O. Clinton, *J. Am. Chem. Soc.* 1950, **72**, 3987-3991.

21 I.P. Dubovik, M. M. Garazd and V.P. Khilya, *Chem. Heterocycl. Compd.* 2007, **43**, 277-281.

22 L. Berti, P.S. D'Agostino, K. Boeneman and I.L. Medintz, *Nano Res.* 2009, **2**, 121-129.

23 T.S. Seo, Z. Li, H. Ruparel and J. Ju, *J. Org. Chem.* 2003, **68**, 609-612.

24 Y.-J. Cherng, *Tetrahedron*, 2000, **56**, 8287-8289.

25 M. Millard, J.D. Gallagher, B.Z. Olenyuk and N. Neamati, *J. Med. Chem.* 2013, **56**, 9170-9179.

26 E.E. Langdon-Jones, D. Lloyd, A.J. Hayes, S.D. Wainwright, H.J. Mottram, S.J. Coles, P.N. Horton and S.J.A. Pope, *Inorg. Chem*. 2015, **54**, 6606-6615.