Development and Application of Fluorescent Labels based on 7-diethylaminocoumarin

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"The journey of a thousand miles begins with one step"

Lao Tzu

My fiancé and my family

Acknowledgement

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Thank you!!!

Tamara Doroshenko

Abstract

7-diethylaminocoumarins are famous fluorescent dyes due to their low pH-dependence, high quantum yields, strong intensity of fluorescence and photostability. They are widely used in multi-coloured fluorescence investigation.

Within the scope of this thesis the synthesis of fluorescent dyes based on 7-diethylaminocoumarin moiety was developed and their application was studied. Furthermore, various labelling methods were developed by attaching coumarins to amino acids and peptides by bioorthogonal reactions for the biomolecular imaging. The fluorescent tags were attached to the *C*-terminus, *N*-terminus and an amino acid backbone. It was found that the triflate derivative of coumarin is a good coupling partner for various transition metal-catalyzed cross-couplings, like for instance Sonogashira, Suzuki-Miyaura and Stille coupling. The amino acid backbone was labelled through Pd-catalyzed allylic alkylation. Furthermore, Michael acceptors based on the coumarin moiety were developed. The labelling reactions proceeded smoothly, under mild conditions, furnishing molecules with fluorescent tags in good to excellent yields. All compounds were characterized by fluorescent spectroscopy in the group of Prof. Jung.

Kurzfassung

7-Diethylaminocumarine sind bekannte Fluoreszenzfarbstoffe aufgrund ihrer niedrigen pH-Abhängigkeit, hohe Quantenausbeuten, Fluoreszenz starken Intensität und Photostabilität. Sie werden in der mehrfarbigen Fluoreszenzspektroskopie verwendet.

Im Rahmen dieser Arbeit wurde die Synthese von fluoreszierenden Farbstoffen auf Basis von 7-Diethylaminocumarin Einheit entwickelt und deren Anwendung untersucht. Weiterhin wurden verschiedene *Labels* in bioorthogonalen Reaktionen zum Fluoreszenzmarkierung von verschiedenen Aminosäuren und Peptiden untersucht. Die Fluoreszenz-Markierungen gelangen sowohl an *C*-Terminus, als auch an *N*-Terminus und an der α-Position der Aminosäuren. Es wurde festgestellt, dass die Triflatderivat von Cumarin ein guter Kupplungspartner für die verschiedenen Übergangsmetall-katalysierten Kreuzkupplungen darstellt, so zum Beispiel für Sonogashira-, Suzuki-Miyaura- und Stille-Kupplung. Das Aminosäure-Rückgrat wurde durch Pd-katalysierte allylische Alkylierung markiert. Außerdem wurde ein Michael-Akzeptor ausgehend aus Cumarin entwickelt. Die Labelingsreaktionen verliefen glatt und unter milden Bedingungen, die Verbindungen mit Fluoreszenzmarker konnten in guten bis ausgezeichneten Ausbeuten isoliert werden. Alle Verbindungen wurden Fluoreszenzspektroskopisch in der Gruppe von Prof. Jung charakterisiert.

Contents

Abbreviations

abs.	absolute
Abs	absorption
Ac	Acetyl, [CH_3–CO]
Ar	Aromatic
Bn	Benzyl, [C_6H_5–CH_2]
Boc	*tert*-Butyloxycarbonyl, [$(CH_3)_3$C–O–CO]
BODIPY	Boron-dipyrromethene
Bu	*n*-Butyl, [CH_3–$(CH_2)_2$–CH_2]
*t*Bu	*tert*-Butyl, [$(CH_3)_3$C]
*n*BuLi	*n*-Butyllithium, [$H_9C_4^+$ Li^-]
cat.	catalyst
CDI	1,1`-Carbonyldiimidazole
CI	Chemical Ionization
Cy	Cyclohexyl, [C_6H_{11}]
d	day
DCC	Dicyclohexylcarbodiimide
DCM	Dichlormethane
DIAD	Diisopropylazodicarboxylate, [$(CH_3)_2$CH–O–CO)$_2N_2$]
DIPA	Di-*iso*-propylamine
DIPEA	Di-*iso*-propylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EA	Ethyl acetate, [CH_3–COO–CH_2–CH_3]
Em	Emission
Et	Ethyl, [C_2H_5]
Eq	equivalent
EWG	Electron-withdrawing group
Ex	Exitation
FRET	Fluoreszenz-resonanz-energie-transfer
GFP	Green fluorescent protein
GP	general experimental procedures
g	gram
h	hour
Hal	Halogen
Hex	Hexyl, [C_6H_{13}]
HMDS	Hexamethydisilazane
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
J	Coupling constant
LCMS	Liquid chromatography–mass spectrometry
LDA	Lithiumdi-*iso*-propylamide
LHMDS	Lithiumhexamethyldisilazane, [Li^+ $((CH_3)_3Si)_2N^-$]
M	Molar

MA	Michael-Additon
Max.	Maximum
M.Pt.	Melting point
Me	Methyl, [CH_3]
MHz	Megahertz
min	minute
mg	milligram
mmol	millimole
mol%	Mole percent
N	Normality of solution
NMR	Nuclear Magnetic Resonance
nm	nanometer
Nu	nucleophile
on	overnight
PE	Petrolether
PG	Protection group
Ph	Phenyl, [C_6H_5]
ppm	parts per million
ppts	Pyridinium p-toluenesulfonate
*i*Pr	Isopropyl, [$(CH_3)_2CH$]
Pr	Propyl
R_f	Retention factor
s	second
sat	saturated
rt	room temperature
t	reaction time
T	Temperature (in °C)
Tf	Triflate
TFA	Trifluoracetyl, [$F_3C–CO$]
THF	Tetrahydrofuran, [C_4H_8O]
THP	Tetrahydropyranyl, [C_5H_9O]
TLC	thin-layer chromatography
UdS	Saarland University (Universität des Saarlandes)
UV	Ultraviolett

1

Preface

Fluorescence spectroscopy is a unique analytical technique in detection and analysis of various biochemical and biophysical properties as well as morphology of cells and tissues. The recent development of fluorescent microscope allows by fluorescent labeling of biomolecules, observing and recording the progress of molecular or cellular processes for biochemical, biological, diagnostic or therapeutic application. This biomolecular imaging made it possible to study mechanisms of the process and identify the targets; therefore the starting points for drug development can be found. [12, 19]

Fluorescent dyes are the most important participants in fluorescence spectroscopy. There are known various fluorescent probes with different characteristics [9], like for instance coumarin, fluorescein, BODIPYs, Alexa dyes and others. Therefore based on the experiment requirements a specific fluorescent tag can be chosen. Fluorescent dyes based on the 7-aminocoumarin moiety are popular

due to their low pH-dependence and high quantum yields. They became also target of this research.

The most commonly labelled molecules are usually antibodies, proteins, amino acids and peptides. Among the labelling methods for biomolecules chemical as well as enzymatic labelling protocols are widely used.[56] The targets for fluorescent tags are usually *N*- or *C*-termini, and also the backbone of the protein.[19] Among the methods, which were used in this work for bioorthogonal labelling, are Pd-catalyzed cross-coupling reactions (Suzuki-Miyaura, Sonogashira), Buchwald-Hartwig amination with and without CO-insertion, Pd-catalyzed allylic alkylations and Michael additions. The methods developed in this work are shown in Figure 1.1 and Figure 1.2.

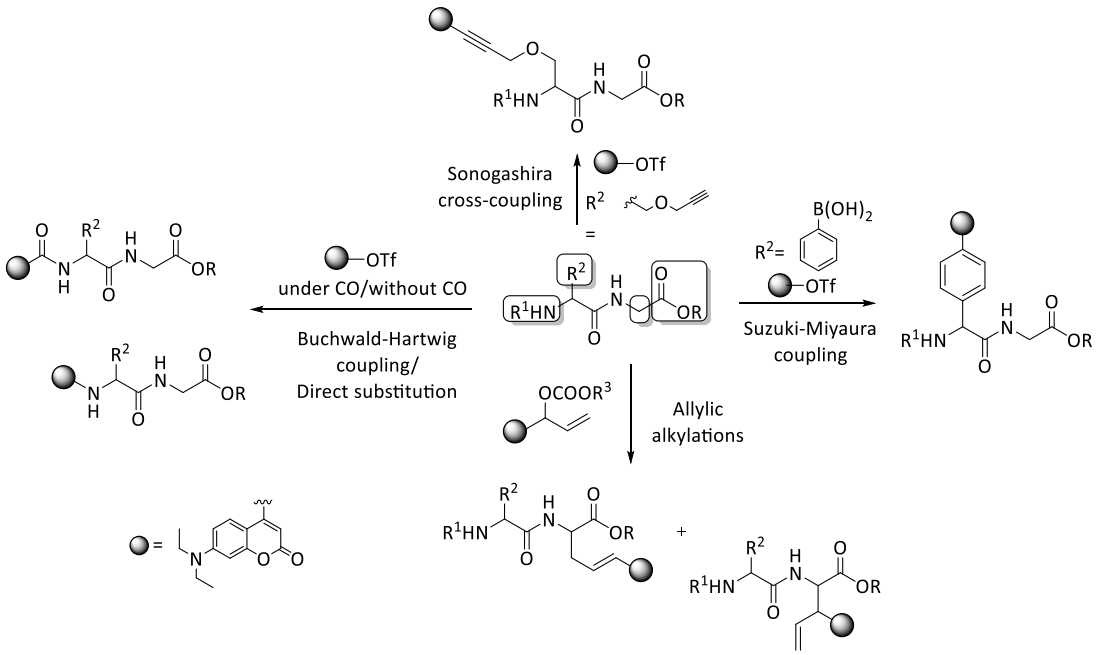


Figure 1.1: Bioorthogonal labelling methods developed in scope of this work.

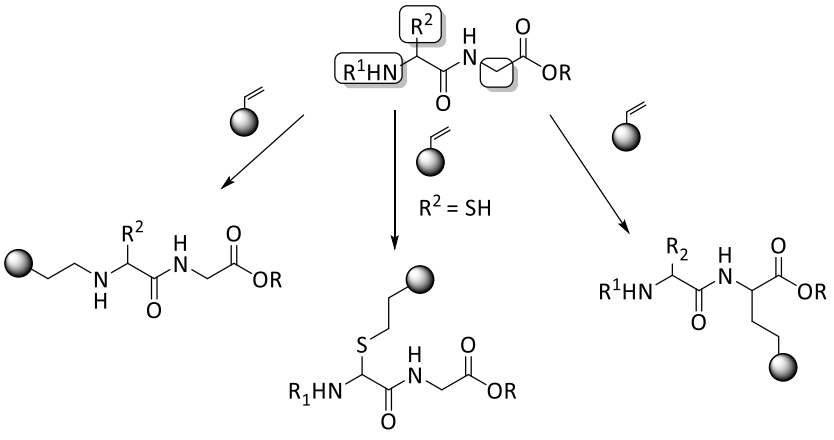


Figure 1.2: Bioorthogonal labelling through Michael addition**.**

2

Introduction

The term fluorescence derives from the name of the mineral fluorite (calcium difluoride)[1] (Figure 2.1).[2] This phenomenon was first described by Monardes in 1565, which he observed in a Mexican medicinal wood; in Europe it was known as *Lignum nephriticum.* The compound which causes this fluorescence is matlaline – an oxidation product of one of the flavonoids found in this wood.[3] For many centuries the fluorescence remained a mystery for science.

At the beginning of 19th century, Edward D. Clarke[4] and René Just Haüy[5] noticed fluorescence in minerals fluorites. The phenomenon was also observed in chlorophyll[6] and in quinine.[7]

In 1852 George Gabriel Stokes described the ability of fluorspar and uranium glass to change from light invisible to human eye into blue light. He named this phenomenon *fluorescence*: "I am almost inclined to coin a word, and call the

appearance *fluorescence*, from fluor-spar, as the analogous term *opalescence* is derived from the name of a mineral".[8] Some samples of fluorite contain traces of europium, which acts as the fluorescent activator to emit blue light.

In nature there are a lot of species, like fishes, butterflies, parrots, plants, corals, etc., which contain fluorescenting substances and therefore exhibit these phenomena.

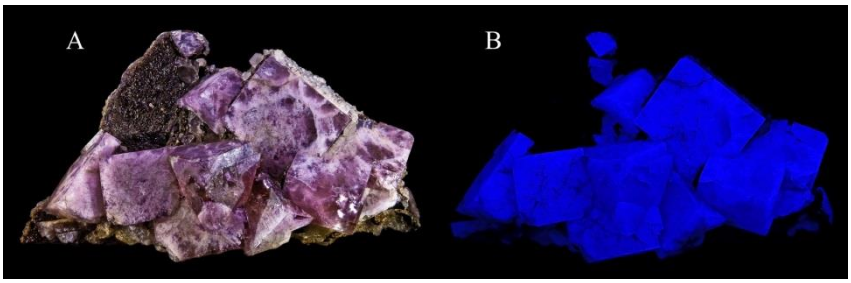


Figure 2.1: Fluorescing fluorite from Boltsburn Mine, Weardale, North Pennines, County Durham, England, UK.[2]

A fluorophore is a molecule that is capable of fluorescing. Typical fluorophores usually contain aromatic and heterocyclic ring. In Figure 2.2[9] are shown some examples of these compounds. Fluorophores allow scientist to detect and visualize different components of complex biomolecules, study the mechanisms of chemical and biological processes, even in case of low concentrations.

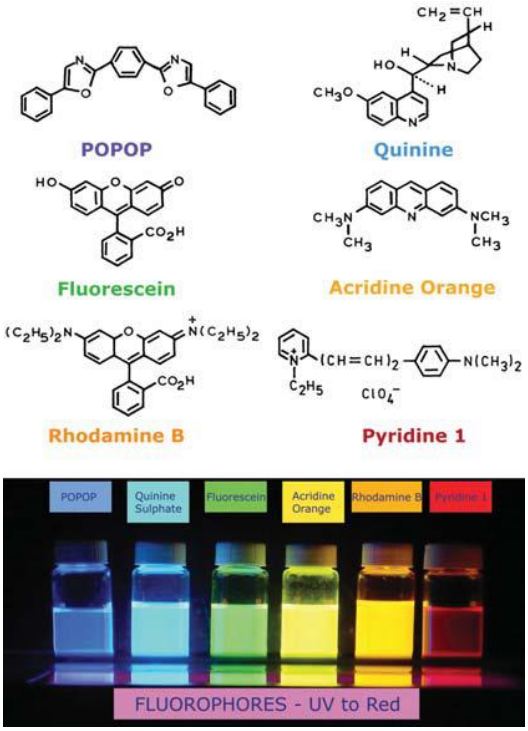


Figure 2.2: Some typical fluorescenting compounds.[9]

One of the well-known fluorophores is quinine, which can be found in tonic water. A slight blue fluorescence can be observed if tonic water is lit by sunlight at a specific angle. This phenomenon of quinine solution was first described by Sir John Fridrich Herschel. Quinine is still widely used as a fluorophore in fluorescent spectroscopy, as well as a drug due to its antipyretic (fever-reducing), antimalarial, analgesic (painkilling), and anti-inflammatory activities. It was widely used during the World War II. Furthermore quinine fluorophore inspired the development of the first spectrofluorometers.[9]

2.1 Basics of Fluorescence

Some molecules are capable of being excited by absorption of light energy to a higher energy state – excited state. These energy states are not stable and energy cannot be sustained for a long period; it decreases by emission of light. This process is called photoluminiscence. Photoluminiscence is an ability of a substance to glow, which is caused by transfer of the electrons of the molecule from an excited state to its ground state, if the excitation is carried out by absorption of photons of electromagnetic radiation in the optical range. Depending on the nature of the energy levels involved in the formation of the radiation, photoluminescence is divided into fluorescence and phosphorescence. The fluorescence results from the optical transition between the two levels of the same multiplicity (usually between the first excited S_1 and singlet ground state S_0 of a molecule) and is characterized by a high probability of spontaneous transition - short lifetime τ of electronically excited singlet state. Phosphorescence unlike fluorescence is due to optical transitions between the levels of different multiplicity (typically between the lowest excited triplet T_1 and singlet ground S_1 state of the molecule), and has a very low probability of transition (long lifetime of the triplet excited state T_1).[10]

In its ground state, a fluorophore molecule has a relatively low energy and is in its stable configuration, it does not fluoresce. If light of an external source hits the molecule, the fluorophore can absorb the energy light, thus getting a higher energy level – *excited state*. This process is called *excitation*. The fluorophore can reach different excited states, depending on the energy of the external light source. Since the high energy level cannot be maintained for a long time, the molecule goes to a lower energy level. The time during which the molecule is in excited configuration is called an *excited lifetime*, and it lasts for a very short time (10^{-15} to 10^{-9} seconds). Then the molecule rearranges from metastable to the ground state, the excess energy is emitted in a form of light. This light has a lower energy and longer wavelength then the absorbed light. This means that the colour of the light emitted

differs from the one absorbed. This entire absorption-emission process can continue repeatedly.[11]

In general, the excited molecules lose their excess energy not only through radiative transitions, but also through nonradiative processes. Nonradiative transitions between electronic states of the same multiplicity are called internal conversion, and between states of different multiplicity - intersystem crossing

The scheme of intramolecular processes occurring after the optical excitation of molecules is shown in Figure 2.3. It is called the Jablonski Diagram.

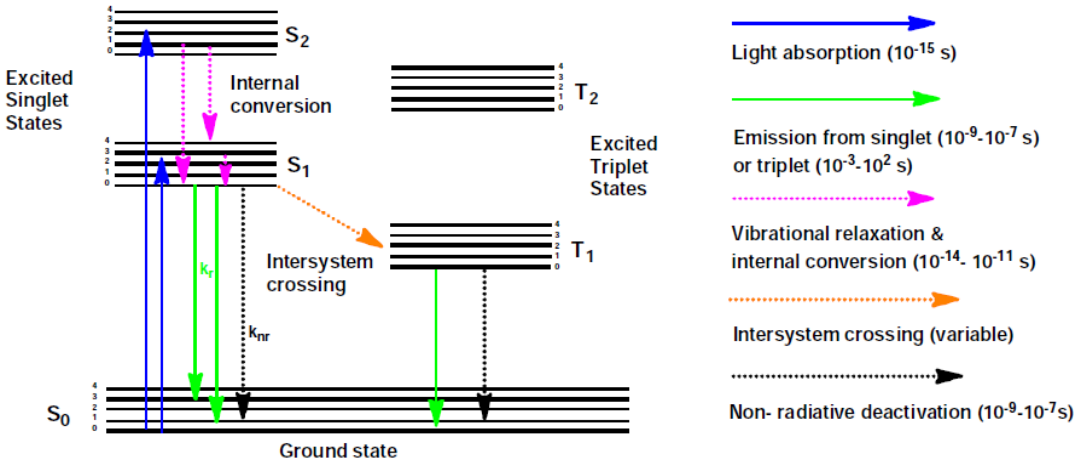


Figure 2.3: Jablonski Diagram.[2]

The entire process can be summarized as follows:

1. The fluorophore is excited by absorption of the light energy;
2. Excited lifetime, fluorophore reminds in the excited state;
3. The fluorophore returns to its ground state emitting light (fluorescence).

Molecules which undergo such electronic transitions and result in fluorescence are called fluorescent probes, fluorochromes, or dyes. *Fluorochromes* which are covalently bonded to macromolecule, like a nucleinic acid, lipid, enzyme, serving as a marker are called *fluorophores*. Fluorophores can be intrinsic and extrinsic. *Intrinsic* fluorophores are naturally occurring compounds like aromatic amino acids, neurotransmitters, flavins, quinine, pophyrins, nicotinamide adenine dinucleotide (NADH), adenosine triphosphate (ATP), etc. *Extrinsic* fluorophores are synthetic dyes, like fluorescein, rhodamine, ethidium bromide, etc. There are as well fluorescent proteins, which are “genetically” attached to the target protein.[12]

Properties of excited molecules

Lifetime and quantum yield

Once a molecule (fluorophore) absorbs light (photons) it is important to know the most crucial processes that can take place. Fluorescence lifetime and quantum yield are two the most important features. The *fluorescence quantum yield* (Φ) is the ratio of photons absorbed to photons emitted by fluorescence. The molecules that possess large quantum yields have the brightest emissions. The quantum yield for some process ***x*** can be found from the following formula:[10]

$$\Phi x = \frac{kx}{\sum k}$$

In other words it is a rate of some process x divided by the sum of all rates. For instance, if quantum yield of some fluorescence is Φ = 0.70, it means that once a photon is absorbed by a fluorophore, 70% of the molecules will irradiate the absorbed energy in form of fluorescence. On the other hand, quantum yield for intersystem crossing Φ_{ISC}, which results the degradation of the fluorophore, should be as small as possible. The lifetime of fluorescence defines the time during which a molecule is in its excited state.

Stokes Shift

As it was mentioned above the energy of the light that fluorophore absorbes differs from the energy of the emitted light. This results in difference between absorption and emission spectra. *Stokes shift* is the difference between positions of maxima of the absorption and emission spectra. It is named after Irish physicist George G. Stokes.[13] If the energy emitted is more than the energy absorbed then the difference is called the *anti-Stokes shift* (Figure 2.4).[14]

This feature is especially interesting in case of polar fluorophores in polar solvents. One of the examples is the indole group of tryptophan residue in proteins, [9] which is a sensitive fluorophore towards the environment. Due to this feature the location of tryptophan residue in protein can be determined. If the residue is shielded and thus has less interaction with an environment (aqueous phase), the emission spectra is observed in shorter wavelength. The unfolding of the tryptophan residue results in exposure of the indole residue and subsequently the emission spectra is shifted toward the longer wavelength.

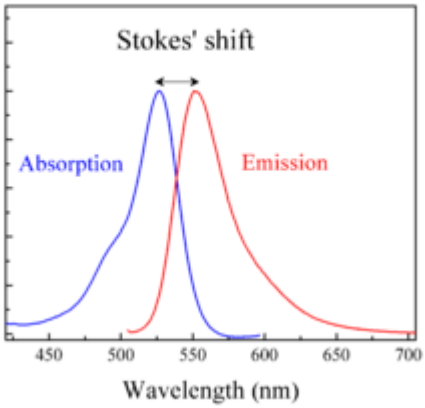


Figure 2.4: Stokes Shift.

Another interesting example is 6-(p-toluidinyl)naphthalene-2-sulfonate (TNS),[9] which reveals very weak fluorescence if water is used as a solvent but a strong fluorescence can be observed once it is bounded to a biomolecule, like apomyoglobin protein. This biomolecule possesses a hydrophobic pocket that binds the heme group as well as other nonpolar molecules. If to a solution of TNS is added apomyoglobin, strong fluorescence can be observed and the emission spectrum is shifted towards shorter wavelengths (Figure 2.5). Therefore by studying the emission spectra of solvent-sensitive fluorophores the binding sites of the biomolecules can be determined.

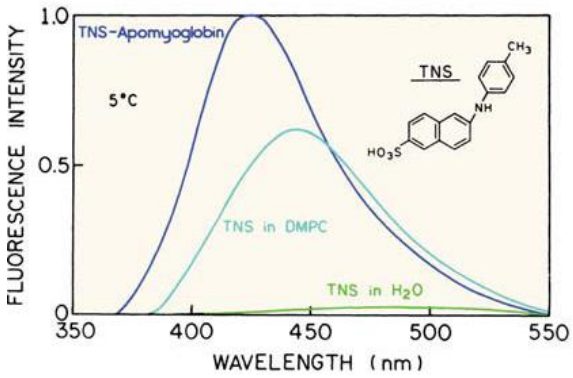


Figure 2.5: Emission spectra of TNS in water, bound to apomyoglobin, and bound to lipid vesicles.

Quenching of fluorescence and photobleaching

Besides emission of light, a molecule can lose its energy by interaction with a *quencher*.[15] A quencher is a molecule that by some sort of interaction with an excited fluorophore decreases the fluorescence. Molecular oxygen, iodide ions and acrylamide[16] are typical examples of chemical quenchers. The chloride ion causes quenching of quinine fluorescence.[17] As mentioned before, many fluorophores have

aromatic and heterocyclic rings, which by interaction with each other *via* van der Waal forces, form nonfluorescenting complexes.[9] Fluorescence quenching is mostly an undesirable process which results in reduction of fluorescence intensity and thus giving incorrect measurements data.

Another undesirable process is photobleaching (or fading) of fluorescence. This dynamic process takes place when a fluorophore loses its ability to fluoresce due to photon-induced chemical destruction and covalent modification caused by excitation light.[18] As shown in the Jablonski Diagram, Figure 2.3, the fluorophore molecule after being excited go from ground singlet state to excited singlet state. By radiative (fluorescence) or non-radiative (internal-conversion) decay they return to the ground state. On the other hand non-radiative intersystem crossing to a triplet state may take place. During these transitions, fluorophores may interact with other molecules from the environment and create irreversible covalent complexes. The lifetime of a triplet state is relatively longer, thus allowing the molecules to undergo different modification. Those photobleached molecules are unable to participate in the excitation-emission cycle. The rate of photobleaching depends on the structure of the fluorophore and the environment.

The phenomena of photobleaching are widely used in fluorescence spectroscopes. The most widely used technic is known as *fluorescence recovery after photobleaching* (FRAP), based on work by Peters *et al*. This is a method of determining the kinetics of diffusion in living cells using fluorescence spectroscope.[12]

2.2 Fluorescence spectroscopy

A fluorescence spectroscope is an electromagnetic spectroscope which analyses fluorescence properties of a sample. A fluorescent spectroscope uses a source of light, usually ultraviolet light, to excite the electrons in molecules of certain compounds. Devices that measure fluorescence are called fluorometers.[9]

In general there are two types of instruments:

- Filter fluorometers, where filters are used for isolation of the incident and fluorescent light.
- Spectrofluorometers, where diffraction grating monochromators are used for isolation of the incident and fluorescent light.

The basic principle of both instruments is shown on the scheme in Figure 2.6. A fluorimeter consists of the light source, which hits the sample. These may be lasers, LED lamps, xenon arc lamp or mercury-vapor lamps. Dissadvantage of the laser

lamps is that it does not allow the change of the wavelength by much. The advantage of a xenon arc is that it has a continuous emission spectrum, high intensity in the range from 300-800 nm. The light goes through a filter or a monochromator, in order to decrease the amount of the light with different wavelengths. Then it hits the sample causing the fluorescence of some of the molecules. The light in form of fluorescence goes further through the second filter or monochromator before it reaches the detector. The detector is placed at a 90° angle in order to avoid interference of the excited light. In the end the results are presented in graphical form.

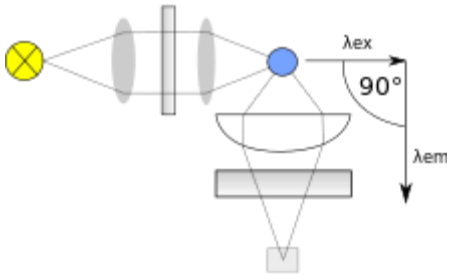


Figure 2.6: A basic scheme of a fluorimeter.

Application of fluorescent technologies in *Life Sciences* grew significantlly in the last decades. The reason for this is that in comparison with other measuring methods, like radioactive methods, it is much cheaper, faster, more flexible and more precise.[19,15] The fields where fluorescence spectroscopy can be applied are vast. It can be used for solution of many problems in lifesciences, especially chemical and biological sciences. Fluorescence technologies can give information about different reaction mechanisms, a wide range of molecular processes, conformational changes, etc. The progress in fluorescent technogies made it inexpensive and unique for diverse analytics.

New fluorescence technologies

Developments in fundamental physics and particularly in spectroscopy have enabled the expansion of luminescent labeling and detection in the life sciences. Nowadays instrumentation devised in laboratories is becoming also commercially available.[20]

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.[21]

In 1873 E. Abbe published an equation according to which the microscope would not be able to observe things smaller than half the wavelength of light, apr. 0.2 micrometres (Figure 2.7). Therefore according to this view the observation of a single protein, the size of which is few nanometers, would not be possible.

By studying absorption and fluorescence of molecules, usually millions of molecules are observed at the same time. As a result the experiments represent an average molecule. Scientist working in this field have always dreamt of a microscope able to measure a single molecule, thus giving more understanding of different processes, like for example, the development of a disease.[21] The first experiment was carried out in 1989 by W. E. Moerner; he measured the light absorption of a single molecule. This gave stimulus to many chemists to start investigation of single molecules.

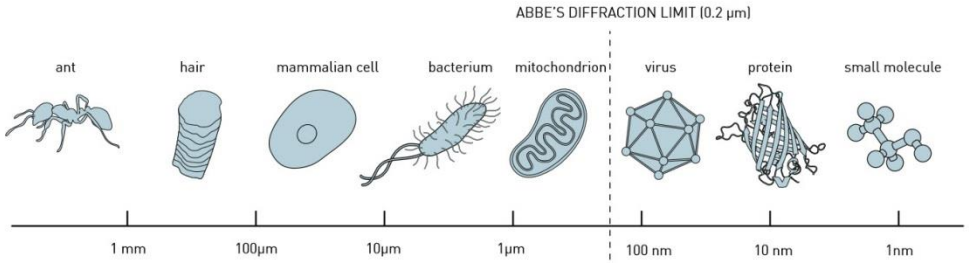


Figure 2.7: Abbe's diffraction limit.

Super-resolution fluorescence microscope is a type of light microscope. The application of fluorescent molecules made it possible to take a light microscope to another level. The principle is the following: all fluorescent molecules are being excited by a light source; meanwhile a laser-beam quenches fluorescence from all fluorophores except those in a nanometre-sized volume. Only this volume is then recorded. By moving along the sample and continuously measuring light levels, it is possible to get a high-resolution image. The smaller the volume taken the better the image can be achieved. Therefore half-wavelength limit no longer applies.

2.3 Fluorescent dyes

As mentioned above, fluorophores are devided into naturally occurring and synthetic. Among the major characteristics that should be considered when choosing a fluorescent dye are absorbtion coefficient and quantum yield, emission spectra (in order to avoid mistakes because of autofluorescence), solubility, reactivity and labelling technic. *Molecular Probes Handbook of Fluorescent Probes and Research Products*[11] gives a very good information about many synthetic dyes.[12] An "ideal" fluorophore should possess further characteristics:[12]

- reacts only with the target molecule, cell, etc;
- be non-toxic;
- be bright;
- not be photobleachable;
- be dissociable;
- be small

Organic fluorophores are the first fluorescent compounds that have been used in biological research. Today they are the most commonly used fluorescent labels due to their flexible design and the small size, which allows binding of the fluorophore to a macromolecule without loss of functionality, as well as the specific labeling of certain molecular positions. Among other advantages are their low price, good availability and the presence of established methods for conjugating the dyes to biomolecules. Organic fluorophores are, for instance, classical chromophores for fluorescence resonance energy transfer measurements (FRET). FRET measurements can be regarded as spectroscopic ruler, with which the distance between two fluorescently labeled, functional groups (donor and acceptor) can be determined. The organic fluorescent dyes have polymethine, xanthene or coumarin ring.

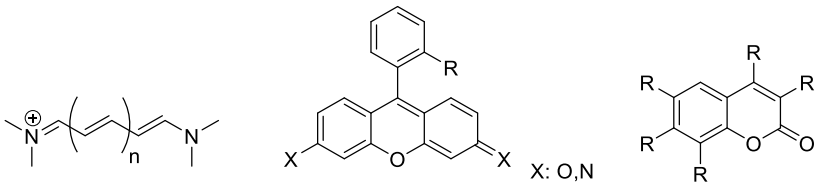


Figure 2.8 Basic structures of organic fluorophores.

Coumarin dyes

Coumarins or *2H*-chromen-2-ones (Figure 2.9) are a class of heterocyclic organic compounds which are widely spread and exhibit different biological activities against cancer, HIV, as well as anticoagulant, spasmolytic and antibacterial activities.[22,23] Its name derives from tonka bean, coumarou, from which it was first isolated in 1820. It also occurs naturally in vanilla grass, woodruff, mullein, lavender, strawberries, apricots, cinnamon and has a peculiar flavour which reminds vanilla.[23]

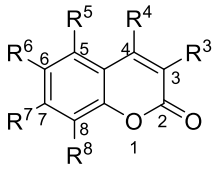


Figure 2.9: Structure of coumarin.

Besides their pharmacological properties, coumarins exhibit fluorescence ability as well.[19] They belong to the group of blue fluorophores, i.e. they absorb light at the λ_{abs} = 354-423 nm range and emission occurs at the λ_{em} = 420-590 nm range. Simple, unsubstituted coumarin does not reavel fluorescence properties, it is necessary to create a "push-pull" – system using different substituents. A fluorescenting coumarin should possess electron-donating groups in positions 6 and 7 (R^7 and R^6 – substituents with +M-effect), and electron-withdrawing groups in position 3 and 4 (R^3 and R^4 – substituents with -M-effect). Fluorescence properties of coumarin changes drastically with different substituents and expansion of the coumarin ring: [24]

- electron-donating groups in position 6 and electron-withdrawing groups in position 3 influence the emission of fluorophore;
- electron-donating groups in position 7 and electron-withdrawing groups in position 2 influrence the fluorescence intensity;
- electron-withdrawing substituents in position 4 cause the shifting of emission maxima.[15]

Due to blue fluorescence of coumarin it is easy to distinguish its light from green, yellow and red; it is widely used in multi-coloured fluorescence investigation, for instance, immunfluorescence, nucleinic acid and protein-microassays. Furthermore because of its fixed *trans*-conformation of a carbon-carbon double bond due to a lactone structure, it helps to avoid the *trans-cis* transformation under UV-irradiation, which results in strong intensity of fluorescence, high quantum yield and photostabilitity of coumarin derivatives.[25] Among the coumarin derivatives the most important are 7-aminocoumarins (Figure 2.10) as well as 7-hydroxycoumarin,[19] which is widely used in fluorescence spectroscopy.[26]

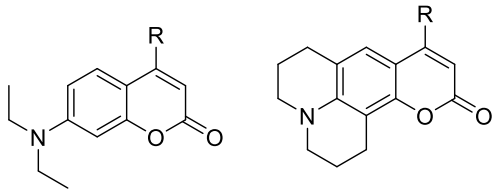


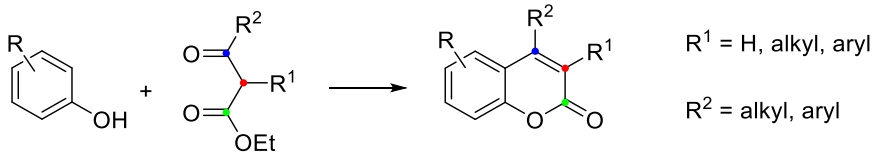
Figure 2.10: 7-aminocoumarins.

Application of 7-hydroxycoumarin and its derivatives is limited because of its pH dependence. It exhibits fluorescence maximum at pH 10 or higher, and only in its anionic form.[27,28] Therefore, for investigation of enzymes which reveal their catalytic activity at pH 7 or lower, 7-hydroxycoumarin, in these conditions neutral and nonfluorescent, cannot be applied.[15]

In comparison with 7-hydroxycoumarin and Pacific blue (6,8-difluoro-7-hydroxycoumarin), 7-aminocoumarin reveals low pH-dependence, it is active at a wide range of pH; 7-aminocoumarins exhibit high fluorescence intensity and excellent fluorescent quantum yield. It is an excellent dye candidate for fluorescent investigation; on the other hand, its synthesis is not as trivial and requires a novel synthetic route.

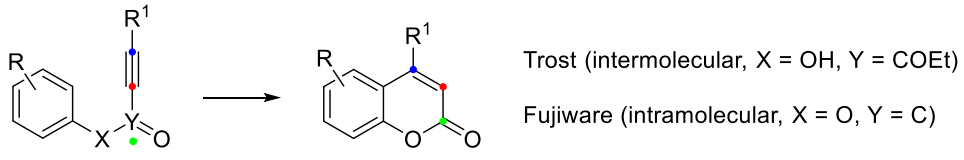
Synthesis of coumarins

Because of all the properties mentioned above, a lot of synthetic routes have been developed to obtain coumarin and its derivatives,[23] like Pechmann,[19] Perkin,[29] Reformatsky[30] and Wittig[31] reactions. The most widely used one is the Pechmann condensation reaction (Scheme 2.1) of phenols with β-ketoesters furnishing the 4-substituted coumarins. Depending on the reagents different catalysts can be used, for instance, sulphuric acid, aluminium chloride, phosphorus pentoxide, as well as Lewis acids.[32] Kazmaier *et al*. reported on Ti-catalyzed Pechmann condensation reaction,[33] which was applied for the synthesis of 7-aminocoumarins giving the desired fluorescent dyes in high yields.



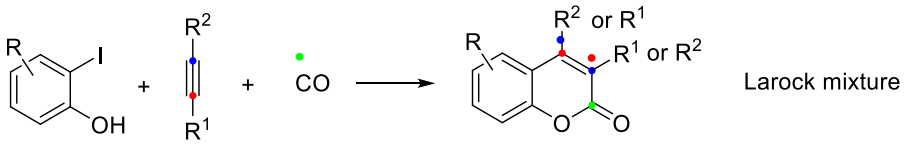
Scheme 2.1: Pechmann condensation reaction.

In recent works Trost et al. developed a Pd-catalyzed C-C bond formation giving 4-substituted coumarins *via* the intermolecular reaction of propiolic acids with phenols.[34,35] Fujiwara and coworkers synthesized coumarins by Pd-catalyzed inter- and intramolecular hydroarylation of aryl propiolates (Scheme 2.2),[32] though this methodology cannot be used in the synthesis of 3-substituted fluorescent dye.



Scheme 2.2: Pd-catalyzed synthesis of coumarin.

Larock et al. synthesized coumarin through Pd-catalyzed annulation of internal alkynes by O-iodophenols in the presence of CO (Scheme 2.3).[36,37] The disadvantage of this method is the formation of regioisomers due to application of unsymmetrical alkyne.



Scheme 2.3: Pd-catalyzed synthesis of coumarin according to Larock.

Fluorescein dyes

Fluorescein which was synthesized for the first time by Bayer in 1871 *via* the Friedel-Crafts reaction between phthalic anhydride and resorcinol in the presence of zinc chloride, is a polycyclic fluorophore which has long absorbtion maxima λ_{abs} = 480-600 nm range and emission wavelength at λ_{em} = 510-615 nm range.[9,19]

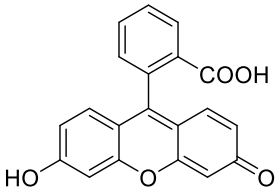


Figure 2.11: Fluorescein.

The most important reactive derivatives are isothiocyanates, iodoacetamides, and maleimides. Iodoacetamides and maleimides are used as labels for sulfhydryl groups, whereas isothiocyantes and sulfuril chlorides are good labels for amines.[9] Fluorescein markers are widely used for labelling antibodies. Fluorescein labelled proteins can be purchased directly and there is a wide application of these proteins in fluorescence spectroscopy and immunoassays.

Fluorescein and its derivatives are characterized by high absorption and high quantum yield (φ = 0.92) at pH > 8,[16] good solubility in water, as well as fluorescein excitation maximum at 494 nm, which is close to the 488 nm spectral line of the argon laser, making it a good fluorophore in confocal laser-scanning microscopy and flow cytometry.[8]

Nevertheless fluorescein and its derivatives have some negative aspects, like photobleachining, pH-sensitivity, a relatively broad fluorescene emission spectrum (which makes them bad candidate in multicolour fluorescent investigation), self-quenching by conjugation with biopolymers, low photostability.[8,33]

There are many known fluorescein derivatives, for instance, fluorescein isothiocyanate, or FITC. This dye reacts with amino group of proteins forming a thiourea linkage.[39]

Rhodamine dyes

Rhodamines are organic heterocyclic compounds, which belong to the family of fluorine dyes. The most important represantives are rhodamine 6G, rhodamine B, rhodamine 123 and texas red (Figure 2.12).[8]

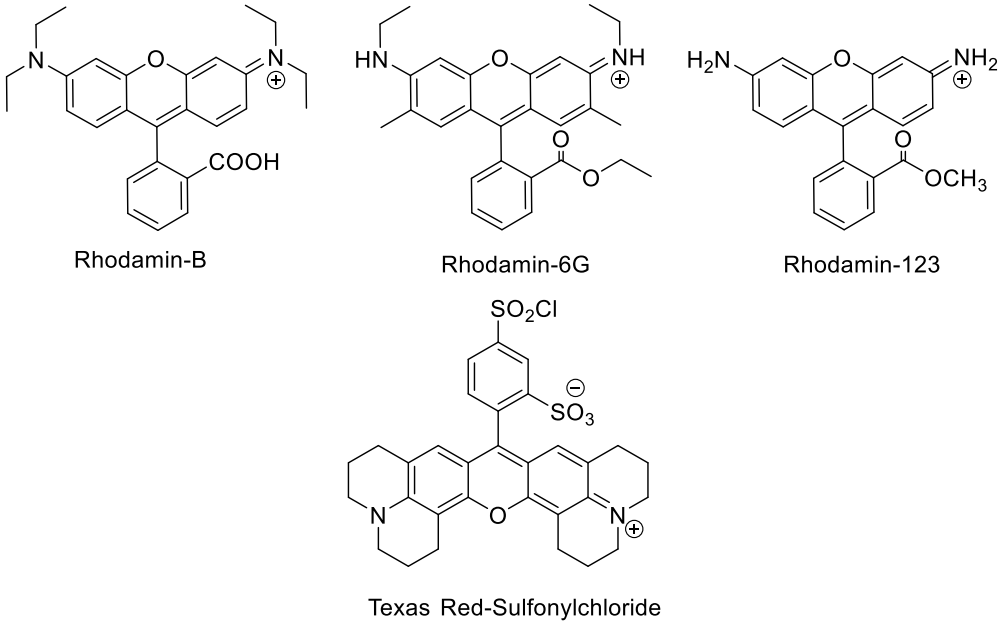


Figure 2.12: Rhodamin dyes.

Rhodamine and its derivatives are usually characterized by high absorption at the λ_{abs} = 470-557 nm range and emission is observed at the λ_{em} = 510-615 nm range.[19] These fluorophores have been reported in different fluorescent studies.[8] The molecule of rhodamine consists of the xanthen nucleus bearing amino-groups in 3- and 6-position, and a benzoic acid in 9-position. By alkylation of amino groups, as well as of xanthen nucleus, different rhodamine derivatives can be obtained, therefore expanding its application in spectroscopy. The expansion of the xanthen skeleton results in bathochromic shifting of absorption spectrum.[15] Usually rhodamines are stable at a wide pH-range (from 4 to 10), are relatively photostable and quenching effect is not as strong as for fluorescein and its derivatives.[15,19] The disadvantage of rhodamine dyes is their poor water-solubility.[39]

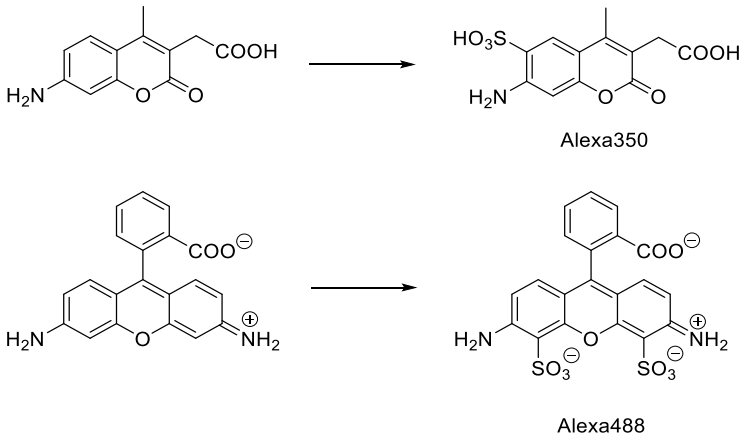
Since the development of BODIPY- and Alexa-fluorescent dyes, the application of rhodamines has been significantly reduced.

Alexa dyes

The Alexa fluor dyes, which were developed by Molecular Probes Inc,[11] are a new series of flurescent dyes which are often used as fluorescent markers in Life Sciences. Their emission and excitation spectra are similar to already known dyes,

like Lucifer Yellow, fluorescein, rhodamine, etc. But unlike other fluorophores they exhibit high photostability and low pH-dependence, and are characterized by strong fluorescence and high quantum yield,[38] on the other hand they are more expensive.

Alexa-dyes were synthesized through sulfonation of coumarin, rhodamine, xanthene and cyanine dyes (Scheme 2.4). The sulfonation reduces the tendency of molecules to self-quench, which can be explained by negative charge and hydrophilicy.[19]



Scheme 2.4: Synthesis of Alexa-dyes.

The name comes from Alex Haugland.[2] Each Alexa-dye is labelled by a number that corresponds its absorption maximum.[15] By modification of coumarin derivatives the blue Alexa-dye (Alexa 350) is obtained, rhodamine and fluorescein give green fluorophores (Alexa 488 and Alexa 430), as well as from rhodamine a red fluorophore can be obtained.[8,15]

BODIPYs

Another interesting class of fluorescent markers are BODIPY-dyes (patented by Molecular Probes). The name comes from abbreviation of ***BO**ron-**DIPY**rromethenes,* which in IUPAC-system is known as *4,4-difluoro-4-bora-3a,4a-diaza-s-indacene.*[39]

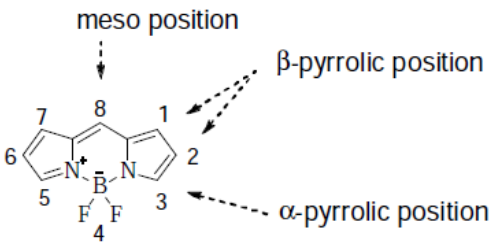


Figure 2.13: Structure of BODIPY-fluorophore.[34]

The first synthesis of these dyes was performed by Treibs and Kreuzer in 1968 but only after 1980 they found their wide range of application. They can be obtained by condensation of acyl chlorides with pyrroles with subsequent complexation with $BF_3 \cdot OEt_2$ in the presence of a base.[40] Depending on its structure, BODIPY-fluorophores exhibit the absorption in the range 504-544 nm, and emission in the range 510-675 nm. Because of their intensive green fluorescence, BODIPY markers are good substituents of fluorescein and rhodamine dyes.[19]

BODIPY dyes are characterized by high fluorescence quantum yield ($\varphi \approx 1.0$) regardless of the solvent (even in water), excellent thermal and photochemical stability, the fluorescence intensity does not depend on pH-value of the environment. They show narrow emission band widths (an advantage in multicolour analysis), high molar absorption coefficients, good solubility, and chemical rebustness. BODIPY dye molecule is neutral, they are widely used as reagents for detection of amine-derivatives in HPLC and capillary electrophoresis because of their high peak intensity.[8,40]

These dyes have been applied as markers for proteins and DNA. Though they have also some disadvantageous characteristics. One example is that most of them emit at less than 600 nm.[41] Through expansion of the chromophore molecule, for instance, annelation of the phenyl ring, results in the bathchromic shift and emmision maximum is observed at 630 nm. The disadvantage of such big structures is a high lipophilicity and therefore they cannot be applied to all the systems.[15]

Squaraine dyes

Squaraine dyes are a class of organic fluorophores characterized by intense fluorescence in the red and near infrared region (λ_{abs} = 630-670 nm, λ_{em} = 650-700 nm). They exhibit intense and sharp absorption spectra in the visible and near-infrared range, as well as sharp and intense fluorescence. These fluorophores are characterized by photoconductivity. Due to their photophysical properties they are widely used in detection of biomolecules. Squaraine dyes are unique four-membered aromatic systems derived from squaric acid.

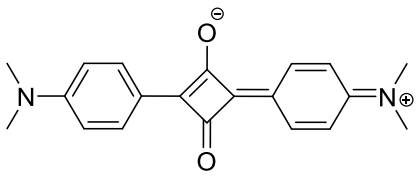


Figure 2.14: Structure of squarylium dye.

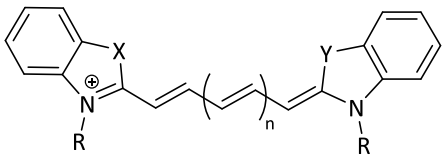
By modification of this dye on the aromatic ring or on the *N*-atom different derivatives can be obtained. The conjugates with protein show higher quantum yields, longer lifetimes and better photostability compared to polymethines. Squaraines dyes can be excited by a red laser as well as by blue light.[8]

Cyanines

Cyanine dyes are another class of fluorescent compounds which have been widely used since their discovery. They are being applied in genetic analysis, DNA sequencing, in vivo imaging and proteomics.[8] The first total synthesis of this compound was reported by Hammer *et al*. in 1930.[15]

The name derives from the Greek word “kyanos” which means a shade of blue-green. The molecule of cyanine is built out of two aromatic or heterocyclic rings which are linked by a polymethine chain with conjugated carbon-carbon double bonds.

Cyanine fluorophores exhibit emission spectra at the range between 600 and 900 nm and absorption close to infrared areas (λ_{em} > 550 nm). The addition of a vinyl group gives further bathochromic shift. These dyes are the main source in long-wavelength spectroscopy.[8] Cyanines are widely used to increase the sensitivity range of photographic emulsions, they are used in CD-R and DVD-R media.[2]



X,Y = O, S, $C(CH_3)_2$ or $C{=}CH_2$
n = 0-4
R = $(CH_2)_xSO_3^-$

Figure 2.15: Basic structure of cyanine dye.

A major advandage of cyanine fluorophores is their large emission wavelengths, and the possibility to control emission spectra by extension or by shortening the polymethine chain. Due to this feature interfering autofluorescence of the tissue can be avoided. In addition, several different fluorescent fluorophores can be used and detected separately, which allows tracking of multiple effects simultaneously.[19]

Cyanine dyes possess also sharp and intense absorbtion bands; they show high stability towards pH (4.0-9.0). Fluorescent quantum yield is in the φ = 0.09 – 0.52 range. Low quantum yield can be explained by thermo- and photoinduced *cis-trans*-isomerisation.[8,15] Furthermore cyanine dyes have short lifetime and in aqueous

solution they build aggregates, which causes low fluorescent intensity. Despite these disadvantages, cyanine fluorophores are still widely used in biological imaging.[15,19]

2.4 Green fluorescent protein

The green fluorescent protein (GFP) is a protein which consists of 238 amino acid residues and is characterized by bright green fluorescence when exposed to high frequency light. It was isolated for the first time from the jellyfish *Aequorea Victoria* (Figure 2.16). Its major excitation peak is observed at 395 nm and minor at 475 nm. Emission occurs at 509 nm, which is in the lower green segment of the visible spectrum. The fluorescent quantum yield is 0.79.[42,43]

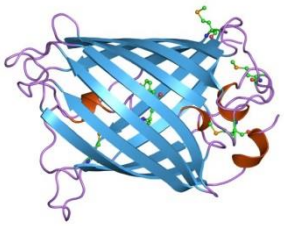


Figure 2.16: Structure of the 'Aequorea victoria' green fluorescent protein.[44]

The GFP gene is extensively applied as a reporter of expression.[45] The use of genetically encoded fluorescent proteins has grown tremendously in modern biological research in recent years. Only thirty years after the isolation by Shimomura (1962) the GFP found its value in biotechnology as a marker of gene expression and protein targeting in intact cells and organisms.[43] The 2008 Nobel Prize in Chemistry was awarded to Martin Chalfie, Osamu Shimomura and Roger Y.Tsien for the discovery and development of the green fluorescent protein.[21]

Modified forms of GFP were used to produce biosensors. The GFP gene can be introduced into organisms and maintained in their genome through breeding, injection with a viral vector, or cell transformation. The GFP has been already introduced so far in many bacteria, yeast and other fungi, fish, plants, flies, as well as mammalian cells, including human.[43]

The biggest advantage of GFP is its heredity, i.e it can be transferred with genetically encoded protein. Furthermore, it is easy to visualize and detect GFP just by shining light (Figure 2.17). Also, GFP is a relatively small and inert molecule, so it does not have any significant impact on the target molecule. If GFP is used with a monomer it is able to diffuse readily throughout cells.[46] It is worth mentioning that GFP does not have any toxic effect on the living cells while illuminated.

GFP can also be expressed in a wide range of structures making morphological distinction possible. In this case, the gene for the production of GFP is introduced into the genome in the region of the DNA which is responsible for the target proteins; consequently the gene's regulatory sequence now controls also the production of GFP. Therefore only those cells in which the tagged gene is expressed, or the target proteins are produced, will reveal fluorescence. This process made it possible to understand many biologically important processes like protein folding, protein transport, etc.[47]

Figure 2.17: Mice expressing GFP under UV light (left & right), compared to normal mouse (center).[2]

GFP has been used as well in cancer research to label and detect cancer cells. GFP-labelled cancer cells have been used to model metastasis.[48]

2.5 Nanoparticles as fluorescent labels

In spite of the large amount of known fluorophores, there are still some limitations of fluorescent detection in bioanalytics. Besides the labelling reaction difficulties, the fluorescent dyes themselves have photobleaching problems, pH sensitivity, low quantum yields and short lifetimes; they very often exhibit narrow excitation bands, broad emission spectra, small Stokes shift, etc. The recent developments in nanosized luminescent agents may be the solution of these problems. Nanoparticles (like quantum dots and gold nanoparticles) may change drastically bioanalytical fluorescence measurements.[49,12]

Unlike classical dye, nanoparticals could overcome much chemical and spectral limitation, and they are becoming commercially available. The newly developed nanoparticles can be applied as well for absorbance and emission in the near-infrared region.[49]

An advantage of nanoparticles is that they can access even hidden target molecules (e.g proteins), through conjugation of specific targeting groups on their surface. Furthermore, due to their optoelectronic properties they could be detected

with high sensitivities.[49] Inorganic nanoparticles based on noble metals and semiconductor quantum dots seem to be mostly interesting in bioanalytical measurements. Gold nanoparticles are widely used in *Life Sciences.*

Quantum dots

Quantum dot is a nanocrystal made of semiconductor materials. Its size is small enough (2- to 10-nm) to possess quantum mechanical properties. It is similar to the size of proteins and DNA (Figure 2.18).[12] Its excitions are confined in all three spatial dimensions.[50] The properties of quantum dots are inbetween bulky semiconductors and discrete molecules.[51,52] quantum dots were discovered by Alexey Ekimov in 1981 [53] and later by Louis E. Brus in 1985.[54] They can be applied in transistors, solar cells, LEDs, diode lasers, as well as agents for bioanalytical and medical imaging.

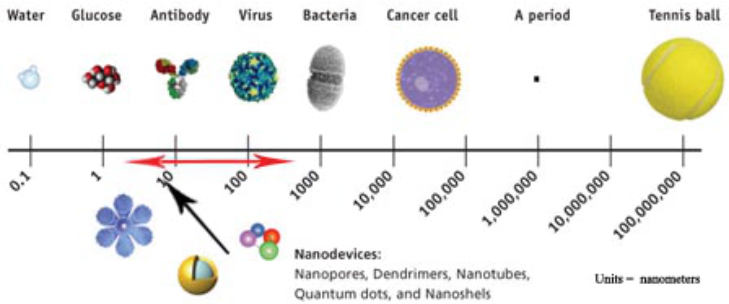


Figure 2.18: Relative size of nanoparticles.[2]

The properties of quantum dots depend on many factors. One of them is the material they are made of (for example, CdSe, CdS, CdTe), which influence the wavelength of emitted light. Another factor is the size, namely decreasing size results in increasing of the frequency of emitted light. This means that the colour changes from red to blue. Since the size of quantum dots is tunable it allows using them as highly multiplexed markers to label different parts of the target molecule with different nanoparticles.[12]

Furthermore, these photostable semiconductor crystals have sharp and symmetrical emission peak, which allows the simultaneous excitation of different quantum dots with one excitation source.

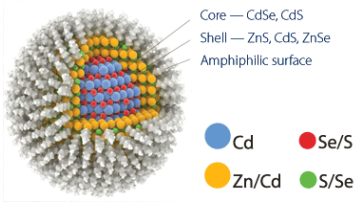


Figure 2.19: Structure of quantum dot.[2]

Nevertheless quantum dots have one major drawback: their lack of biocompatibility. The heavy metal-containing crystals are toxic and may interfere in biological processes.[19]

2.6 Fluorescent labeling techniques of biomolecules

Nowadays the number of known fluorophore dyes is vast; it is possible to choose a specific dye with optimal characteristics for the given experiment. However a lot of work is still needed in the development of new dyes and improvement of the existing ones (e.g. relaxing of limitations). In design and synthesis of fluorophores as well it is important to include a functional group which is capable to bond selectively to a specific functional group of a biomolecule.[8] In the last decades many protein-labelling methods have been developed.[19] Inspite of the fact that a lot of modification methods of biomolecules are known, scientist are still developing methods which are more specific and can be applied for a particular residue of the protein.[55]

The fluorophore can be attached to the target molecule chemically (covalently or non-covanlently) or biologically (genetic combination of unnatural amino acids, fusion of biomolecules with a fluorescent peptide, or enzyme catalysis). Classical protein labeling methods which allow the coupling of small organic fluorophores substantially comprise simple reactions, which act selectively on the functionality in the side chains of the canonical, proteinogenic amino acids.[56] Cysteine and lysine are the most commonly modified residues. Labelling of amines proceedes usually very fast and furnishes amide or secondary amine bond in high yield.[55]

2.6.1. Chemical labelling

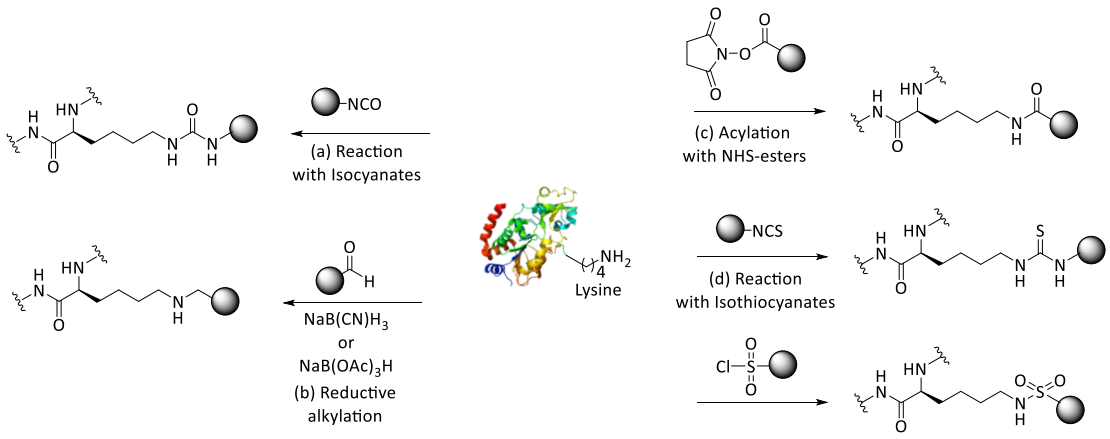
Amine labelling

It is essential that a fluorescent dye reacts selectively with the target molecule, and at the same time does not influence its biological activity, i.e. maintaining both protein integrity and function. The “ideal” reactions are those which require the same conditions as proteins, like functional group tolerance compatibility, selectivity, water as a solvent (or pH ~ 7), room temperature, high reaction rates, low reactant concentration, nontoxic reagents. Methods that meet these requirements can be perfect for *in vivo* analysis.[56]

The lysine residue of the protein is one of the most important targets due to high nucleophilic properties of the amino group [55] and its natural abundance. NHS-esters (*N*-hydroxysuccinimide ester) are most widely used reagents for labelling lysine

residue (Scheme 2.5c).[57] Among the other classical reagents are isocyanates (which are available from acyl azides using the Curtius rearangement), isothiocyanates (Scheme 2.5a and d), as well as sulfonyl chlorides (Scheme 2.5e).

Another possible labelling method is reductive alkylation (Scheme 2.5b), which can be carried out by interaction of the protein and aldehydes and consequent reduction with $NaB(CN)H_3$ or $NaB(OAc)_3H$. Francis and McFarland developed the reductive alkylation of proteins using a water stable Iridium complex ([Cp*Ir-(bipy)(H_2O)]SO_4 for transfer hydrogenation.[58] In comparison with the alkylation using sodium boronhydride, which proceeds at low pH, this method gives the desired product in high yield also at pH > 7.4.[19]



Scheme 2.5: Labelling of Lysine-residue.

The kinetically controlled labelling approach is a new attractive labelling method reported.[59] A single lysine moiety of a biomolecule reacts with an activated biotinylation reagent, which requires mild conditions, physiological pH and temperature, giving the desired product in high yield. Amino group of lysine residues serves as good nucleophiles for the aza-Michael addition reaction.[60] Cyclohexene sulphonamide derivatives have been used for selective labelling of Lys in human serum albumin (HSA).[63]

Thiol labelling

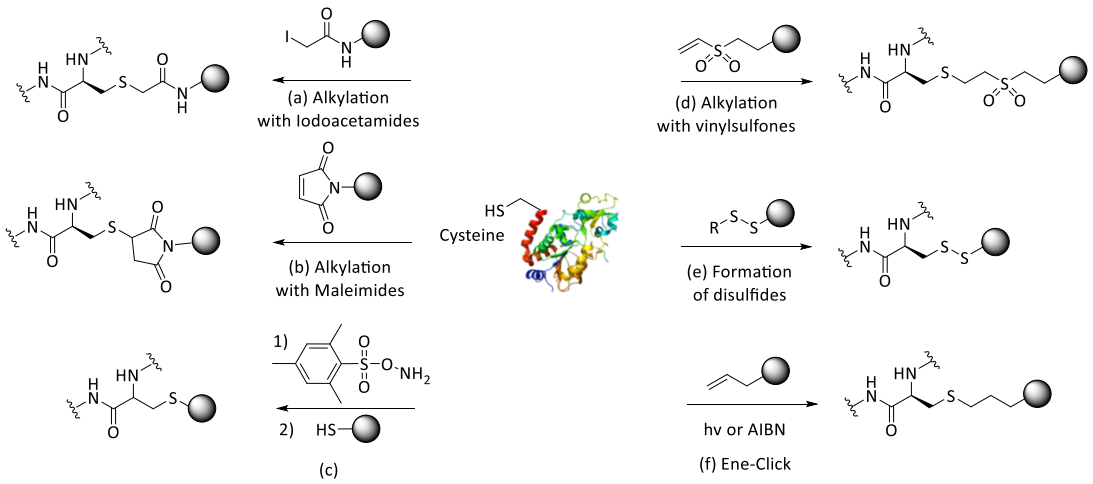
Cysteine is in comparison with lysine a rare amino acid. Introduction of a uniquely reactive cysteine is possible by means of genetic methods. As a relatively rare amino acid cysteine is often used for single-site modification.[56] Reagents used in modification of cysteine are usually commercially available. By deprotonation of the thiol group of cysteine ($pK_a \sim 8$) a thiolate nucleophile is generated which reacts with "soft" electrophiles, like iodoacetamides (Scheme 2.6a), maleimides (Scheme 2.6b), acrylamides, and vinyl sulfones (Scheme 2.6d). As a result a new carbon-sulfur bond

is generated. Many of these reagents can modify lysine residue as well. The concurrent reaction can be avoided at relatively low pH, which causes the protonation of amino group. Furthermore the thiol group of cysteine by exchange of disulfide group with exogenous disulfides forming disulfides on the protein surface (Scheme 2.6e). The efficiency of the reaction can be improved in case of application of asymmetric disulfides. By coupling cysteine-alkylating groups to small molecules that bind to specific sites on a protein an additional level of labeling selectivity can be achieved.[56]

Among the modern modification methods of cysteine the one developed by Davis *et al* should be mentioned.[61] It is a two-step process where cysteine is converted first to dehydroalanine by reaction with O-mesitylenesulfonylhydroxyl-amine under basic conditions. Afterwards the dehydroalanine reacts with thiol reagent forming thiol ether (Scheme 2.6c). The Michael addition is not stereospecific resulting in formation of mixture of diastereomers.

Another modern technique for labelling of cysteine is based on Thiol-En-Click-Chemistry (Scheme 2.6f).[56] The reaction proceeds by a radical chain (UV- or initiator-induces) giving usually quantitative yields. Thiol-En-Click reactions are wide in scope, stereospecific and usually require mild conditions.[62,63]

Reactions with thiols are usually fast and produce compounds bearing stable thiother or disulphide bonds in high yields.



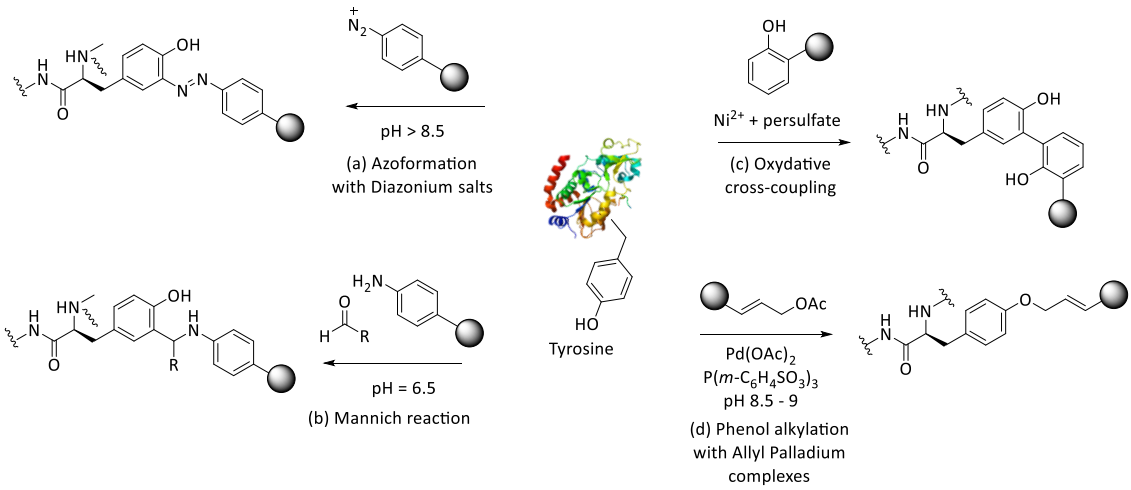
Scheme 2.6: Labelling of Cysteine-residue.

Chemical reactions of tyrosine residues

Reagents used for modification of lysine residues can also be coupled to hydroxyl (-OH) groups of the amino acid. The reaction is basically carried out in organic solvent like DMSO which absorbs the formed water molecule and thus hydrolysis can

be avoided. For labelling reactions hydroxyl groups are not very specific because of the existence of multiply hydroxyl groups in biomolecule (serine, threonine, and tyrosine).[55]

In Scheme 2.7 are shown the most important modification reactions of tyrosine residue. One of the well-known labelling methods is the reaction with diazonium salts (Scheme 2.7a) resulting in the formation of azo compounds.[64] The pH range should be 8-10 for the formation of a phenolate anion. Another method is the three-component Mannich-type reaction (Scheme 2.7b), which involves the condensation of an aniline derivative with aldehydes followed by the nucleophilic addition of tyrosine with formation of a new carbon-carbon bond. Aliphatic amines do not participate in this reaction; therefore side reaction with proteins can be avoided.[65]



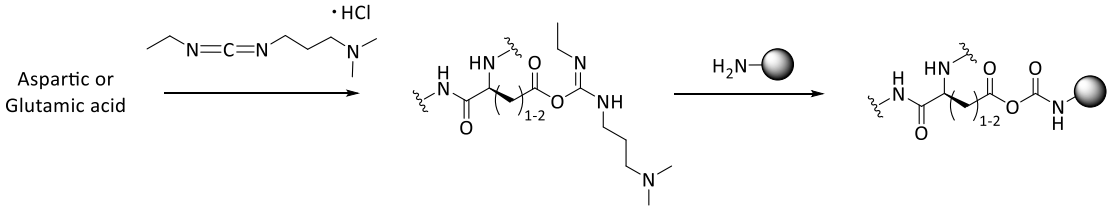
Scheme 2.7: Labelling of Tyrosine-residue.

Examples of metall-catalyzed modification reactions are the oxidative coupling of two phenol groups and the palladium-catalyzed alkylation of the phenol. In the first method (Scheme 2.7c) a persulfate ion oxidizes the Ni(II) ion bind to $(His)_6$tags to Ni(III). The metal ion takes an electron from a tyrosine and creates a radical intermediate, which couples with another tyrosine thus forming a dityrosine link. During the second approach (Scheme 2.7d) Palladium(0) complexes activate allylic substrates to form electrophilic π-allyl-complexes. At pH 8-10 these complexes react with the phenolate anions of tyrosine residues under formation of an aryl ether and the Pd(0) catalyst is regenerated.[57]

Chemical labelling of other amino acids

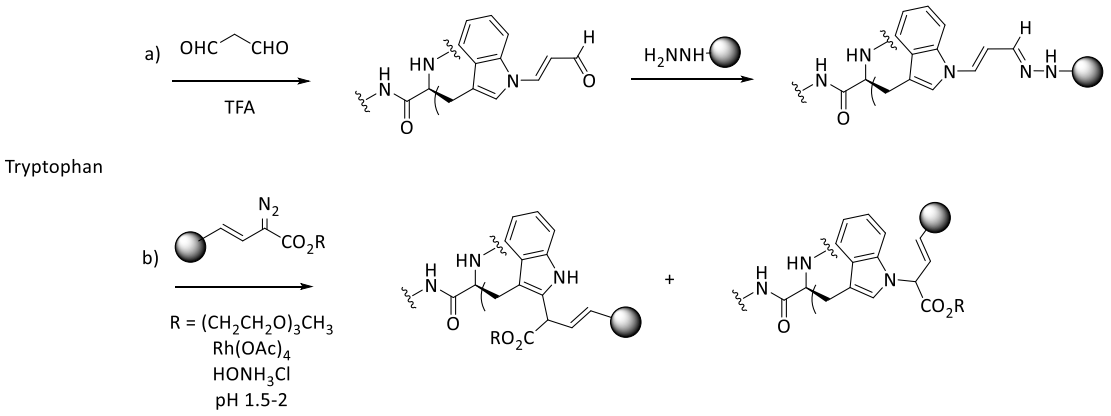
Besides the residues described above, the surface of various proteins has many aspartic and glutamic residues. The carboxylate groups can be activated with as *N* -

ethyl-3-*N*',*N*'-dimethylaminopropyl carbodiimide (EDC), (Scheme 2.8). EDC forms an O-acylisourea intermediate which reacts with amine forming amides.[57]



Scheme 2.8: Labelling of aspartic/glutamic acid.

Several strategies have been developed for tryptophan chemical labelling. Tryptophan is the most fluorescent of the native amino acids. The chemical modification of this residue allows the control of the features, like emission wavelength and fluorescent intensity.[57] A recent method reported is the modification of a nitrogen atom of the indole ring with malonyl dialdehyde derivatives (Scheme 2.9a).[66] The aldehyde can further react to hydrozone formation. The transition metal catalysed modification of tryptophan residues proceed with high chemoselectivity.[57] By degradation of vinyldiazo compounds by rhodium-salts metallcarbenoid intermediates are formed, which react with indole residue forming a mixture of N- and 2-substituted products (Scheme 2.9b).



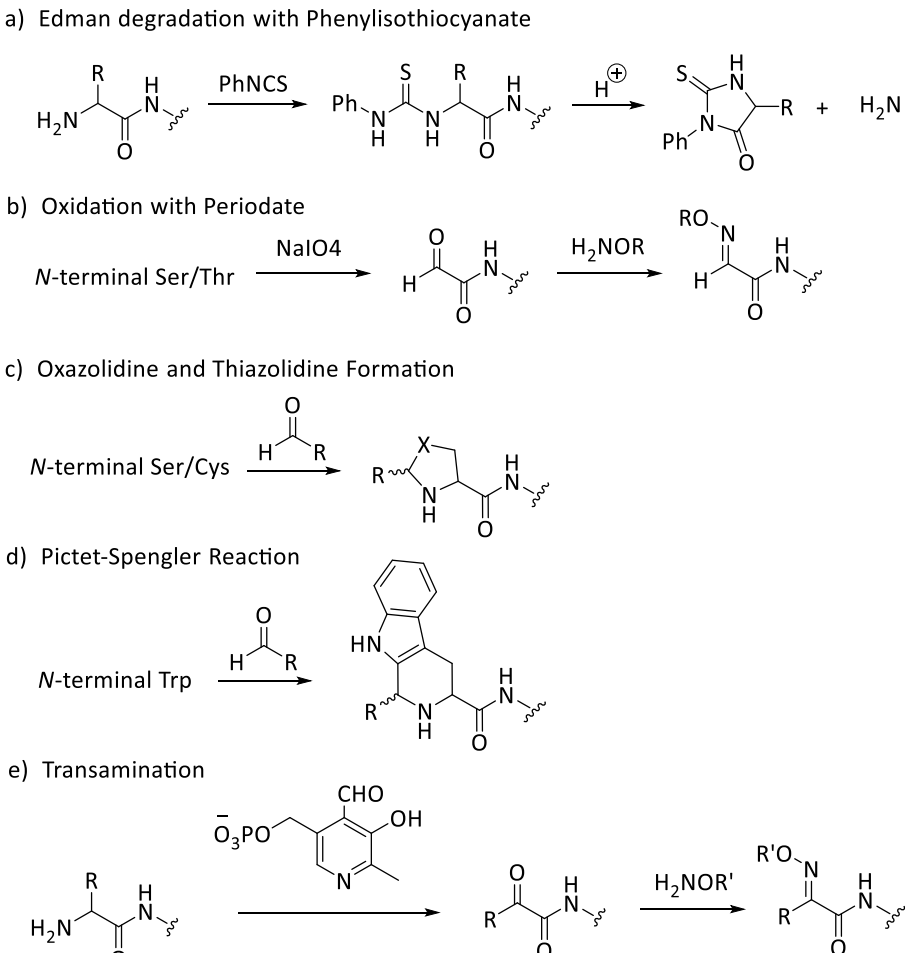
Scheme 2.9: Chemical labelling of tryptophan.

Modification of the *N*- and *C*-Termini

Often in the protein chain several rare amino acids are present the labelling of which is not possible using the above described methods. To overcome this problem several methods have been developed that are applicable to the unique chemical reactivities of the polypeptide termini. These methods have been used to form native-like peptide links.[57]

The *N*-terminal amino group of the peptide chain is less basic [pKa = 6–8] than lysine amino groups, which makes single-site modification possible.[67] Due to its relative lower pKa value in comparison with the amino group of the lysine side chains, it can be selectively acylated or alkylated in the presence of many lysine residues.

One of the most known methodologies to modify the *N*-terminus is the Edman degradation (Scheme 2.8a). In this protocol the *N*-terminal amino group reacts with phenylisothiocyanate to furnish a thiourea. The following acidification results in the removal of the first amino acid from the polypeptide chain. The thiazolidinone rearranges forming a stable thiohydantoin.

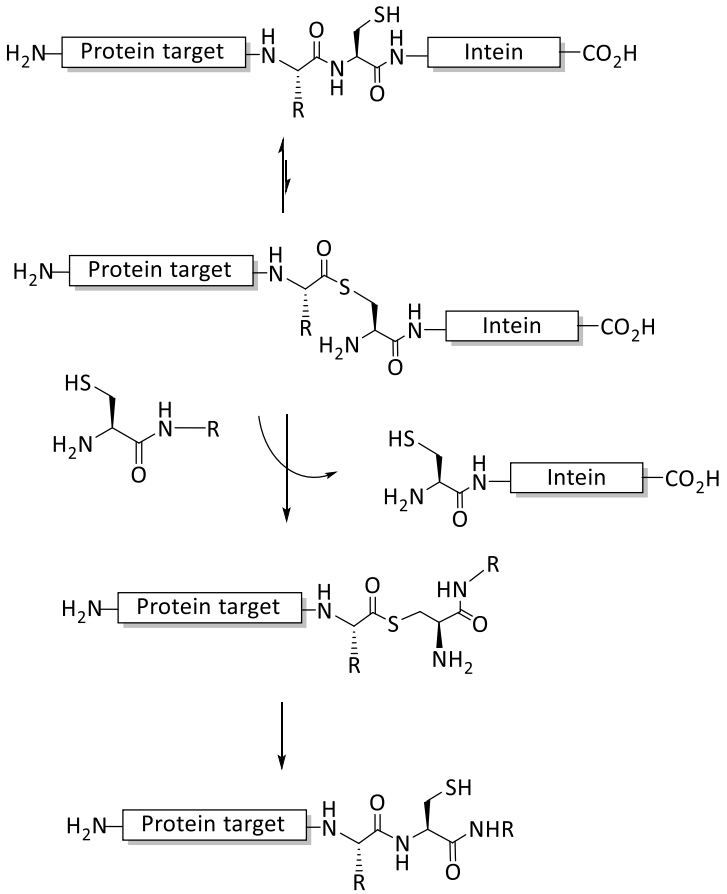


Scheme 2.10: Modification of *N*-terminus.

Among the more selective methods is the oxidation of β-amino alcohols of *N*-terminal serine and threonine residues, which results in the formation of the aldehyde group, which can be further, modified (Scheme 2.10b). The condensation of N-terminal serine and cysteine residues with aldehydes gives oxazolines and thiazolidines, respectively (Scheme 2.10c). *N*-terminal tryptophan reacts with aldehydes giving Pictet-Spengler products in a high yield (Scheme 2.10d).

Transamination is the most important method for the selective modification of *N*-terminus. The first significant transamination that proceeds under physiological conditions without the addition of metals or bases was described by Francis *et al*.[68] Through the condensation of the *N*-terminal amine with pyridoxal-5-phosphate and subsequent hydrolysis the pyruvamide is formed. The resulting pyruvamide-keto group can react with hydrazine- or aminooxyreagents allowing further modifications of the protein. The best results with this method produce amino acids such as alanine, glycine, aspartic acid, glutamic acid and asparagine in *N*-terminal position. *N*-terminal residues can also participate in native chemical ligations with thioesters.

Native chemical ligation or NCL is an important extenstion of the chemical ligation to construct large polypeptide chains by reaction of two or more unprotected peptides.[69] NCL is an important and widely used method for extension of the peptide backbone or modification. In general it is a ligation between two peptides having a *C*-terminal thioester and *N*-terminal cysteine, respectively, which forms a "native" amide bond (Scheme 2.11).



Scheme 2.11: Modification of the C-terminus through native chemical ligation (NCL).[57]

The *N*-terminal portion of the target peptide is connected directly to the intein domain. The intein catalysed an *N*-to-*S* acyl shift forming a thioester between the

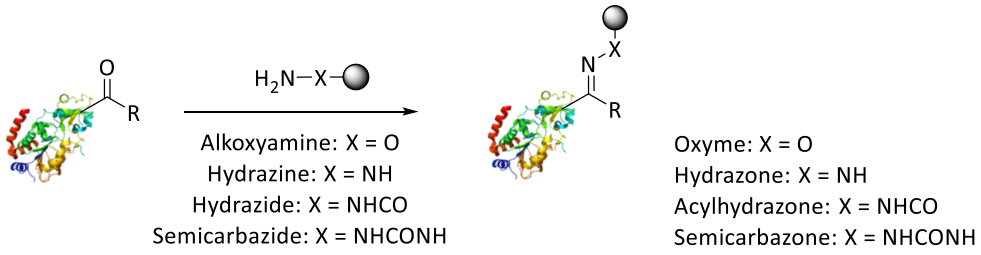
peptide target and a cysteine thiol at the end of the intein sequence. During this process a transthioesterification takes place joining two coupling parts of the protein and releasing the intein. A final *S*-to-*N* acyl shift forms the native peptide bond to yield the desired product.[57]

The NCL method has been widely used to link biologically interesting molecules to the *C*-terminus of proteins, like lipids and polysaccharides, as well as to attach fluorophores, affinity tags, and metal chelating agents.[70]

Bioorthogonal labelling of protein

Naturally occurring proteins usually contain a wide range of different functional groups and it is important to target a unique functional group to label only a goal biomolecule. The "bioorthogonal" labelling reactions made it possible to insert functional groups into biomolecules, which afterwards react selectively with a fluorophore or biomolecular label.

Aldehydes and ketones functionalities are attractive functional groups in bioorthogonal labelling due to their small size. These groups can be introduced into a biomolecule through the periodate oxidation of *N*-terminal serine residues,[71] transamination,[72] or carbohydrate oxidation with periodate.[73] Under the mild acidic conditions (pH = 5 ~ 6), carbonyl group reacts with a primary amine to form a reversible Schiff base where equilibrium typically favors the free carbonyl form. Aldehydes and ketones are virtually inert towards other endogenous groups at neutral pH; by condensation with hydrazine and alkoxyamine derivatives they form hydrazine- and oxime-type compounds, respectively (Scheme 2.12).

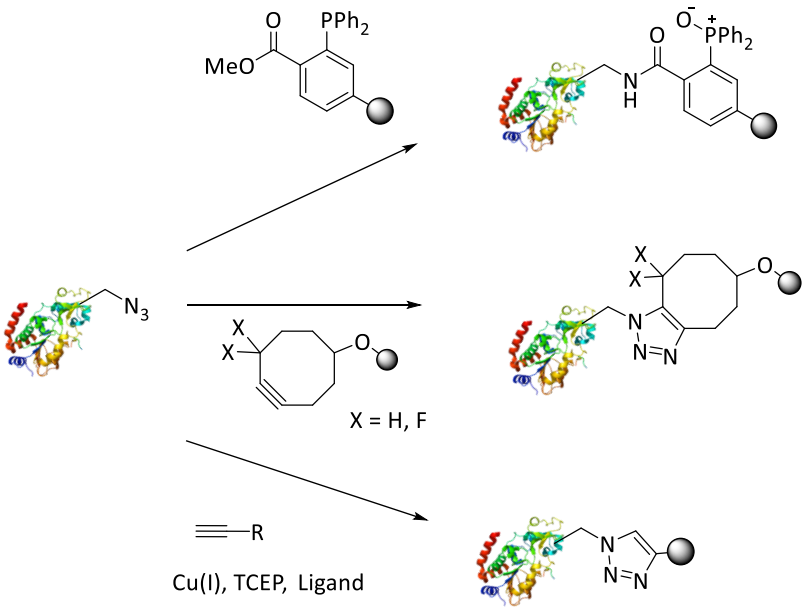


Scheme 2.12: Modification of ketones and aldehydes.

These reactions proceed in mildly acidic condition (pH 6.5) in order to accelerate the dehydration step without full protonation of nucleophilic agents. This method is chemoselective, though the presence of competing ketone and aldehyde metabolites does not allow protein labelling in crude cell lysates.[57]

The azide group does not occur naturally in amino acids and therefore cannot be found in proteins. On the other hand, this group can be incorporated into

biomolecules using different synthetic methods. The azide group is small and therefore has minimal impact on the substrate. Recently azide groups became very popular targets for chemoselective labelling of proteins. Among the bioorthogonal reactions with azides the most widely used are the Staudinger ligation with phosphines, copper(I)-catalyzed cycloadditon with terminal alkynes and strain-promoted cycloaddtion with cyclooctynes.[74] One of the classical applications of azides in bioorthogonal labelling is the Staudinger ligation with triphenylphosphines (Scheme 2.13).[75]

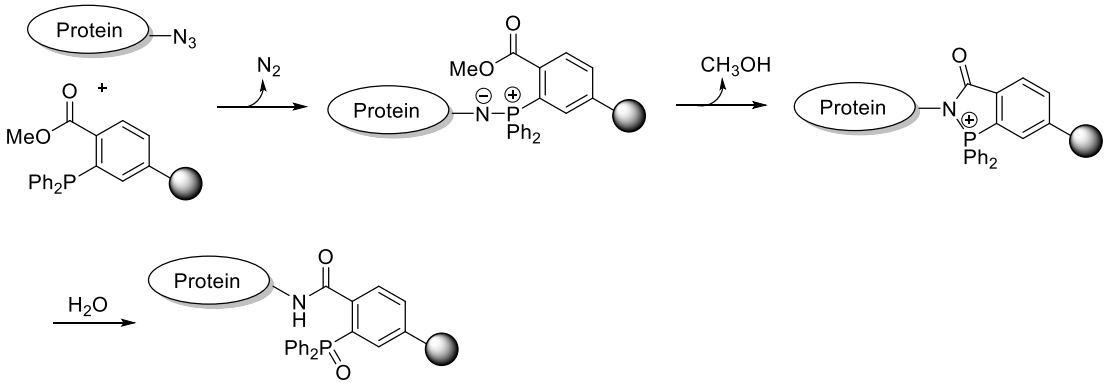


Scheme 2.13: Chemoselective modification strategies targeting non-native functional groups.

The Staudinger-Ligation is a variation of the classical Staudinger-reduction. As shown in Scheme 2.14, the substitution of aryl substituents of the phosphine (1) with ester functionality results in the formation of aza-ylide intermediate (2), which undergoes the intramolecular amineformation (3) releasing the methanol molecule. The subsequent hydrolysis results in formation of the ligation product 4, which contains phosphanoxid unit.

This method found its application in many biological systems. Bertozzi et al. engineered the surface of Jurkat cells with azide functional group, and subsequently rendered the cell surfaces with biotin using Staudinger ligation method.[75] Furthermore, Staudinger ligation has been used for enrichment of glycoprotein in the cell lysates,[76] addition of new functionalities to proteins and biological systems,[77] for attachment of fluorescent marker[78] and detection of active proteasome in living cells.[79] Staudinger ligation is a very good method to modify biomolecules under mild conditions; the reaction proceeds with high chemo-

selectivity. Naturally, it has some disadvantages, like oxidation of phosphine in air or by metaboilic enzymes, which can be avoided by using an excess of phosphine reagent. Another disadvantage is a slow reaction rate. [80]

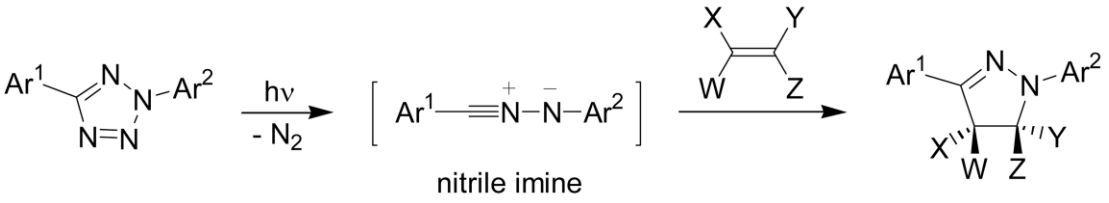


Scheme 2.14: The Staudinger-Ligation.

Another application of azide groups is a [3+2] cyclization reaction with acetylenes to yield triazoles, which was described by Huisgen more than four decades ago.[81] Usually the classical reaction proceeds termally, but the reaction with the terminal alkynes can be accelerated using copper salts (Cu-catalyzed azide-alkyne cycloaddition CuAAC). This CuI-catalyzed azide-alkyne 1,3-dipolar cycloaddition reaction, now known as “click chemistry”, proceeds extremely efficiently under physiological conditions at neutral pH.

Click chemistry found ample application on fast reaction kinetics and tolerance of other functional groups. It has been used in virus surface remodelling,[82] immobilization of nucleic acid and selective protein modification.[83] The main advantage of click chemistry is its fast rate, though application of this method is limited due to cytotoxicity of copper.[56] For this reason several metal-free azidealkyne cycloaddition methods have been developed.[84] Bertozzi *et al.* used the ring strain present in cyclooctyne to accelerate the reaction with azide at room temperature (Scheme 2.14).[56]

About four decades ago, Huisgen and co-workers reported a photoactivated 1,3-dipolar cycloaddition reaction between 2,5-diphenyltetrazole and methyl crotonate. [85] According to the proposed reaction mechanism the diaryltetrazole undergoes a facile cycloreversion reaction upon photoirradiation to release a nitrogen molecule and generate *in situ* a nitrileimine dipole which then cyclizes spontaneously with an alkene dipolarophile to afford a pyrazoline cycloadduct (Scheme 2.15).



Scheme 2.15: Photoinduced cycloaddition reaction.

Further studies showed that this photoinduced cycloaddtion can be applied as well to modify the tetrazole-containg proteins. Because the terminal alkenes do not exist in live systems, this photoclick chemistry has a high degree of bioorthogonality. The main advantage of this method is that it is light induced (allows the control over the reaction initiation) and mild conditions.

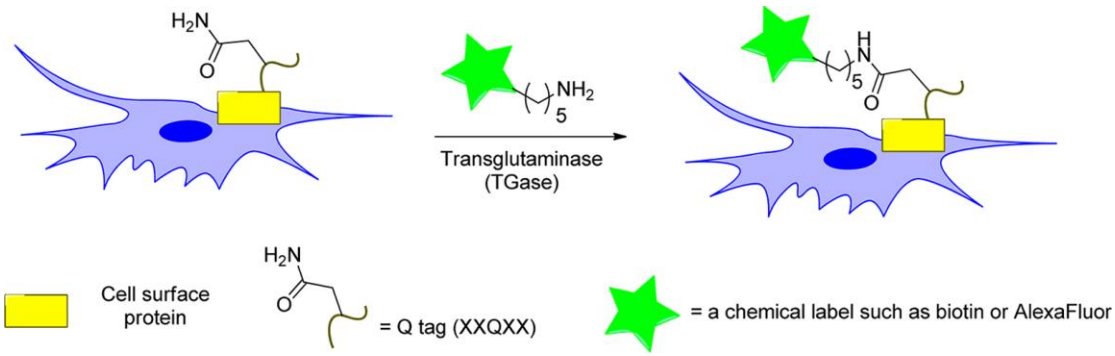
2.6.2 Enzymatic labelling

In comparison with chemical labelling methods, enzymatic labelling has several advantages. They are usually fast, efficient and more selective, rendering them good candidates for application *in vivo* and *in vitro.* Among the disadvantages is their bulkiness or application of a large polypeptide tag, which can result in changing of biological properties of the target molecule. Enzymatic labelling also proceeds with lower yields in comparison to chemical labelling.[74]

In general there are two basic types of enzymatic labelling. In first case, the enzyme acts as a catalyst (for example, Transglutaminase), and in the second case, the enzyme undergoes self-modification before labelling the target protein.

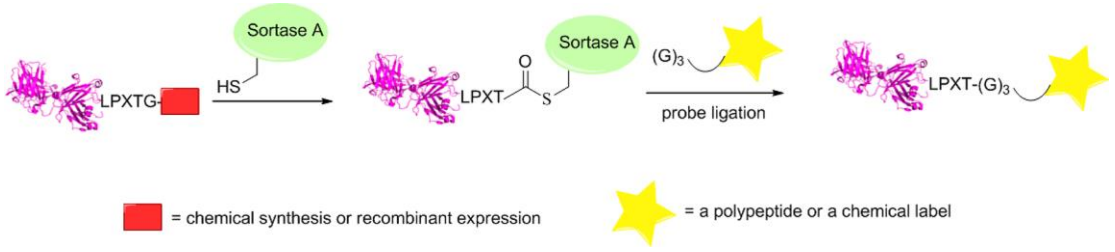
Labelling catalysed by post-translational enzyme modification

Transglutaminases also known as TGases are widely used as catalyst for the fluorescent labelling of cells by forming an isopeptidebond between Gln and Lys residues.[86] During the reaction an acyl group is transferred from γ-carboxamide group of a glutamine residue of a biomolecule to the ε-amino group of a lysine residue, which is connected to the fluorophore. A new ε-(γ-glutamyl)lysine isopeptide connection between the protein and a fluorescent marker is formed (Scheme 2.16).[74]



Scheme 2.16: 1 Enzymatic labelling of protein catalysed by transglutaminase.[87]

Sortase Enzyme with transpeptidase activity is used to label proteins with fluorescent marker connected to an amine group. The main task of sortase is to anchor important cell surface proteins to peptidoglycans as a part of the cell wall of gram-positive bacteria. The enzyme catalyzes hydrolysis of the peptide bond between Thr and Gly and formes consequently a new peptide bond between the carboxyl group of the Thr and amine residue of penta- or tri-glycine (Scheme 2.17). Enzymatic fluorescent labelling using sortase as a catalyst allows site-specific labelling of the biomolecule, though only at the C-terminus of the target molecule.

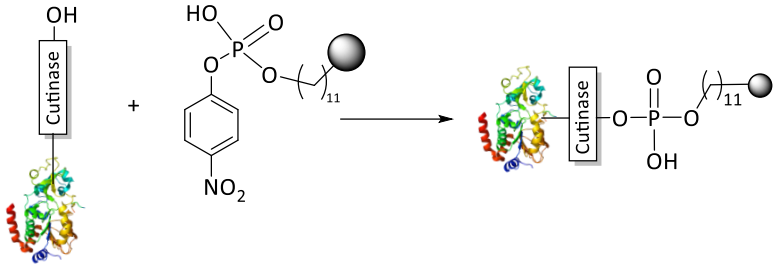


Scheme 2.17: Enzymatic labelling of protein catalysed by sortase.[87]

Labelling with self-modified enzymes.

Cutinase is a serine esterase with the native activity of cutin. During the fluorescent labeling, an alkyl phosphonate, which is connected to a fluorescent marker, forms a covalent adduct with cutinase on conjugation with the Ser residue in the enzyme active site. The mechanism of this process is the following: at the beginning the target molecule is fused to cutinase and afterwards the fused molecule couples with p-nitrophenyl phosphonate, which is connected to a fluorophore (Scheme. 2.18). Enzymatic labelling using cutinase is widely applied for targeting proteins, which are immobilized on a self-assembled monolayer.[88] This method also has some drawbacks, the first of which is the large size of cutinase itself, which limits the labelling of small proteins. Also there is a possibility that after

cutinase is fused with the biomolecule it may change the biological activity of the target protein.[74]



Scheme 2.18: Labelling with self-modified enzymes.

Inteins are segment of a protein that are expressed in frame with flanking *N*- and *C*-terminal polypeptides (exteins) and are engineered to carry out selective labeling in the protein segments (Scheme 2.11). Intein is able to join the remaining portions (the exteins) with a peptide bond during the process known as protein splicing.[89] The mechanism of this native chemical ligation was described before (See Chap. 2.6.1). The main advantage of this process is the site specificity and the lack of non-specific labelling, though it cannot be used to label the loops inside the protein.[74]

2.7 Pd-catalyzed cross-coupling reactions

The 2010 Nobel Prize in Chemistry to Richard Heck, Ei-ichi Negishi[90] and Akira Suzuki[91] was a significant event in the world of chemistry. Their discoveries started a new chaper in the field of palladium-catalyzed cross-coupling reactions. Nowadays palladium-catalyzed cross-coupling reactions are still attractive in different fields of research both in academic research and in industry.

The general mechanism is shown in Figure 2.20.[92] The first step – oxidative addition of the aryl halide (or pseudohalide) to the catalytically active L_nPd^0 species – is the same in case of both coupling reactions. In case of Mizoroki-Heck coupling, the reaction proceeds by coordination of an alkene to the Pd^{II} and consequent *syn* migratory insertion. The regioselectivity depends usually on the nature of the alkene, the catalyst, and the reaction conditions. The formation of the alkene product occurs through *syn*-β-hydride elimination; L_nPd^0 catalyst is regenerated by subsequent base-assisted elimination. In the Negishi and Suzuki-Miyaura reactions (as well as related Corriu-Kumada, Stille, and Hiyama couplings) after oxidative addition occurs, transmetalation of an organometallic species and Pd^{II} intermediate with two organic coupling partner fragments is generated. During the following reductive elimination a new C-C bond is formed and Pd-catalyst is regenerated.[93]

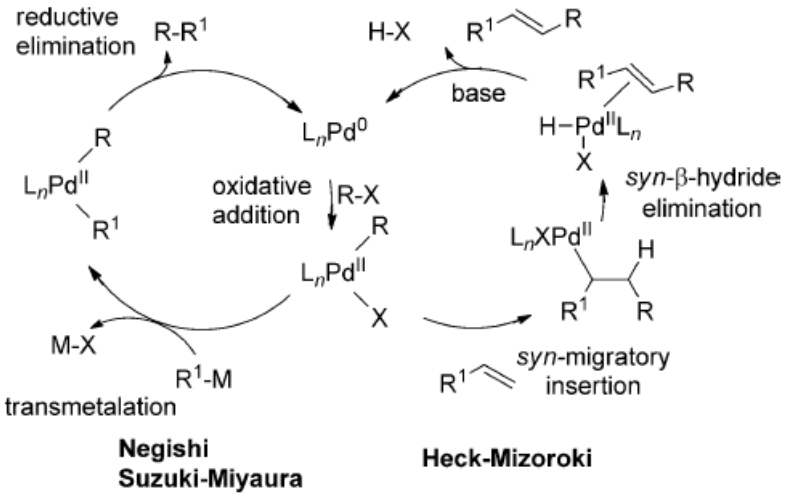


Figure 2.20: General mechanism for Palladium-catalyzed cross-coupling reactions.

The interest towards these reactions is incredible. As a result many Pd-catalyzed reactions have been developed which require milder conditions and lower amount of calatyst, using more efficient catalytic systems, as well as different ligands. Today palladium-catalyzed reactions play a major role in modern organic synthesis. The idea of applying this type of reactions on the living systems as well is very fascinating, though Pd-catalyzed modifications on proteins were not widely spread. The success of CuAAC reactions on protein stimulated the use of the Pd-chemistry, as well. [94]

Pd-catalyzed protein biorthogonal labelling

The application of palladium-catalysts in investigations of biological processes has seen fast growth in the past few years. The first reactions suffered from harsch conditions, low yields, and lack of bioorthogonality. In recent years great progress has been made in this field. In 2007, Yokoyama *et al*. used for the first time Pd(OAc)2-triphenylphosphine-3,3',3''-trisulfonate complex for Sonogashira coupling reaction between the iodophenylalanine-encoded Ras protein and propargylic biotin to yield the desired product with 25% yield.[95] Francis and co-workers managed to label a tyrosine residue using the π-allyl palladium complex, though with only 30% yield. [96] Schultz *et al*. were the first to perform the Suzuki cross-coupling on biosynthetic proteins.[97] The biggest disadvantage of this reaction were high temperature (70 °C) and low yield (around 30%), limitating its wide application.

With the discovery of an effective ADHP (2-amino-4,6-dihydroxypyrimidine, L2)-based catalyst system $[Pd(OAc)_2(L2)_2]$ full conversion has been achieved between an aryl iodide group incorporated into the protein with different aryl-/vinyl-boronic

acids.[98] Chen *et al*. reported a ligand free Sonogashira-coupling reaction for fluorescent labelling of protein. They used $Pd(NO_3)_2$ which was able to catalyse the cross-coupling between alkyne-encoded GFP and rhodamine-conjugated phenyl iodide.[94] Due to high efficiency, chemoselectivity and biocompatibility, the cross-couplings found broad application in protein modifications and labelling. Though only few of them have so far proved to work under living conditions.

Davis *et al*. used Pd-cross coupling reactions in conjugation with "tag and modify" stragedy to label an outer membrane of protein. By metabolic and genetic incorporation they obtain bacterial strain with four "tags" with good accessibility. They managed to label the bacteria with boronic acid-functionalized fluorescein (BAF) *via* Suzuki-Miyaura cross-coupling reaction (Figure 2.21).[99]

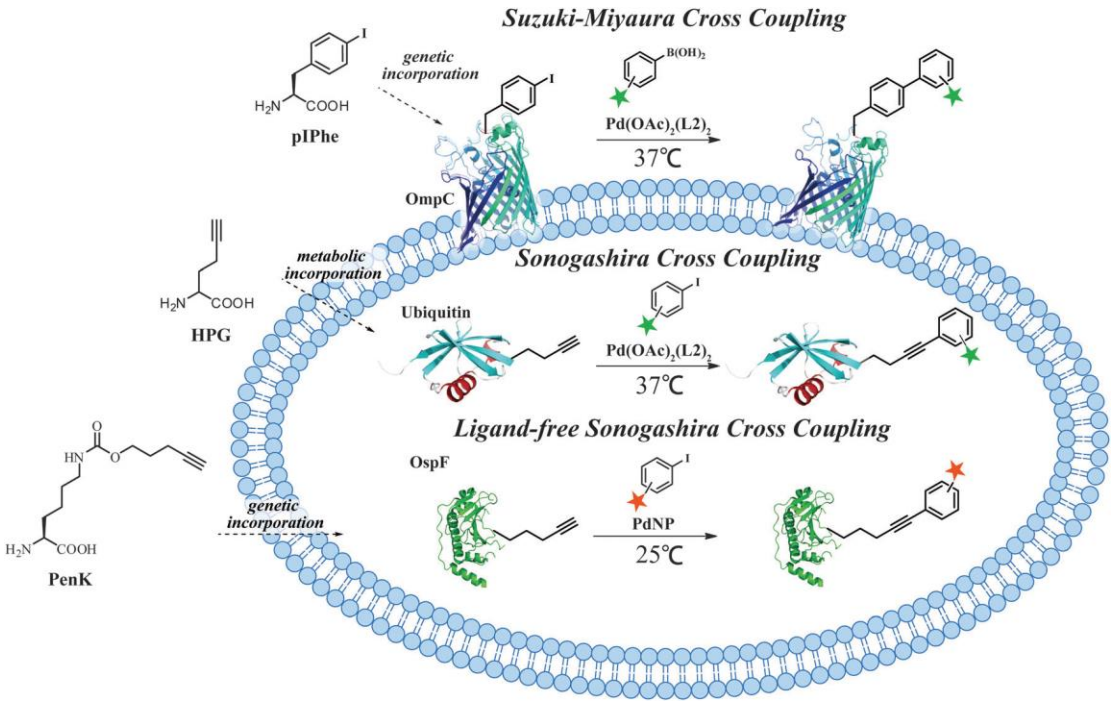


Figure 2.21: Palladium-catalyzed cross-coupling labelling of living Gram-negative bacterial cells.[81]

Good results promoted further investigations and optimizations of these reactions. Lin *et al.* developed water-soluble $[Pd(OAc)_2(L3)_2]$ complex to catalyse the copper-free Sonogashira coupling reactions on the living cell (Figure 2.21). All these results clearly show that cross-coupling reactions can be readily used on the living cells, since they require mild conditions, are biorthogonal and give the desired transformations in high yields. The main advantages of these methods are (a) high tolerance of the functional groups, (b) less toxicity, (c) chemical bioorthogonality, (d) air- and moisture-stable ligands allow to conduct reactions without inert atmosphere and water-free solvents, (e) irreversibility of the reactions.

2.8 Pd-catalyzed allylic alkylation reactions

Besides the known labelling methods of *N*- and *C*-termini which were discussed above, in the group of Prof. Kazmaier[19] a labelling method of the peptide backbone through Palladium-catalyzed allylic alkylation reaction was developed.

For many years the group of Prof. Kazmaier has been concentrated on the stereoselective synthesis of amino acids. The main target are chelated ester enolates of amino acids that can be used as highly reactive nucleophiles in C-C-bond formation reactions like esterenolate Claisen-Rearrangement, Aldol reaction or Michael addition, as well as Palladium-catalyzed allylic alkylation reaction, which gives the γ,δ-unsaturated amino acids with high regio- and stereoselectivity.

The biggest advantage of these reactions is the formation of C-C, C-O, C-N and C-S-bonds, as well as mild conditions and tolerance of many functional groups. Among them is the Palladium-catalyzed allylic alkylation reaction or the Tsuji-Trost reaction.[100,101] The Tsuji-Trost reaction involves an allylic substrate with a leaving group. The mechanism of the reaction is as follows[102]: in the first step the double bond of the allylic substrate coordinates on Pd(0)-complex forming a η^2-Olefin-Palladium complex **A**. The further oxidadive addition of Palladium to the allylic C-X bond (X = carboxylate, carbonate, halogenid, phosphate, etc.) results in formation of η^3-Allyl-Palladium(II)-complex **B**, which is usually neutral. In the next step through the exchange of anionic ligands X with the neutral ligands L (for instance, phosphine), the positively charged complex **C** (η^3- complex) is formed, which due to its electrophilic nature can react with different nucleophiles. As a result of reductive elimination η^2-Olefin-Palladium complex **D** is built again. After its dissociation Palladium-catalyst is regenerated.

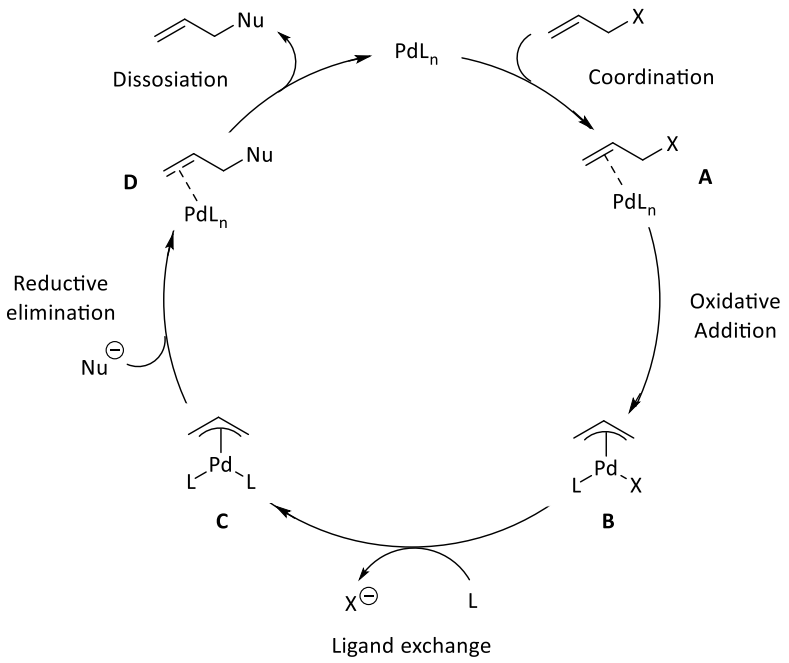
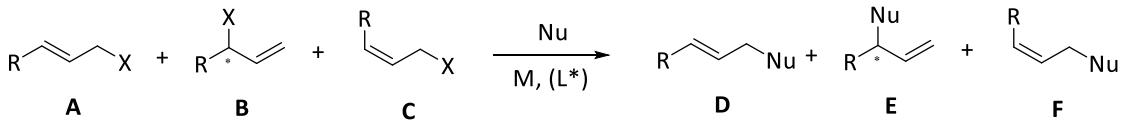


Figure 2.22: Mechanism of Palladium-catalyzed allylic substitution.

Regioselectivity in allylic substitution

Monosubstituted allyl substrates **A**, **B** and **C** are the most interesting substrates for allylic substitution (Scheme 2.19). In the course of the substitution they give rise to following regioisomers **D**, **E** and **F**. The control of the regioselectivity in these types of reactions is very important. In the formation of the regioisomer **E** as well as enantioselectivity should be considered.



Scheme 2.19: Transition metal-catalyzed allylation with monosubstituted substrates.

There are different ways of controlling the regioselectivity of allylic substitution. The most important is the choice of transition metal catalyst. Furthermore, electronic and/or steric properties of the transition metal can be controlled by application of certain ligands or additives.

Generally three different possibilities can be observed in regioselectivity:

1. Formation of linear products is favoured: Pd,[103] (Ru,[104] Fe).[105]

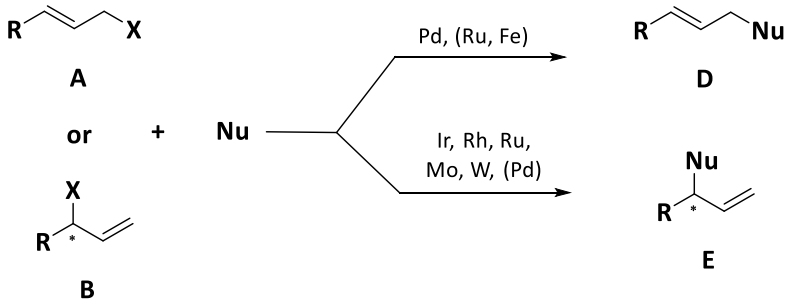
Linear and branched allyl substrates, **A** and **B**, give rise to a linear product **D** (Scheme 2.20);

2. Formation of branched products is favoured: Ir,[106] Rh,[107] Ru,[108] Mo,[109] W,[110] (Pd).[111]

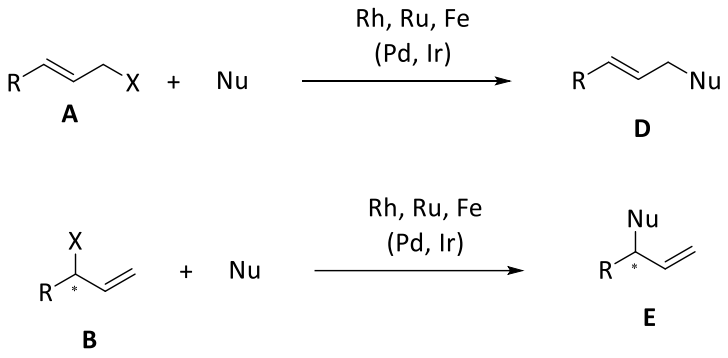
   In course of a reaction with linear and branched allyl substrates, **A** and **B**, the branched product **E** is formed preferably (Scheme 2.20);

3. Regioretention (regiochemical Memory-Effect): Rh,[112] Ru,[113] Fe,[114] (Pd,[115] Ir).[116]

   The nucleophile attacks from the side of the leaving group. Linear substrate **A** gives the linear product **D** and branched substrate **B** results in formation of the branched product **E** (Scheme 2.21).[117]



Scheme 2.20: Regioselectivity in transition metal-catalyzed allylic substitutions.[117]



Scheme 2.21: Regioretention in transition metal-catalyzed allylic substitutions.[117]

The backbone labelling *via* palladium-catalyzed allylic alkylation.

In the work of L. Wirtz[19] a new method for fluorescence labeling of amino acids and peptides was developed. This labeling technique of the peptide backbone is based on the palladium-catalyzed allylic substitution reaction. Different fluorescent labels with allylcarbonate side chain based on 7- aminocoumarin (Figure 2.23) were synthesized using Titan-catalyzed Pechmann condensation reaction.

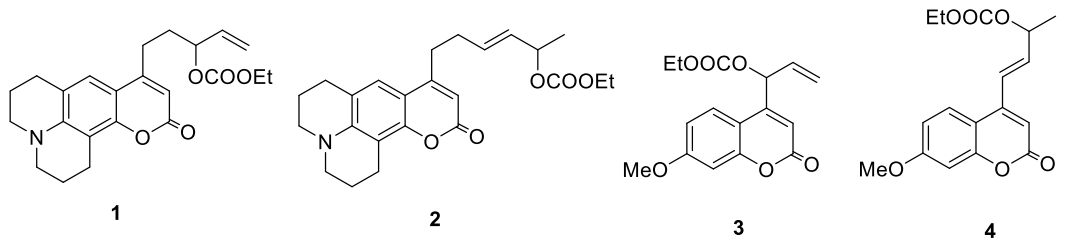
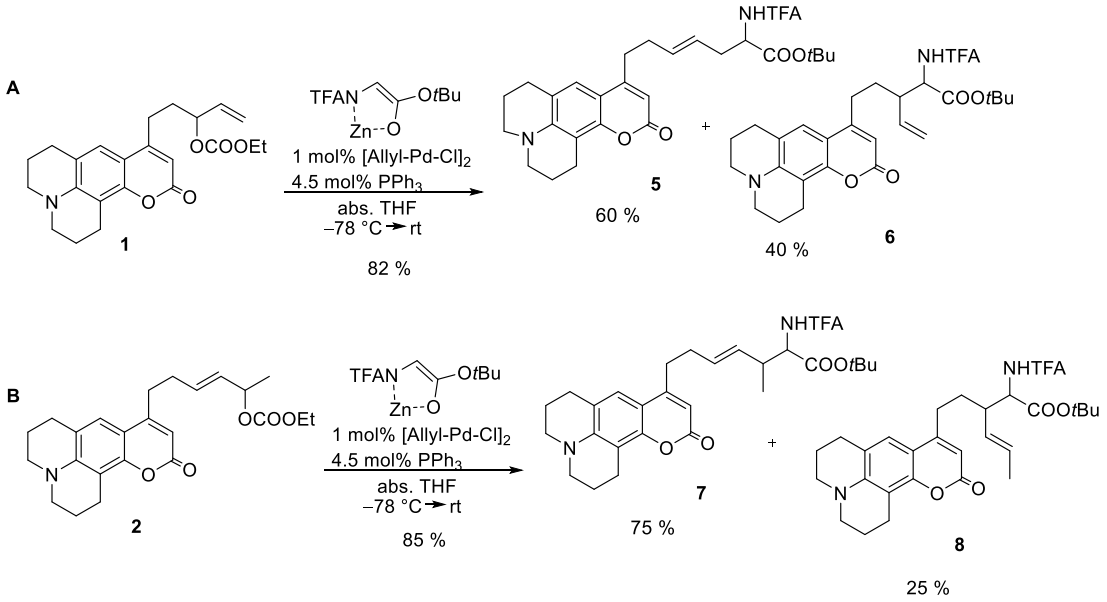
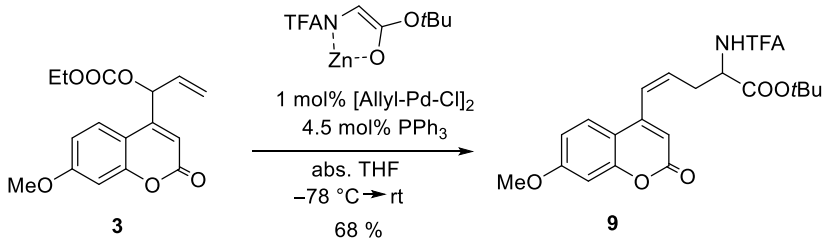


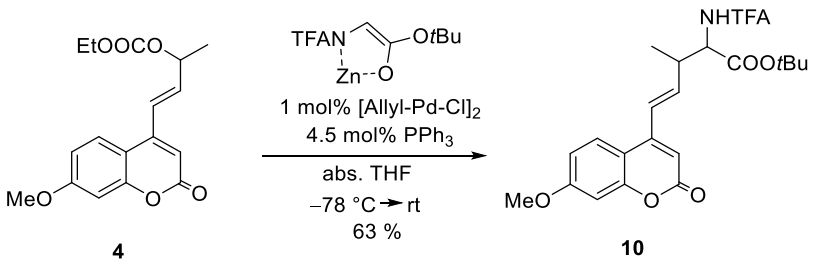
Figure 2.23: Coumarin-based fluorescent labels for allylic alkylation reactions.

The synthesized allyl carbonate labels were applied for fluorescence labeling of TFA-glycine-*tert*-butyl ester. The palladium-catalyzed allylic alkylation proceeded in moderate to good yields. The allylation with 7-aminocoumarin labels **1** and **2** resulted in the formation of two regioisomers **5, 6**, **7** and **8** (Scheme 2.22), while while 7-methoxycoumarin labels **3** and **4** gave rise to only one regiosomer **9** and **10**, respectively (Scheme 2.23).



Scheme 2.22: Backbone labelling through palladium-catalyzed allylic alkylation with fluorescent dyes **1** and **2.**





Scheme 2.23: Backbone labelling through palladium-catalyzed allylic alkylation with fluorescent dyes **3** and **4.**

3

Results and Discussion

3.1 The aims of the thesis

In recent years labelling reagents (dyes and fluorophores) became an essential component for the detection of biomolecules. With the development of fluorescent spectroscopy, fluorescent labelling has been employed in a variety of areas like environmental analysis, disease diagnostics, pharmaceutical screening, proteomic and genomic studies.[49]

Green fluorescent protein (GFP) was the first fluorescent dye which was genetically introduced into the target protein. The main disadvantages of GFP like its size and limited windows of fluorescent spectra do not allow its wide application in fluorescent labelling techniques. Contrariwise, synthetical dyes are smaller in size, do not usually have any effect on the target compound, often possess improved photochemical properties, as well as offer a wide range of colours. For this reason a

lot of investigations have been done for the development of new fluorescent dyes (Chap. 2.3) and the synthetic work is still ongoing. Coumarins with an electron-donating subtituent at 7-position are characterized by strong fluorescence. For instance, 7-aminocoumarins reveal strong fluorescence, low pH-dependence, they are active at a wide pH range, as well as exhibit high fluorescence intensity and excellent fluorescent quantum yield.[118] 7-dialkylaminocoumarins and derivatives were synthesized in the group of Prof. Kazmaier with good yield and were further used for bioorthogonal labelling of amino acids and peptides (Chap. 2.8).[19]

Encouraged by previous results, we decided to continue investigations in this area. Therefore the aims of the presented thesis were:

1. Synthesis of further 7-dialkylaminocoumarin and its derivatives;
2. Study their application in bioorthogonal labelling of biomolecules.

The possible sites for the bioorthogonal labelling are *N*-/*C*-termini, as well as the backbone of the peptide (Figure 3.1). The methods chosen for the studies were:

1. C-C cross-coupling reactions, for instance Sonogashira, Suzuki-Miyaura, Stille couplings.[152]
2. Buchwald-Hartwig C-N coupling under CO-insertion
3. Michael addition with different nucleophiles, amines and thiols, as well as C-nucleophiles.
4. Pd-catalyzed allylic alkylation.[19]

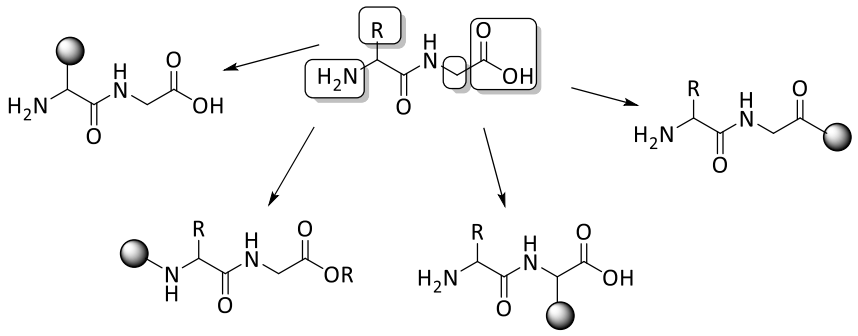
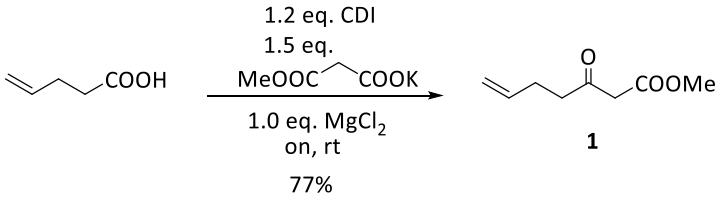


Figure 3.1: Possible sites of the protein for fluorescent labelling.

3.2 Synthesis of 7-dialkylaminocoumarin

Among the different methodologies developed for the synthesis of coumarins (Chap. 2.3), the most important and popular is the Pechmann reaction,[119] which is based on condensation of phenols with malonates or β-oxo esters in the presence of strong acids, such as H_2SO_4. Though application of strong acids in the synthesis of aminocoumarins is limited because of protonation of the amino group and therefore deactivation of the aromatic system.[33]

For the development of new aminocoumarins the work started with the synthesis of β-ketoester, which was performed according to the method developed by Hashiguchi (Scheme 3.1).[120] 4-pentenoic acid was activated with cabonyldiimidazole (CDI) to form a mixed anhydride. The attack of the released imidazole on the mixed anhydride results in the formation of an imidazolide, which was converted by action of a magnesium chelated monomethylmalonate and subsequent elimination of CO_2 to the β-keto ester **1**.



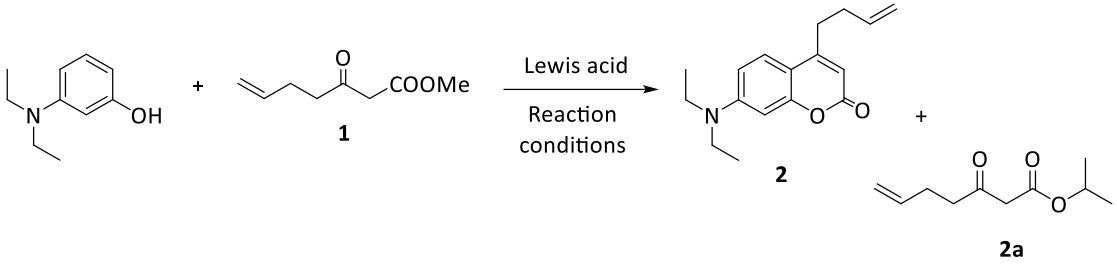
Scheme 3.1: Syntheis of β-keto ester from 4-pentenoic acid according to Hashiguchi.[120]

For the synthesis of the aminocoumarin scaffold the Pechmann-condensation was applied and different reaction conditions were screened. S. Frère *et al* reported that the synthesis of aminocoumarin using concentrated sulfuric acid as a catalyst does not proceed smoothly.[121] As it was expected no product could be isolated in the first experiment (table 1, entry 1). Therefore, milder conditions were chosen and instead of strong Broensted acids, Lewis acids were employed as catalyst. The reaction was allowed to reflux in toluene overnight in the presence of 5 mol% Bismuthchloride[122] (Entry 2), though only traces of the fluorescent product could be observed. Kazmaier *et al*.[33] could isolate 7-dialkylaminocoumarins in almost 80% yield with 2 eq. of $Ti(O\textit{i}Pr)_3Cl$. Under these conditions the target product was isolated only in 25% yield and a significant side reaction was observed – the transesterification of the keto-ester. Titanalkoxides are widely known as good catalyst for transesterification reactions.[123] In synthesis of 7-diethylaminocoumarin derivative J. Burkhart[157] observed as well the formation of the side product. Furthermore the condensation was performed at room temperature and at 60 °C (entries 4 and 5) but in none of the cases the formation of the target compound was

observed. The ammonium metavanadate[124] catalysed reaction yielded only traces of the desired product (entry 6 and 7). Also Ti(O*i*Pr)$_4$ as catalyst did not give any satisfying results (entry 8).

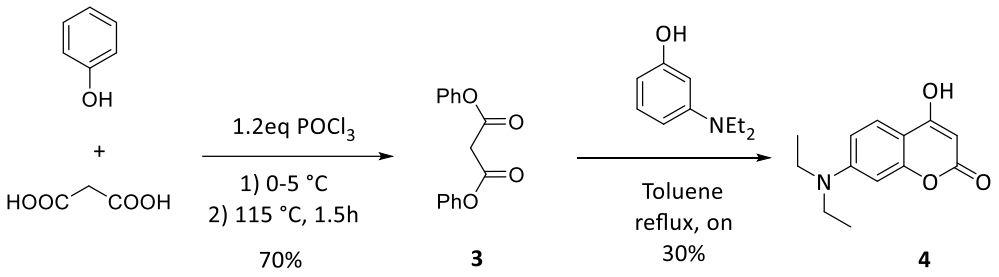
Microwave accelarated condensations (30 min, 90 W, 130 °C) with 75 wt.-% montmorillonite and 4-5 drop conc. H_2SO_4, respectively (entries 9 and 10) did not afford the target coumarin **2**. Ti(O*i*Pr)$_3$Cl (entry 11) yielded **2a** as a main product (65% yield) and only 15% of **2** could be isolated. Bismuth chloride afforded only traces of the desired compound.

Table 3.1 Synthesis of 7-diethylaminocoumarin *via* Pechmann-condenstion reaction:



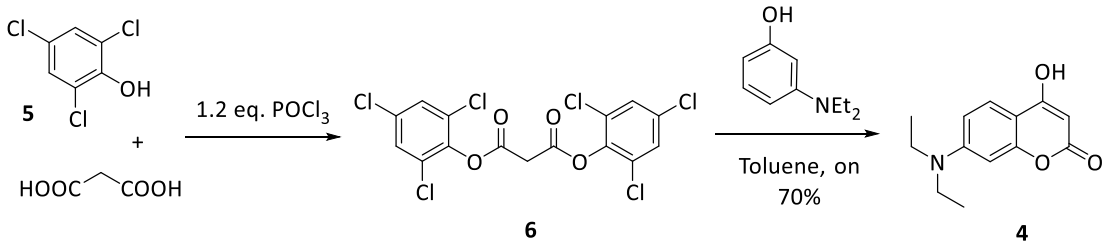
	Catalyst	Conditions	Yield [%]
1	1.0 eq. conc. H_2SO_4	1 h, 75 °C	---
2	5 mol-%. $BiCl_3$	on, 130 °C, toluene	traces
3	2.0 eq. Ti(O*i*Pr)$_3$ Cl	on, reflux, toluene	25 (2)/ 65 (2a)
4	2.0 eq. Ti(O*i*Pr)$_3$ Cl	on, rt, toluene	---
5	2.0 eq. Ti(O*i*Pr)$_3$ Cl	on, 60 °C, toluene	---
6	10mol% NH_4VO_3	on, 60 °C, toluene	traces
7	10mol% NH_4VO_3	on, reflux, toluene	traces
8	1.0 eq. Ti(O*i*Pr)$_4$	on, reflux, toluene	---
9	75 wt.-% Montmorillonite	30 min, 90 W, 130 °C	---
10	4-5 drop conc. H_2SO_4	30 min, 90 W, 130 °C	---
11	2.0 eq. Ti(O*i*Pr)$_3$Cl	30 min, 90 W, 130 °C	25 (2)/ 65 (2a)
12	10 mol-%. $BiCl_3$	30 min, 90 W, 110 °C	traces

Since all the methods applied could not provide a preparative ammount of the coumarin **2** for further modifications, 4-hydroxy-7-diethylaminocoumarin **4** was prepared according to a reported procedure.[125] As shown in scheme 3.2 malonic acid was converted to the activated bisphenylester **3**, which was subsequently condensed with commercially available 3-diethylaminophenol. Unfortunaly only 30% of the target molecule could be isolated probably due to an intramolecular condensation as a side reaction.



Scheme 3.2: Synthsis of 4-hydroxycoumarin **4.**

In order to avoid the formation of this side product trisubstituted phenol **5** was used to form activated bis(2,4,6-trichlorophenyl) ester **5**, which after condensation and purification furnished the target compound **4** in 70% yield. Analytically pure compound was applied directly for further modifications.



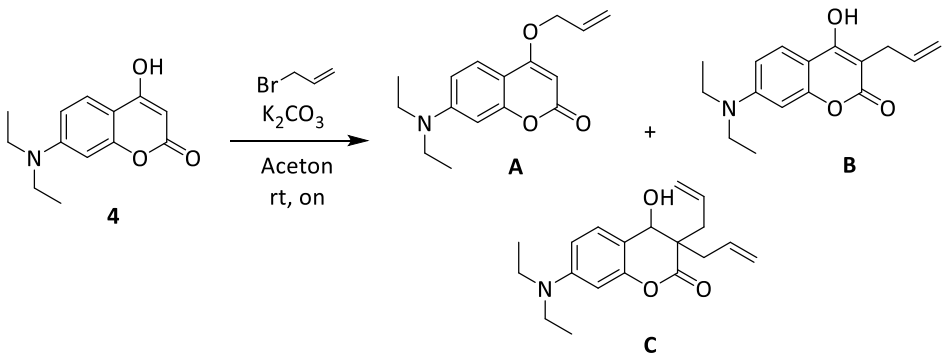
Scheme 3.3: Syntheis of 4-hydroxycoumarin **4.**

3.3 Modifications of 4-hydroxy-7-diethylaminocoumarin

As it was discussed in the introduction (Chap. 2.6.1) for the design and synthesis of fluorophores it is important to include a functional group which is capable of creating selectively a covalent bond with a target molecule. For further modifications of 4-hydroxy-7-diethylaminocoumarin **4,** it was subjected to allylation reactions and to studies towards the development of a methodology for selective *O*-allylation, as well as *C*-allylation in 3-position.

In the first test reaction a protocol described for 4-hydroxycoumarin[126] (Table 3.2, entry 1) was applied. Due to high C-H acidity of position 3 only 34% of the *O*-allylated compound were obtained and traces of the mono *C*-allylated product. As a main product *C*-diallylated coumarin was isolated. In THF as solvent the formation of the same compounds (entry 2) was observed. NaH as a base provided a mixture of all three products (entry 3).

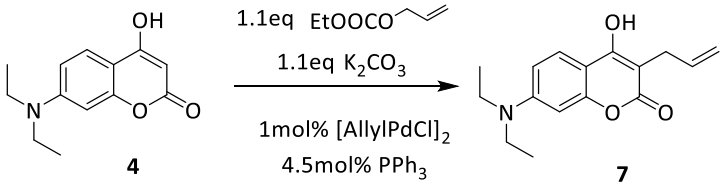
Table 3.2 Allylation of aminocoumarin **4**:



	Base	Solvent	Yield Product A [%]	Yield Product B [%]	Yield Product C [%]
1	K_2CO_3	Acetone	34	traces	56
2	K_2CO_3	THF	15	traces	70
3	NaH	DMF	Mixture of all three products (TLC control)		

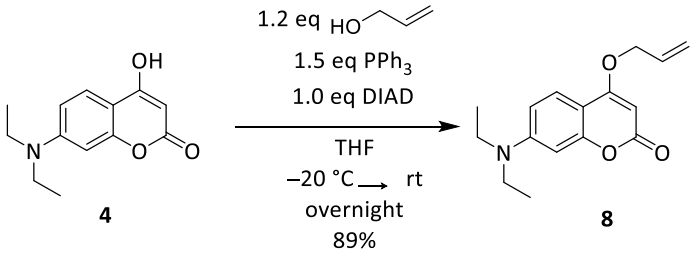
Since the conditions employed above could not provide selective allylation, palladium-catalalyzed alkylations were applied, using allylpalladium chloride dimer in combination with triphenyphosphine as a ligand (Table 3.3). The test experiments were performed under microwave irradiation. Reactions carried out in THF as a solvent, at room temperature and 60 °C, respectively, yielded only traces of *C*-monoallylated product (entries 1 and 2). Dimethylformamide at 80 °C gave similar results (entry 3). The addition of K_2CO_3 as a base for deprotonation changed the reaction course drastically, yielding 84% of the target molecule **7**. The same reaction was upscaled and performed overnight at 90 °C furnishing the *C*-monoallylated product in 87% yield.

Table 3.3 Palladium-catalalyzed alkylation of **4**:



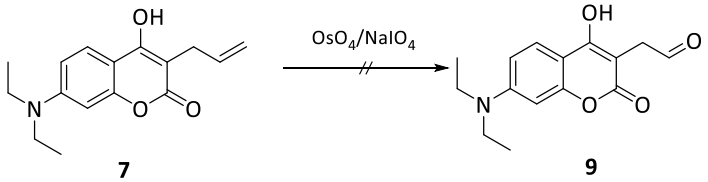
	Base	Solvent	Conditions	Product	Yield
1	-----	THF	25 °C, 90W, 15 min	C-monoallylated product + Starting material	Traces
2	-----	THF	60 °C, 90W, 30 min	C-monoallylated product + Starting material	Traces
3	-----	DMF	80 °C, 90W, 30 min	C-monoallylated product + Starting material	Traces
4	K_2CO_3	DMF	90 °C, 90W, 30 min	C-monoallylated product	84%
5	K_2CO_3	DMF	90 °C, overnight	C-monoallylated product	87%

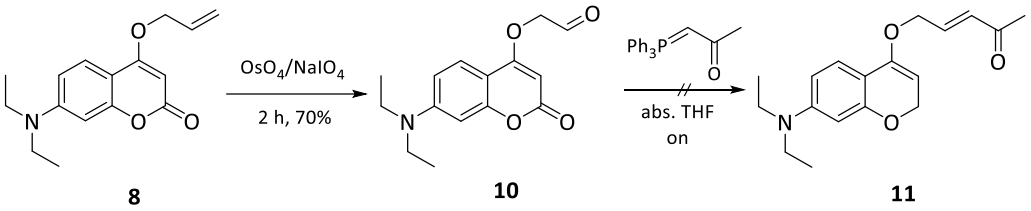
O-allylations with high selectivity under Mitsunobu conditions using PPh_3 in combination with either diethyl azodicarboxylate (DEAD) or 1,1'-(azodicarbonyl)dipiperidine (ADDP) were reported by Jacobsen *et al.* [127] Following this methodology, it was possible to isolate the target *O*-allylated molecule **8** in 89% yield (Scheme 3.4).



Scheme 3.4: *O*-allylation under Mitsunobu conditions.

Unfortunately, further attempts of oxidative cleavage of the allylic double bond to yield the corresponding aldehydes did not afford the desired compounds (Scheme 3.5). No formation of product **9** was observed, and compound **10** decomposed the following day.



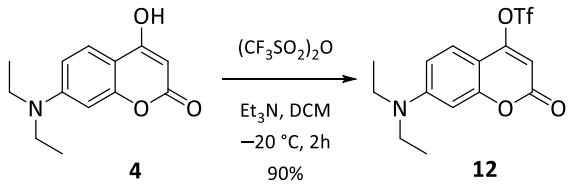


Scheme 3.5: Studies towards the oxidative cleavage of the allylic double bonds of compounds **7** and **8**.

3.4 Pd-catalyzed cross-coupling reactions

In modern synthetic chemistry the application of transition metall-catalyzed-coupling reactions has grown incredibly in the past few years.[90,91,128] During the last two decades they have been also widely used for bioorthogonal labelling of biomolecules.[99] These reactions usually proceed under mild conditions and tolerate many functional groups (Chap. 2.7).

In order to incorporate such a functional group which would be appropriate for different types of cross-coupling reactions, 4-hydroxycoumarin **4** was transferred into a triflat according to a reported methodology[129] using trifluoromethane-sulfonic anhydride (Scheme 3.6), which gave the functionalyzed coumarin **12** in 90% yield. It should be mentioned, that the yield depends highly on the quality of anhydride. Only newly purchased reagent provided high yields.



Scheme 3.6: Synthesis of 7-diethylamino-4- trifluoromethylsulfonyloxy-coumarin **12.**

Vinyl triflate **12** was employed in different cross-couplings as possible labelling methods of amino acids and peptides. In Figure 3.2 possible residues for cross-coupling reactions are depicted.

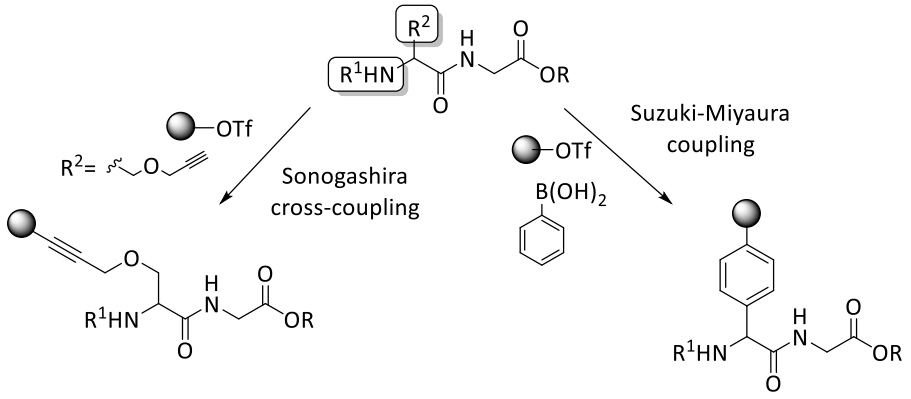


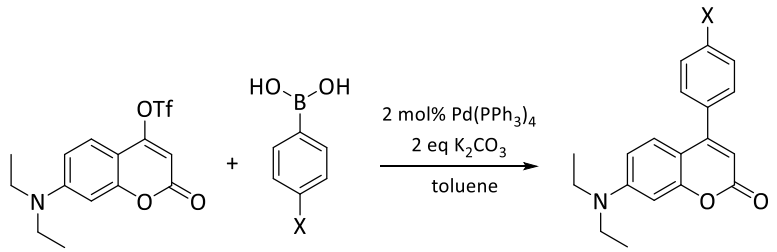
Figure 3.2: Possible sites for Pd-catalyzed cross-coupling labellings.

3.4.1 Suzuki-Miyaura coupling

Among naturally occurring coumarins, 4-arylcoumarins (neoflavones) are an important class of flavonoids, which possess a wide range of pharmacological activities[130], like anticancer,[131] antimalarial,[132] antibacterial[133] and cytotoxic activities.[134] Classically, these compounds are synthesized by condensation of phenols with carbonyl compounds. Arylations of coumarin using Suzuki-Miyaura coupling have been recently reported.[135] In a one-pot reaction of 4-trifluoromethyl-sulfonyloxy-6-bromocoumarin with various arylboronic acids 4-arylcoumarins could be isolated in high yields.[129] Davis *et al*. reported about the labelling of bacteria with boronic acid-functionalized fluorescein (BAF) *via* a Suzuki-Miyaura cross-coupling reaction (Figure 2.21).[99] Since boronic acid residue can also be incorporated into biomolecule,[136] the Suzuki-Miyaura coupling was applied as one of the labelling methods.

The reactions were performed in toluene at reflux overnight using K_2CO_3 as a base and tetrakis(triphenylphosphine)palladium(0) catalyst. Different boronic acids were screened. The results are summarized in the table 3.4. The yields in all cases were close to quantitative. The low yield in case of (2,4,6-trimethylpyridin-3-yl)boronic acid can be explained by its bulkiness.

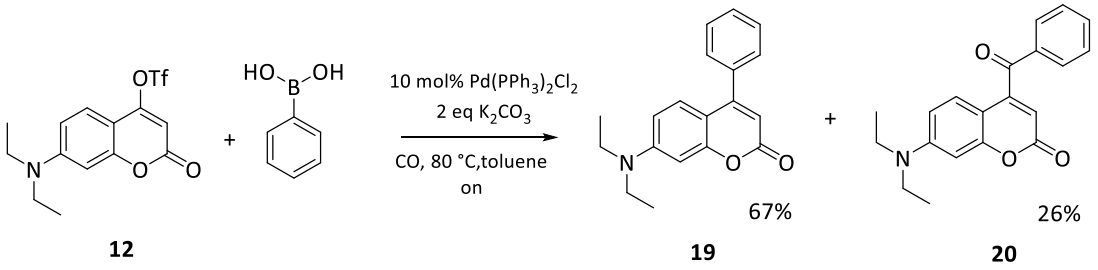
Table 3.4 Suzuki-Miyaura reactions with fluorescent marker **12**:



12 **13-19**

	Boronic acid	Product	Yield [%]
1	3-nitrophenylboronic acid	**13**	89%
2	p-tolylboronic acid	**14**	93%
3	(2,4,6-trimethylpyridin-3-yl)boronic acid	**15**	40%
4	(4-chlorophenyl)boronic acid	**16**	94%
5	(4-fluorophenyl)boronic acid	**17**	89%
6	4-methoxyphenylboronic acid	**18**	95%
7	phenylboronic acid	**19**	quant.

The Suzuki-Miyaura coupling was performed as well under CO atmosphere (15 bar), what afforded only 26% of the target molecule **20**. As a main product was isolated the direct coupling compound **19**.

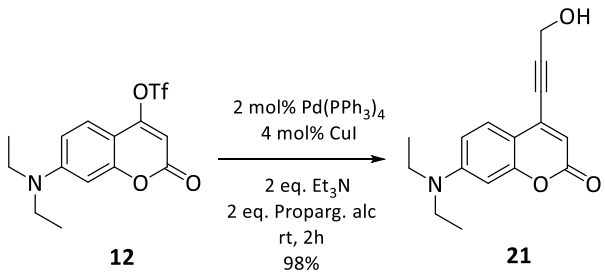


12 **19** **20**

Scheme 3.7: Suzuki-Miyaura coupling with CO-insertion.

3.4.2 Sonogashira coupling

Another transition metal-catalyzed cross-coupling, which became a powerful tool in organic synthesis and has been used as a new labelling method of biomolecules, is the Sonogashira coupling reaction (Chap. 2.7). A test reaction was performed with propargylic alcohol under standard conditions reported.[137] The coupling proceeded smoothly affording strong fluorescenting alcohol **21** in almost quantitative yield (Scheme 3.8).

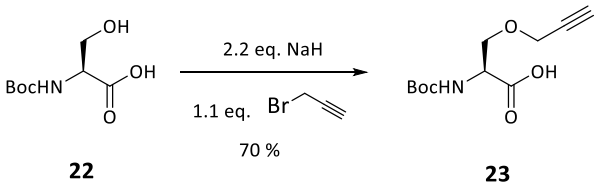


Scheme 3.8: Sonogashira coupling reaction with propargylic alcohol.

Encouraged by this result it was decided to apply this methodology for further direct labelling of several amino acids, as well as peptides. For this reason it was necessary to modify the side chain of amino acids by incorporation of a propargylic residue.

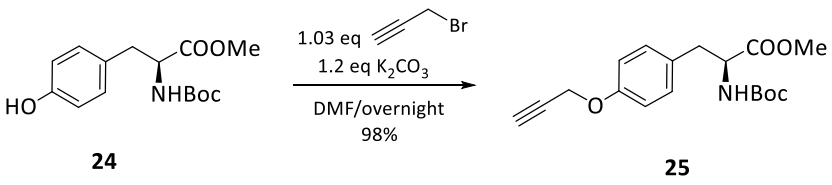
Synthesis and fluorescent labelling of amino acids and peptides with propargylic residue

Serine and tyrosine amino acids that are famous targets for bioorthogonal labelling (Chap. 2.6.1) were chosen for further modifications, which were performed according to the scheme 3.9. The desired propargylic ether of serine **23** could be isolated in 70% yield.



Scheme 3.9: Propargylation of Boc-serine.

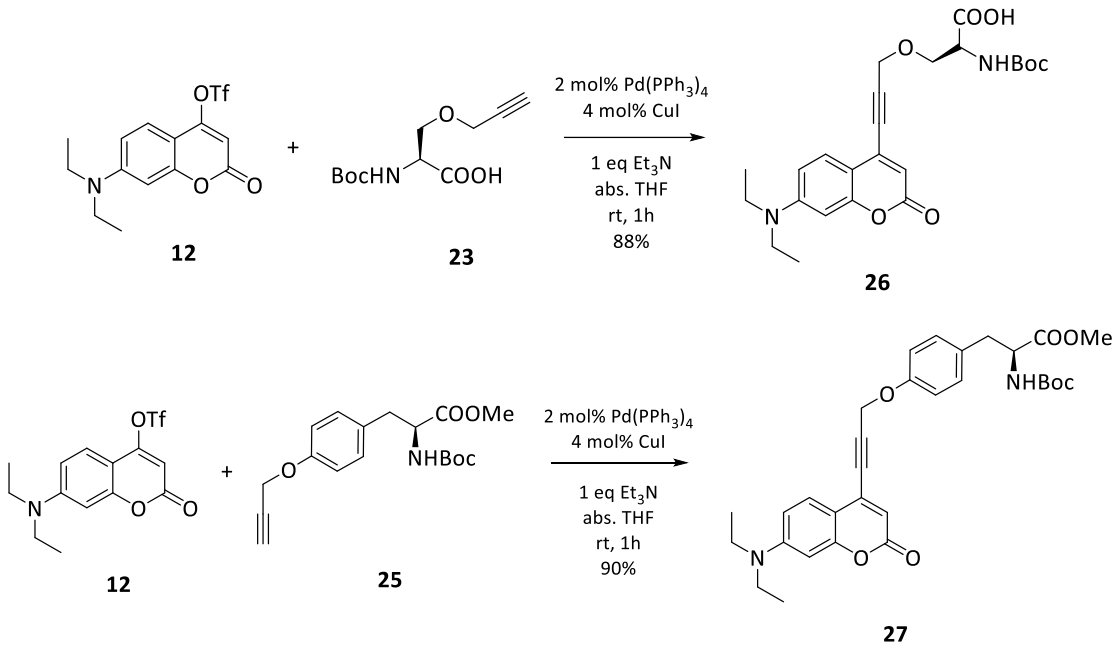
The propargylation of Boc-protected tyrosineester **24** furnished the desired ether **25** in 98% yield (Scheme 3.10):



Scheme 3.10: Propargylation of Boc-tyrosine.

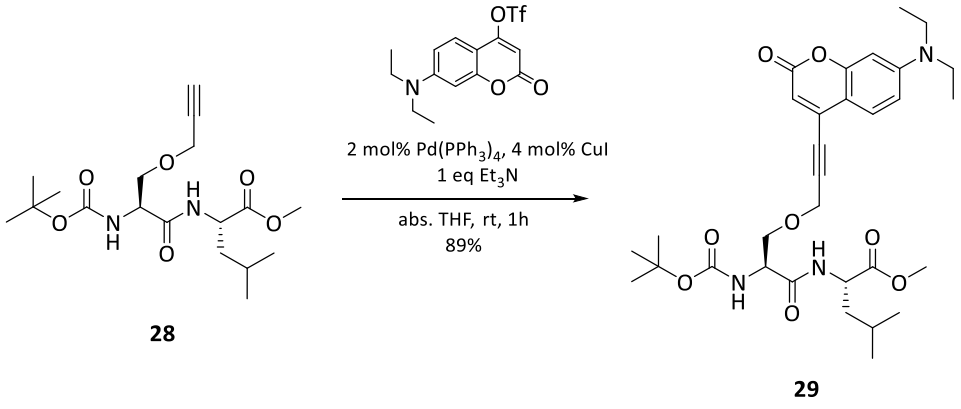
After these modifications the amino acid ethers **23** and **25** were subjected to Sonogashira coupling with the target fluorescent marker **12**. In both cases the

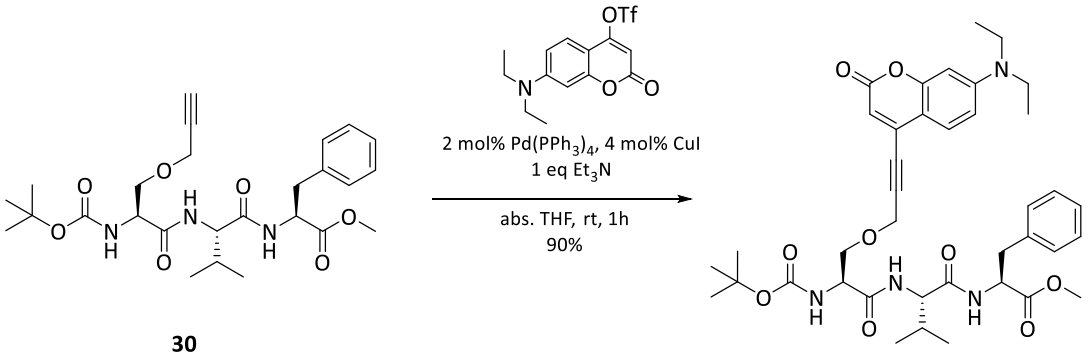
couplings proceeded smoothly furnishing the desired labelled compounds **26** and **27** in excellent yields (Scheme 3.11).



Scheme 3.11: Fluorescent labelling of Boc-Tyr **25** and Boc-Ser **23** *via* Sonogashira coupling.

Since the labelling of amino acids gave very good results, this methodology was applied furthermore for labelling dipeptide **28** and tripeptide **30**, which were previously synthesized and successfully used in cupfer-catalyzed click reactions by L. Wirtz[19]. The desired labelling was achieved after 1 hour under standard conditions yielding **29** and **30** in very good yields (Scheme 3.12).



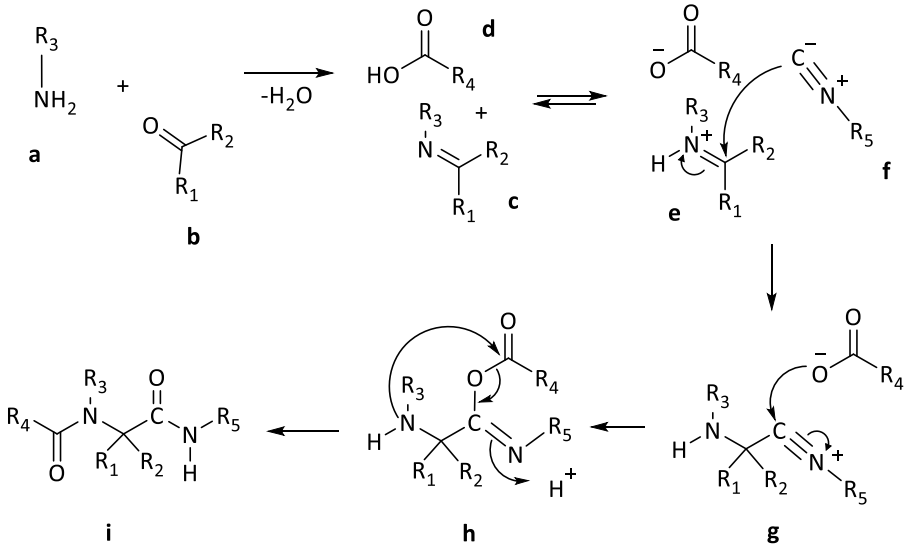


30 **31**

Scheme 3.12: Fluorescent labelling of dipeptide and tripeptide *via* Sonogashira coupling reaction.

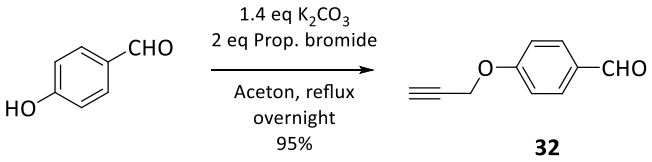
Synthesis of peptides *via* Ugi-reaction using 4-(propargyloxy)-benzaldehyde

The Ugi reaction is a multi-component reaction (MCR) involving an aldehyde, an amine, an isocyanide and a carboxylic acid to form a dipeptide. It is named after Ivar Karl Ugi, who first published this reaction in 1959.[138] MCRs are one-pot reactions which the allow synthesis of complex molecules from relatively simple precursors. A proposed mechanism of this process is shown in Scheme 3.13. In the first step aldehyde **b** condenses with amine **a** forming imine **c**, a Schiff base. Afterwards the acid component **d** protonates the nitrogen atom of imine **c,** therefore increasing the electrophilicity of the C=N bond. The electrophilic iminium ion and the nucleophilic acid anion add to the carbon atom of isocyanide forming adduct **h**, which after the Mumm rearrangement forms a stable Ugi product **i**.



Scheme 3.13: Mechanism of the Ugi-MCR.

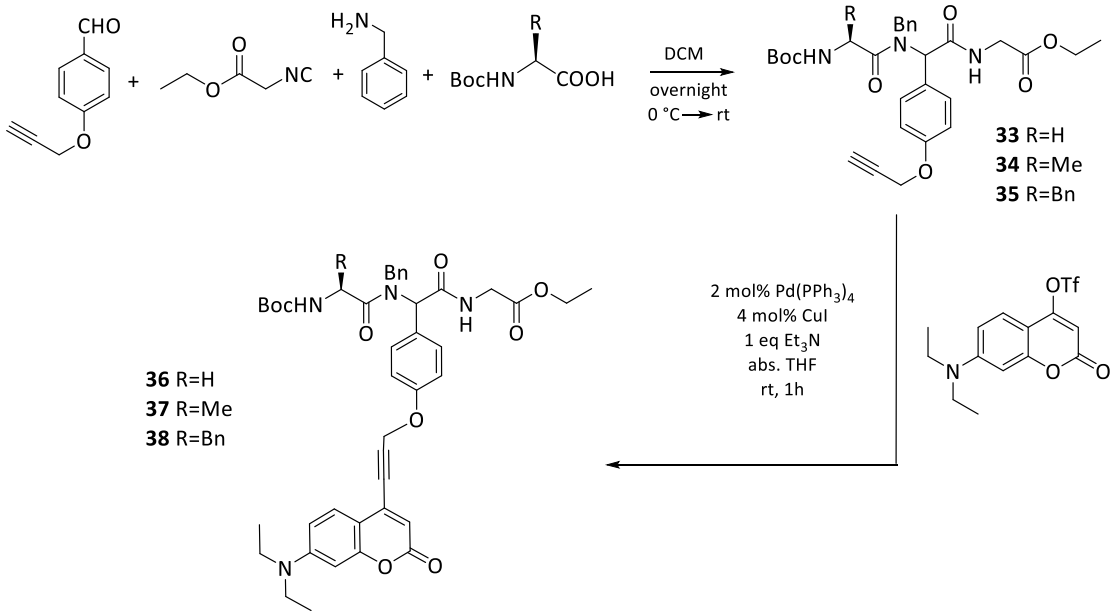
The synthesis of peptides under Ugi conditions was performed using an aldehyde with a propargylic residue. Benzaldehyde was modified according to the literature procedure in 95% yield (Scheme 3.14).[139]



Scheme 3.14: Propargylation of benzaldehyde.

The Ugi multi-component reaction was performed in DCM at 0 °C using benzyl amine, ethyl isocyanoacetate and several Boc-protected amino acids.[140] The target peptides **33-35** were isolated in 58-75% yield, respectively. After chromatographical purification they were directly subjected to coupling with 4-trifluoromethyl-sulfonyloxy-7-diethylamino-coumarin **12.** The labelled compounds **36-38** were formed in excellent yields. The results are summarized in Table 3.5.

Table 3.5 Ugi reaction with subsequent coupling:



	R	Product	Yield [%], Ugi reaction	Product	Yield [%], Sonogashira reaction
1	H	**33**	65	**36**	92
2	Me	**34**	60	**37**	90
3	Bn	**35**	58	**38**	91

3.4.3 Studies towards the biolabeling with coumarine derivatives through cross-coupling with CO-Insertion

Besides the above described direct C-C-couplings it should be possible as well to perform the coupling of diethylaminocoumarin **12** with different nucleophiles through Buchwald-Hartwig C-N cross-coupling with CO-Insertion (Figure 3.3).

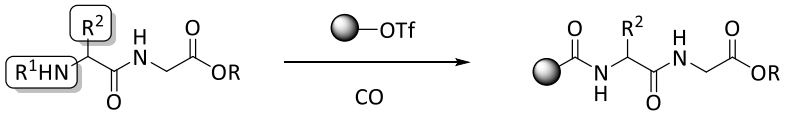
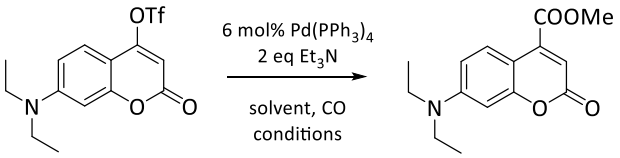


Figure 3.3: Labelling amino functionality through Buchwald-Hartwig C-N cross-coupling.

Initally MeOH was selected as simplified nucleophilic cross-coupling partner for triflate **12**. Treatment of coumarine derivative **12** with catalytic amounts of $Pd(PPh_3)_4$ in methanol under 1 atm of CO provided the desired methylester **39** in only 13% yield (table 3.6, entry 1). In DMF as solvent with stoichiometric amounts of MeOH only small traces of the cross coupling product **39** were observed (entry 2). With $Pd(OAc)_2$ and PPh_3 as precursor for the catalytically active species in MeOH/DMF 1:1 no conversion of the starting triflate occurred (entry 3). In entry 1-3 mostly unreacted starting material **39** was reisolated. However, increasing the CO-pressure to 15 bar afforded the ester **39** in 70% isolated yield (entry 4).

Table 3.6 Buchwald-Hartwig cross-coupling with MeOH under CO-atmosphere:



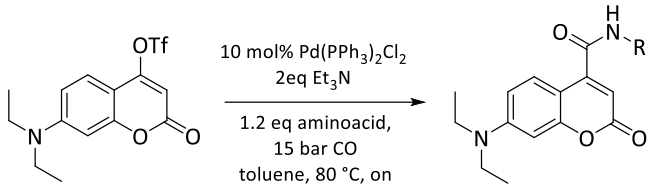
12 **39**

	Solvent	Conditions	Catalyst	Yield
1	abs. methanol	room temperature 2h, CO (1 bar)	6 mol% $Pd(PPh_3)_4$	13% product + starting material
2	abs. DMF 2 eq. methanol	room temperature CO (1 bar)	6 mol% $Pd(PPh_3)_4$	traces of the product, starting material
3	abs. methanol abs. DMF	40 °C, 12h CO (1 bar)	0.1eq $Pd(OAc)_2$ 0.2eq PPh_3	no product formation starting material reisolated
4	abs. methanol	reflux	10 mol%	70%

abs. toluene (1:6)	CO (15 bar)	$Pd(PPh_3)_2Cl_2$

Fortunately, under the optimized conditions of the cross-coupling with MeOH (table 3.6, entry 4, in toluene as a solvent) various amines could be utilized as nucleophiles to give the aspired amides **40-48** in good to excellent yields (table 3.7). Several hydrochloride salts of amino acid and dipeptide esters delivered the coumarine labeled derivatives **40-44** (entry 1-5). Moreover different amines and anilines served as excellent coupling partners for triflate **12** (entries 6-9).

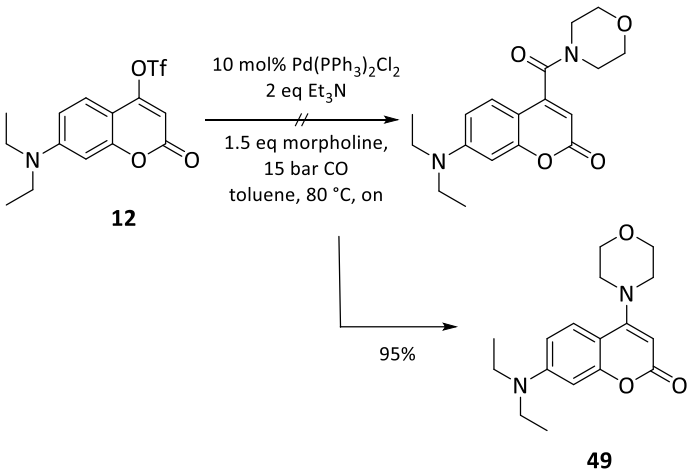
Table 3.7 Cross-coupling reactions with different nucleophiles under CO-atmosphere:



12 **40-48**

	Nucleophile	Product	Yield [%]
1	GlyOMe · HCl	**40**	86%
2	AlaOMe · HCl	**41**	71%
3	PheOMe · HCl	**42**	75%
4	LeuOMe · HCl	**43**	62%
5	PheGlyOMe · HCl	**44**	70%
6	Benzylamine	**45**	83%
7	Aniline	**46**	78%
8	Cyclohexylamine	**47**	92%
9	Anisidine	**48**	quant

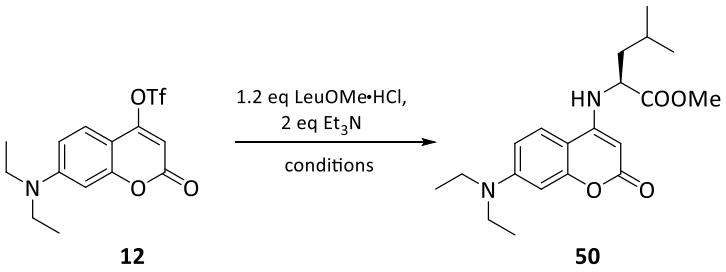
Surprisingly, more nucleophilic amines (e.g. morpholine) provided enamines of type **49** without CO-insertion. Indeed even in the absence of a Pd-catalyst the enamine **49** was formed in an excellent yield. Therefore, the (transition metal-free) conjugate substitution (Michael addition + elimination of TfOH) leading to **49** must be significantly faster than the Buchwald-Hartwig amination including CO-Insertion giving **49** in 95**%** yield (Scheme 3.15).



Scheme 3.15: Buchwald-Hartwig amination.

One of the amino acids, namely LeuOMe hydrochloride, was subjected to direct substitution in absence of palladium species. Firstly THF was used as a solvent and unfortunately after stirring overnight at room temperature no conversion was observed (table 3.8, entry 2). Under microwave accelerated conditions at 60 °C starting material was reisolated (entry 3), whereas in 10 minutes traces of purple fluorescent compound were formed. Furthermore the solvent was exchanged for toluene and amino acid refluxed with **12** overnight, what resulted in substitution product **50** in 62% yield.

Table 3.8 Substitution reaction with amino acid:



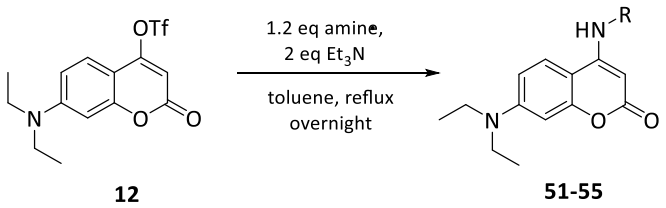
	Solvent	Conditions	Catalyst	Yield
1	abs. toluene	80 °C on, CO (15 bar)	10 mol% $Pd(PPh_3)_2Cl_2$	65%
2	abs. THF	room temperature on	----------	starting material (TLC monitoring)
3	abs. THF	60 °C, 90 W 1 min	----------	starting material (TLC monitoring)

4	abs. THF	60 °C, 90 W 10 min	----------	starting material + traces of purple fluor. comp (TLC monitor.)
5	abs. toluene	reflux, overnight	----------	62%

Coupling under CO-atmosphere resulted in amide formation and no substitution occured (entry 1). It could be visually determined under UV-light whether substitution with or without CO-insertion took place. The direct substitution product is fluorescing purple, whereas the CO-insertion compound (expansion of the π-system and shifting towards longer wave), showed yellow fluorescence.

Various secondary amines were screened under substitution conditions. Pyrrolidine and piperidine (table 3.9, entry 1 and 2) yielded the substitution compounds in almost quantitative yield, whereas diethylamine, dibenzylamine and ephedrine in 68%, 60% and 56%, respectively. The better yields in the case of pyrrolidine, piperidine and morpholine can be explained by their stronger nucleophilic properties, principally because the lone pair is less hindered due to the fixed geometry of the two alkyl "substituents" making the approach to electrophile much easier.[141]

Table 3.9 Substitution reaction with different secondary amines:

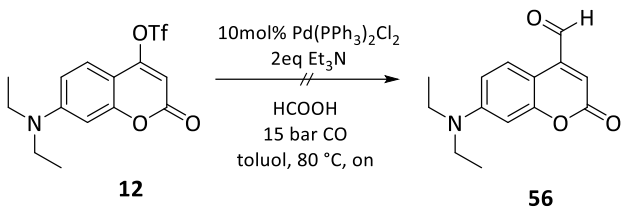


12 **51-55**

	Nucleophile	Product	Yield [%]
1	Pyrrolidine	**51**	97
2	Piperidine	**52**	98
3	Diethylamine	**53**	68
4	Dibenzylamine	**54**	60
5	Ephedrine	**55**	56

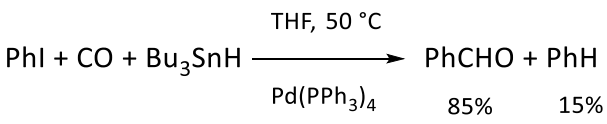
3.5 Synthesis of fluorescenting aldehyde

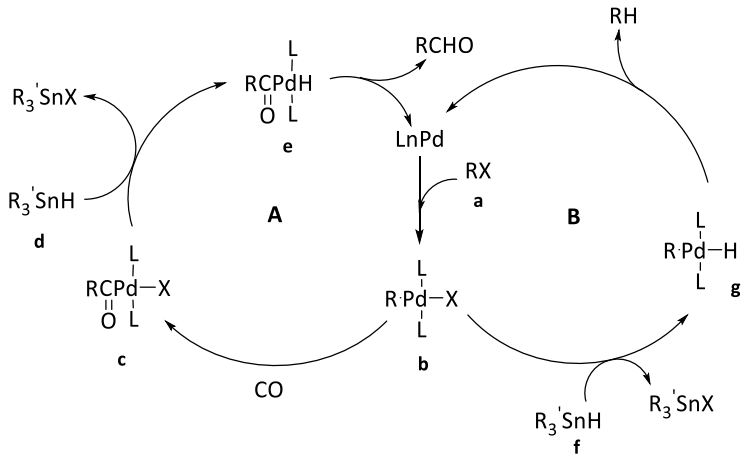
For the synthesis of fluorescenting aldehyde the Pd-catalyzed coupling with CO-insertion was applied (Scheme 3.16). Unfortunately aldehyde **56** was not formed under these conditions. Apparently compound **12** is not stable in presence of formic acid.



Scheme 3.16: Attempt to synthesis of aldehyde **56** *via* Pd-catalyzed reaction with CO-insertion.

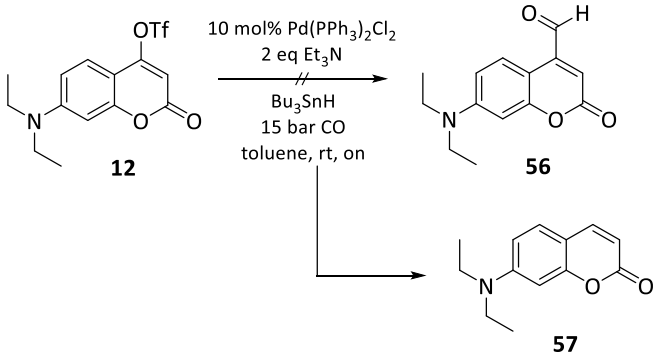
In the literature it was reported that the palladium-catalyzed formylation of a wide variety of organic substrates including vinyl triflates with tin hydride and carbon monoxide gave good yields of aldehydes.[142] The authors observed formation of a side product as well, which can be explained by two overlapping catalytic cycles (Scheme 3.17). In each catalytic cycle first the oxidative addition of **a** to the palladium (0) catalyst occurs to form the alkylpalladium(II) complex **b**, which under CO atmosphere undergoes the carbon monoxide insertion to form the acylpalladium(II) complex **c**. Further transmetallation with tributyltinhydride **d** gives rise to an acylhydridopalladium (II) complex **e,** which by subsequent reductive elimination furnishes the aldehyde and regenerates the palladium(0) catalyst (catalytic cycle A). The undesired side product is formed according to the cycle B. In competition with the carbon monoxide insertion the palladim(II) complex **b** undergoes transmetalation with tributyltin hydride forming an alkylhydrido-palladium(II) complex **g**, which after further reductive elimination forms the reduced product and regenerated palladium(0) catalyst.





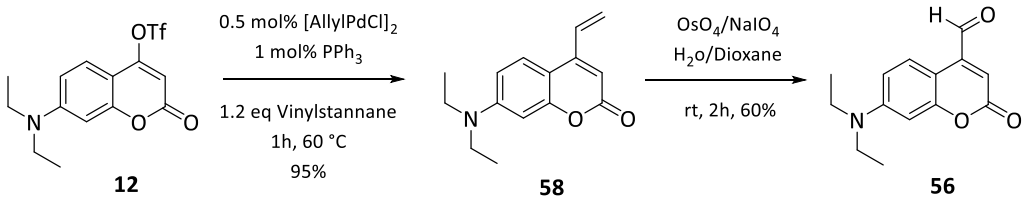
Scheme 3.17: The proposed catalytic cycle.

Unfortunately also under these conditions only reduction of the vinyl triflate occured (the B catalytic cycle) because the Bu_3SnH had to be added slowly in the portion. It was impossible to keep the reaction mixture under the CO-atmosphere and slowly add tributyltin hydride *via* syringe (Scheme 3.18).



Scheme 3.18: Attempted synthesis of aldehyde **56** *via* Pd-catalyzed reaction with CO-insertion.

In the previous chapter 3.4 was shown that 4-trifluoromethylsulfonyloxy-7-diethylamino-coumarin **12** is a good candidate for different metal-catalyzed cross-couplings. Therefore, the vinyl triflate **12** was subjected to Stille coupling with vinylstannene using allylpalladium(II) chloride dimer with PPh_3 as a ligand,[143] what furnished the desired molecule **58** in 95% yield. It should be mentioned that **58** is unstable and undergoes probably photoinduced 1,2-addition reactions. For this reason it should be kept under a nitrogen atmosphere and protected from light. The subsequent oxidation of the double bond with OsO_4 (Lemieux-Johnson oxidation) resulted in formation of aldehyde **56** in 60% yield (Scheme 3.19).



Scheme 3.19: Synthesis of the aldehyde **56** through Stille-coupling reaction and subsequent oxidation.

The obtained aldehyde **56** was successfully used in fluorescence labelling of Miuraenamide *via* aldol addition. [144]

3.6 Labelling backbone of the protein

Most of the methods known for fluorescent labeling of proteins are based on the modification of various side chains and the *N*- or *C*- termini (Chap. 2.6.1). In the group of Prof. Kazmaier also C-C bond forming reactions (namely Pd-catalyzed allylic alkylation and Claisen rearrangement) were successfully applied as possible labeling methods of the backbone of a peptide (Chap. 2.8). Based on the previous results, further allylic fluorescent dyes were developed and studied their application in Pd-catalyzed allylic alkylations.

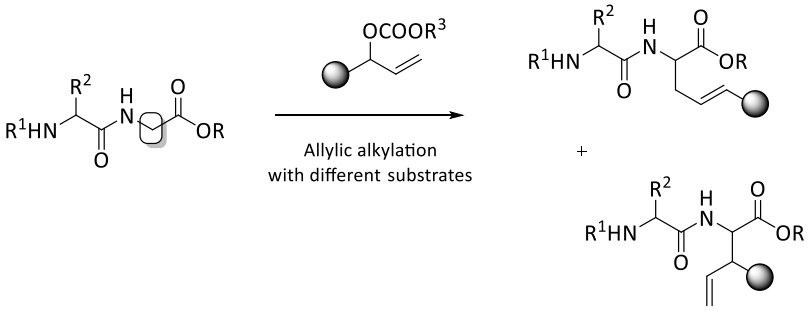


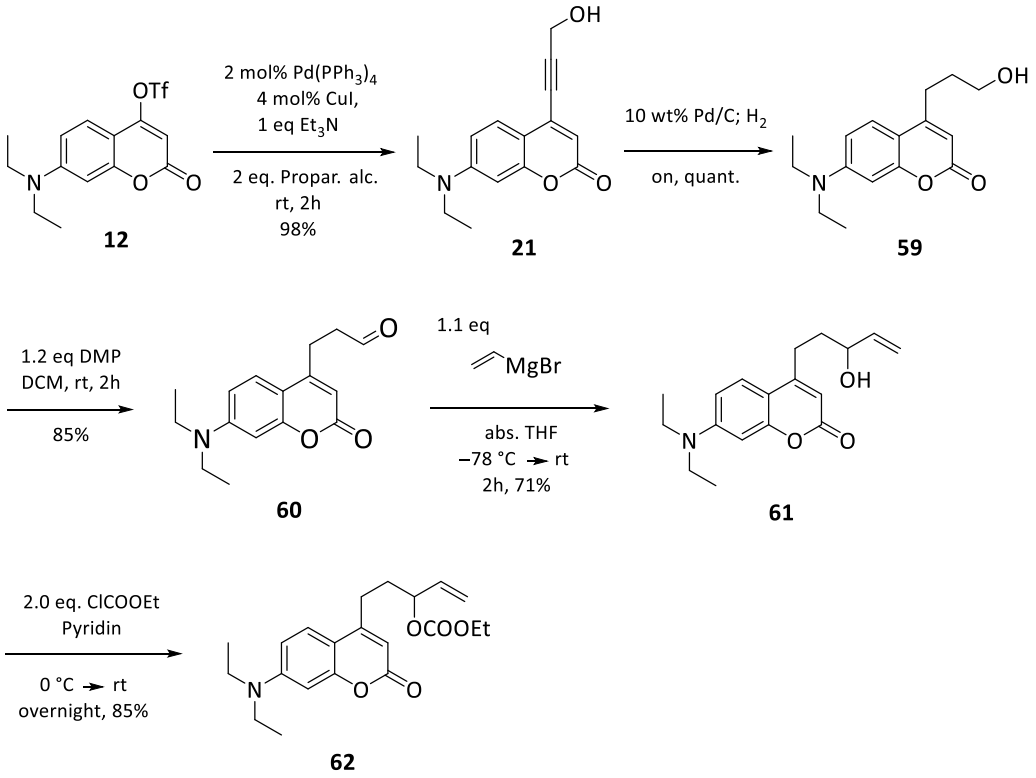
Figure 3.4: Labelling backbone of the peptide using Palladium-catalyzed allylic alkylation reaction.

3.6.1 Fluorescent labelling *via* palladium-catalyzed allylic alkylation

For the labelling amino acids or peptides *via* palladium catalyzed allylic alkylation 7-aminocoumarins with a terminal allylic side chain were developed. Since the methodology developed by L. Wirtz[19] could not be further applied due to the low yield of Pechmann condensation (Table 3.1), a different protocol was developed for the synthesis of allylic dyes.

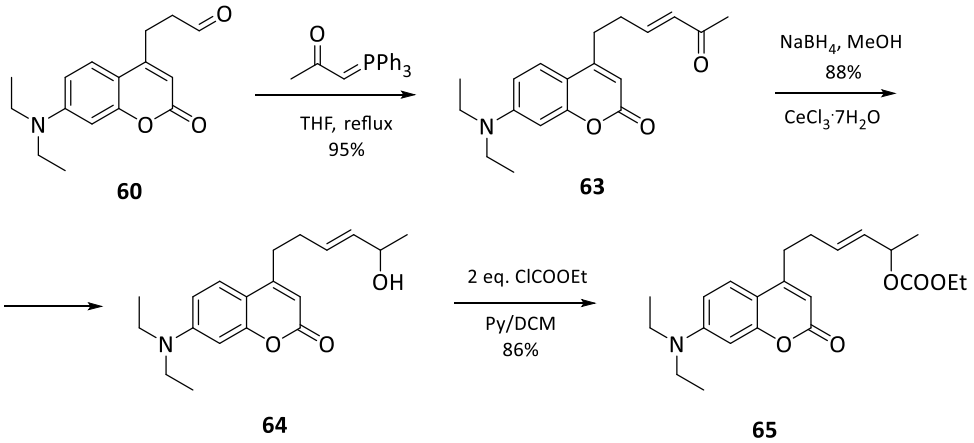
Previously synthesized fluorescent alcohol **21** was subjected to hydrogenation in the presence of palladium on charcoal delivering the desired product **59** in

quantitative yield (Scheme 3.20). Further oxidation of the alcohol **59** to the aldehyde **60** with Dess-Martin periodinane afforded the fluorescenting compound **60** in good yield too. Further Grignard reaction with vinylmagnesium bromide followed by esterification furnished the desired allylcarbonate **62**.



Scheme 3.20: Synthesis of 7-aminocoumarin **62** with allylcarbonate side chain.

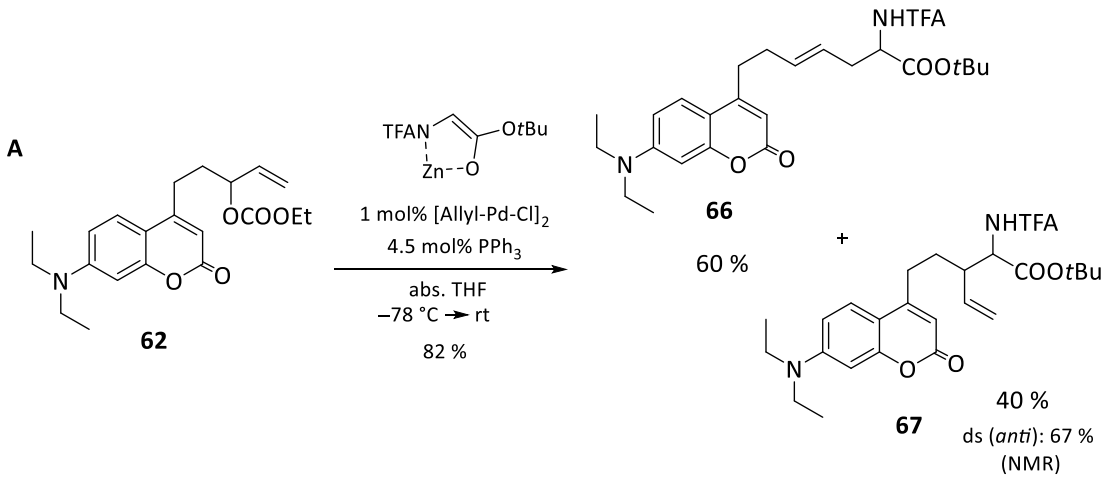
The aldehyde **60** was furthermore transformed to the *trans*-α,β-unsaturated ketone **63** in 95% yield through Wittig reaction. The ketone **63** was subsequently reduced to the racemic alcohol **64** *via* Luche reduction followed by esterification to afford allylic dye **65** (Scheme 3.21).

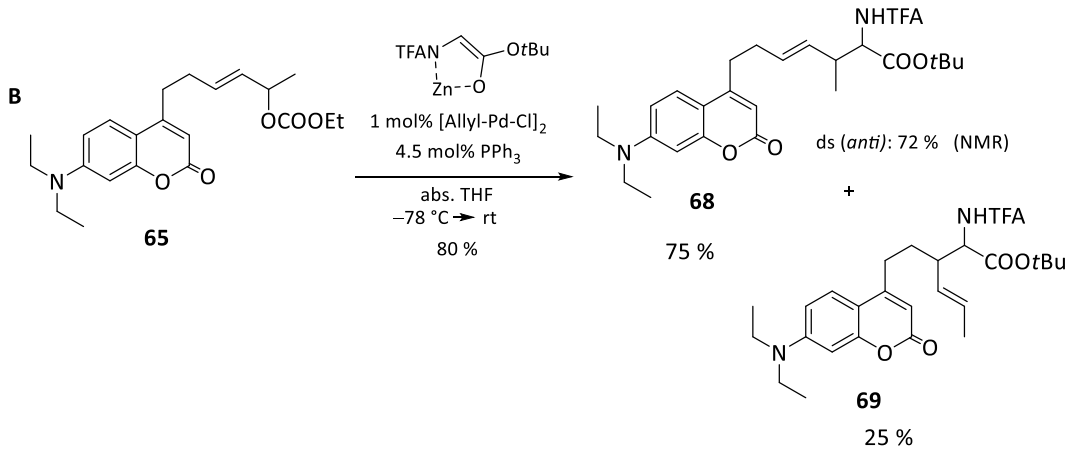


Scheme 3.21: Synthesis of 7-aminocoumarin **65** with allylcarbonate side chain.

The synthesized allylcarbonate-labels **62** and **65** were subjected consequently to palladium-catalyzed allylic alkylation of TFA-glycine-*tert*-butyl ester as a nucleophile, under conditions described by L. Wirtz (Scheme 3.22). Here, the allylic substitution reaction again proved to be an excellent method for fluorescent labeling of amino acid esters. Both allylic alkylations provided the fluorescently labeled amino acid esters **66, 67, 68** and **69** in good yields (around 80%). In reaction with the carbonate **62** the formation of both - linear and branched products **66** and **67** was observed (ratio 60:40), (Scheme 3.22, A). Unfortunately chromatographic separation of the two esters **66** and **67** was not possible. The selectivity could only be estimated from the ^{1}H-NMR spectra. The reaction of the carbonate **65** (Scheme 3.22, B) also provided two regioisomers **68** and **69** with the ratio of 75:25 and again it was not possible to separate both isomers during purification.

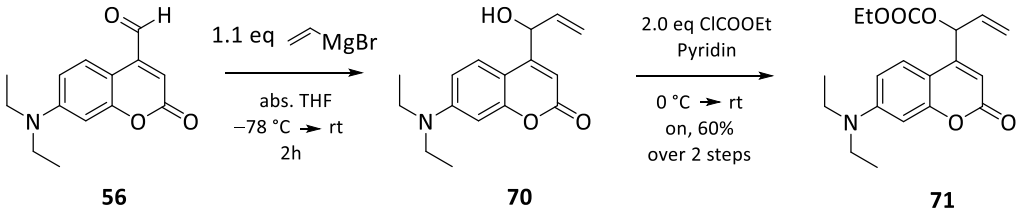
Similar observations were made by L. Wirtz in the allylic substitution of TFA-glycine-*tert*-butyl ester with allylic fluorescent dyes.[19] In studies of monoalkylated, terminal allyl carbonates, such as carbonate **62**, and asymmetrically substituted allyl carbonates (analog carbonate **65**) mixtures of two regiosomers were also obtained. [145] In addition, it was shown in studies of F. L. Zumpe that in allylic alkylation the anti-diastereomer is formed preferably.[146]





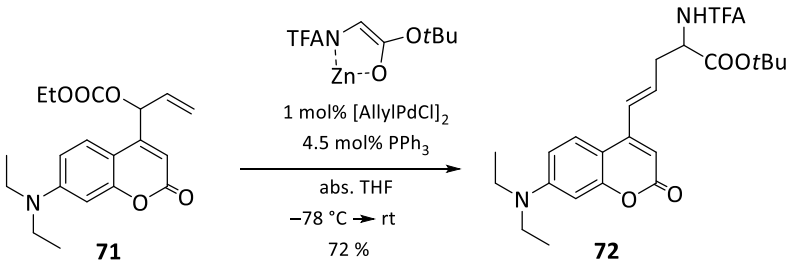
Scheme 3.22: Allylic alkylations of TFA-glycine-*tert*-butylester with 7-diethyl-aminocoumarin-l*abels* **62** and **65.**

In order to improve the regioselectivity a fluorescent dye with a shorter allylic residue was developed. For the synthesis the previously synthesized aldehyde **56** (Scheme 3.19) was used, which was subjected to Grignard reaction and subsequently converted to allylcarbonate **71** in 60% yield over two steps (Scheme 3.23).



Scheme 3.23: Synthesis of 7-aminocoumarin **71** with shorter allylcarbonate side chain.

The obtained allylcarbonate **71** was subjected to Pd-catalyzed allylic alkylation and afforded *E*-configurated fluorescenting ester **72** in 72% yield (Scheme 3.24). Unlike 7-aminocoumarins **62** and **65**, allylic alkylation proceeded with the formation of only one regioisomer. Compound **71** has two CH_2 groups less, what results in formation of only one regioisomer, since the branched position of monosubstituted π-allyl complex is prevented by the coumarin ring for an attack of the nucleophile.



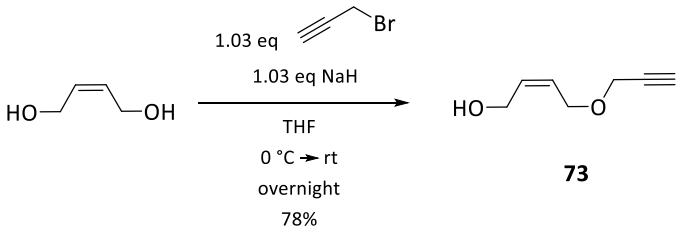
Scheme 3.24: Allylic alkylation with 7-aminocoumarin label **71**.

In the work of L. Wirtz shortened 7-methoxycoumarins were subjected to allylic alkylation[19]. However, the labeling with 7-diethylaminocoumarin has more advantages because aminocoumarins unlike methoxycoumarins exhibit fluorescence and excellent quantum yields at physiological pH.[147] In order to detect a biomolecule marked with a methoxycoumarin, the methyl group should be first cleaved and the resulting alcohol deprotonated at pH > 10. Optimized labelling technique can be further applied for di- and tri-peptides.

3.6.2 Fluorescent allylic label with an expanded π-system

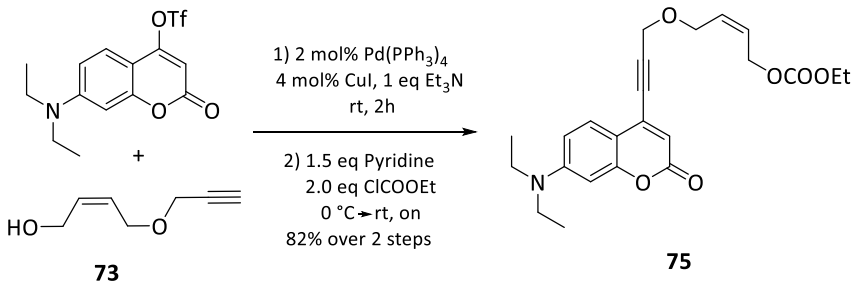
Since 7-diethylaminocoumarins with an allylic carbonate residue gave good results in Pd-catalyzed allylic alkylations of the amino acid, further allylic substrates with an expanded π-system were developed. For the synthesis of allylic dyes Sonogarshira coupling with propargylated diol was applied.

Propargylation of the diol proceeded selectively furnishing the mono-propargylated compound **73** as the main product in 78% yield. The formation of the di-propargylated compound was observed as well (Scheme 3.25).



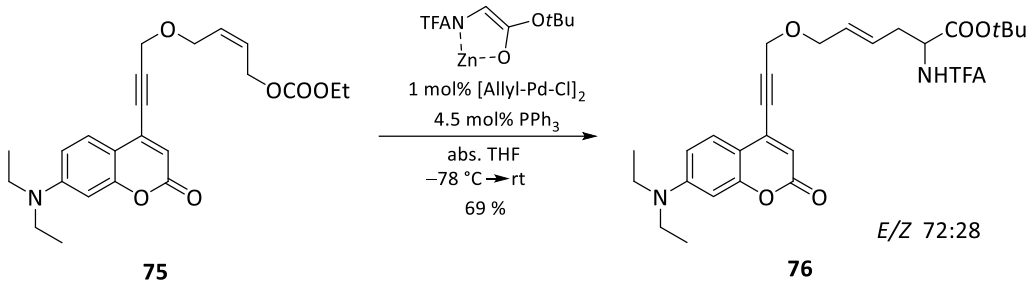
Scheme 3.25: Mono-propargylation of diol.

The following Sonogashira coupling with vinyl triflate **12** and esterification of the formed alcohol provided the new *Z*-configurated fluorescent label in 87% yield over 2 steps (Scheme 3.26).



Scheme 3.26: Synthesis of allylic carbonate label **75** with an expanded π-system.

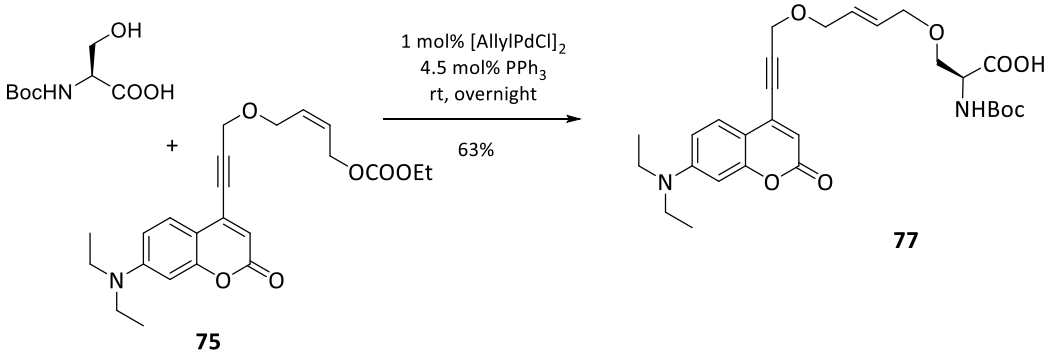
The fluorescent dye **75** was screened in allylic alkylation reactions with various nucleophiles. Pd-catalyzed allylic alkylation of TFA-glycine-*tert*-butyl ester under standard conditions proceeded successfully, forming the mixture of *E/Z*-configurated isomers **76a** and **76b** with a ration 72:28 (NMR) in 69% yield (Scheme 3.27). The problem we faced here was the separation of the starting material from product, since the conversion was not complete and the R_f values of both compounds were identical. The chromatographic separation of the product was only possible after quenching with morpholine, converting the remaining starting material to the corresponding morpholino allylamide, which is more polar.



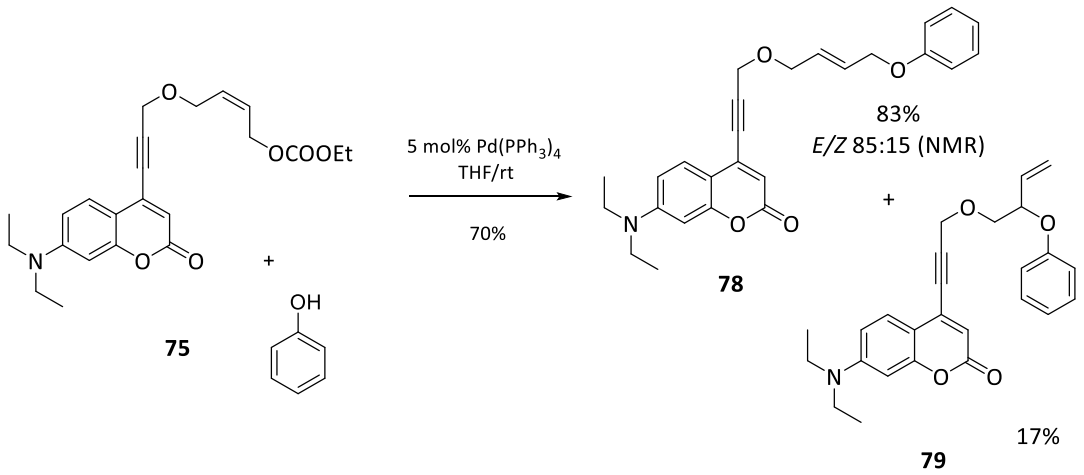
Scheme 3.27: Allylic alkylation of TFA-glycine-*tert*-butyl ester with 7-aminocoumarin label **75**.

Since the synthesis of allylic substrate with an expanded π-system **75** proceeded smoothly, in only two steps and gave the desired fluorescent dye **75** in excellent yield, it was further screened in labelling of various *N*- and *O*-nucleophiles.

As *O*-nucleophiles Boc-protected serine and phenol were chosen. The allylation of Boc-serine proceeded in 63% yield (Scheme 3.28). The modification of phenol provided two regioisomers, linear **78** (*E/Z* 85:15) and branched **79** in a ratio of 83:17 and in an overall yield of 70% (Scheme 3.29). The chromatographic separation of both regioisomers was not possible.



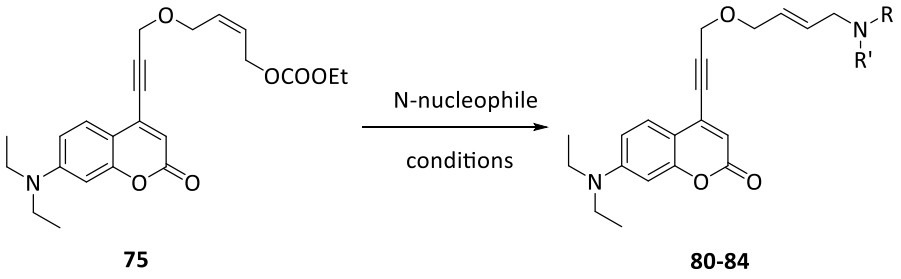
Scheme 3.28: O-allylation of Boc-serine with **75.**



Scheme 3.29: O-allylation of phenol with **75**

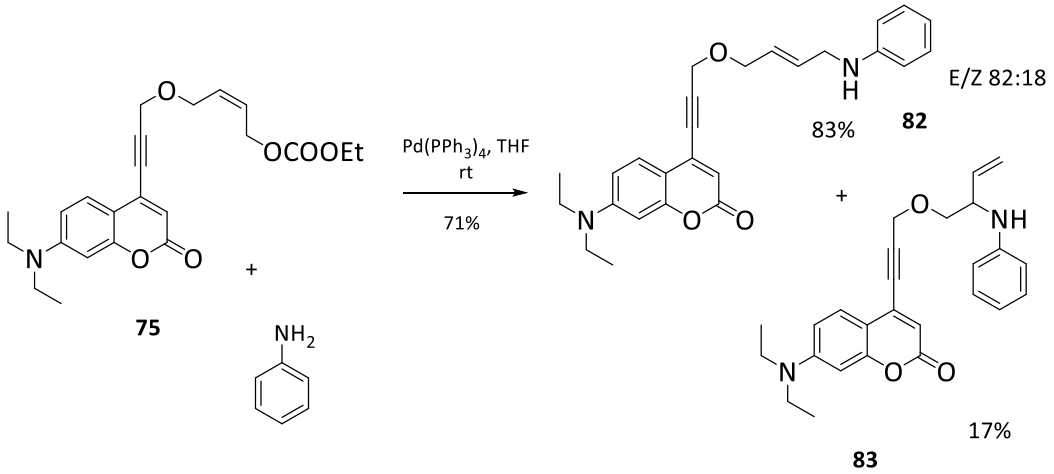
For *N*-alkylation piperidene was chosen as a first candidate. Under the conditions described by Kazmaier *et al,*[148] $[AllylPdCl]_2/PPh_3$ as a catalyst and THF as a solvent, the desired allylated compound **80** (*E/Z* 89:11) was isolated in 73% yield (Table 3.10, entry 1).

Table 3.10 N-alkylation reactions with **75**:



	Nucleophile	Conditions	Product	Yield	E/Z ratio
1	Piperidine	5 mol% $[AllylPdCl]_2$/ 20 mol% PPh_3, THF –78 °C -> rt	**80**	73%	89:11
2	Piperidine	5 mol% $Pd(PPh_3)_4$, DMF –20 °C -> rt	**80**	88%	86:14
3	Morpholine	5 mol% $[AllylPdCl]_2$/ 20 mol% PPh_3, DMF –20 °C -> rt	**81**	84%	93:7
4	Aniline	5 mol% $Pd(PPh_3)_4$, THF rt	**82-83**	71%	82:18
5	N-methylanilin	5 mol% $Pd(PPh_3)_4$, THF rt	**84**	75%	90:10

The yield was significantly increased using $Pd(PPh_3)_4$ and dimethylformamide as a solvent (entry 2), what gave rise to the *N*-alkylated product in 88% yield. The allylation of morpholine proceeded in 84% yield (*E/Z* 93:7) (entry 3). In case of aniline the overall yield was 71% at room temperature (entry 4, Scheme 3.30) and gave rise to regioisomers linear **82** (*E/Z* 82:18) and branched **83** with a ratio 83:17, (Scheme 3.30), which were separated through chromatography. *N*-methylanilin yielded **84** (*E/Z* 90:10) in 75% yield (entry 5).



Scheme 3.30: N-alkylation of aniline with **75.**

Pd-catalyzed allylic alkylations with *Z*-configurated carbonat **72** proceeded in good yields with formation of *E*-configurated compounds preferably. Aniline and phenol gave rise to formation of linear and brached regioisomers, as well.

3.7 Labelling of molecules based on Michael addition reactions

Coumarin derivatives are reported to be good Michael acceptors for ratiometric detection of *in vivo* biothiols.[149] Since Michael additions have been studied for years in the group of Prof. Kazmaier, the development of Michael acceptors based on the coumarin moiety and their subsequent application in fluorescent labelling was an attractive aim of this thesis. The possible sites for fluorescent labelling through Michael addition are shown in Figure 3.5.

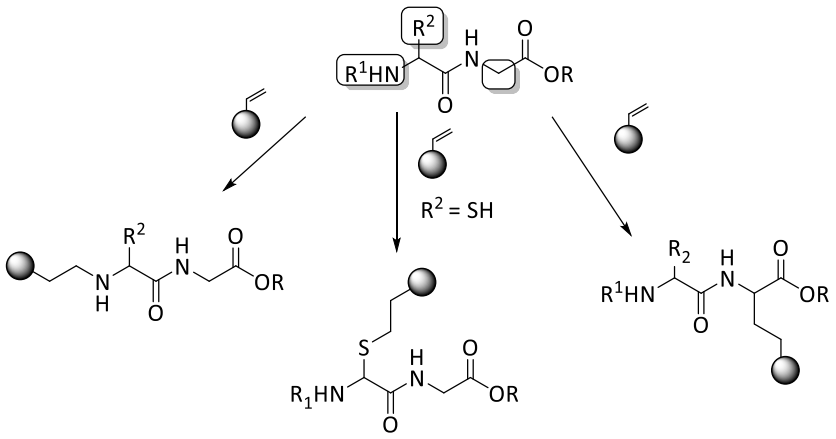
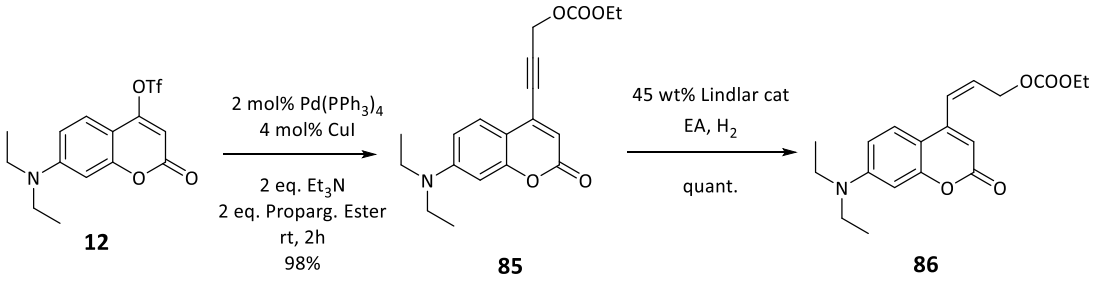


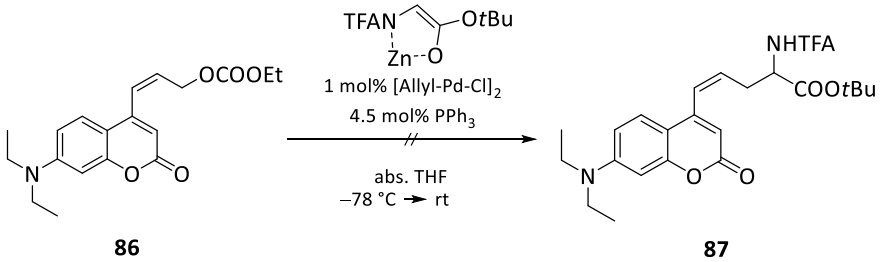
Figure 3.5: Possible sites for fluorescent labelling through Michael addition**.**

In the course of the development of a new possible allylic fluorescent label and its subsequent application in Pd-catalyzed allylic alkylation, no allylation took place. The allylic carbonate was synthesized under Sonogashira coupling conditions with propargylic ester and subsequent hydrogenation of the triple bond using Lindlar catalyst, what furnished the desired *Z*-product **86** in almost quantitative yield (Scheme 3.31).



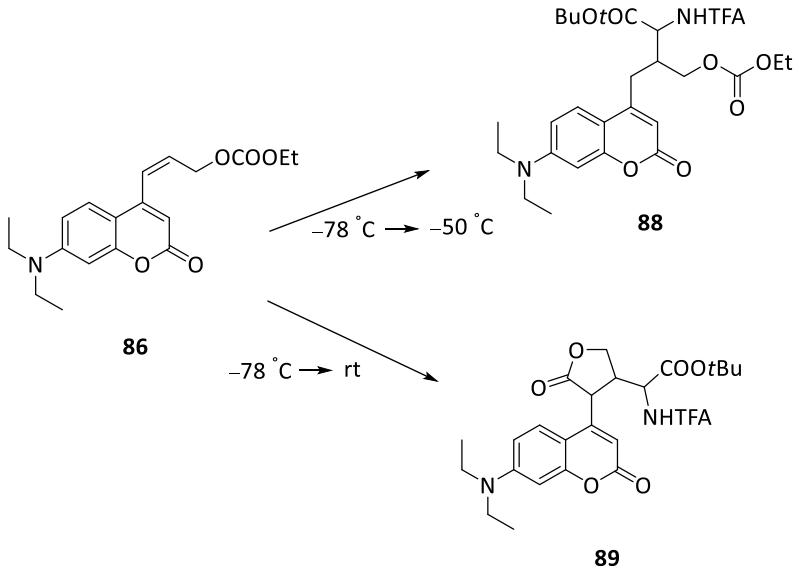
Scheme 3.31: Synthesis of fluorescent allylic substrate **86.**

The new substate **86** was screened in allylic alkylation of the TFA-glycine-*tert*-butyl ester under standard conditions. The reaction proceeded with the formation of a new product the ^{1}H NMR-spectra and LC-MS analysis (Figure 3.6) of which did not correspond the allylation product's structure (Scheme 3.32)



Scheme 3.32: Attempts in allylic alkylation with fluorescent dye **86.**

It turned out that fluorescenting dye **86** represents a good Michael system. The same phenomenon was observed as well by Kazmaier *et al* with other substrates,[150] when instead of palladium-catalyzed allylation a Michael addition took place, since the addition reaction proceeds much faster then the allylation. The Michael addition product **88** is only an intermediate and could be isolated only if the reaction mixture was quenched at –50 °C. If the reaction was allowed to warm up to room temperature the lactone **89** was isolated (Scheme 3.33).



Scheme 3.33: Michael addition with fluorescent dye **86.**

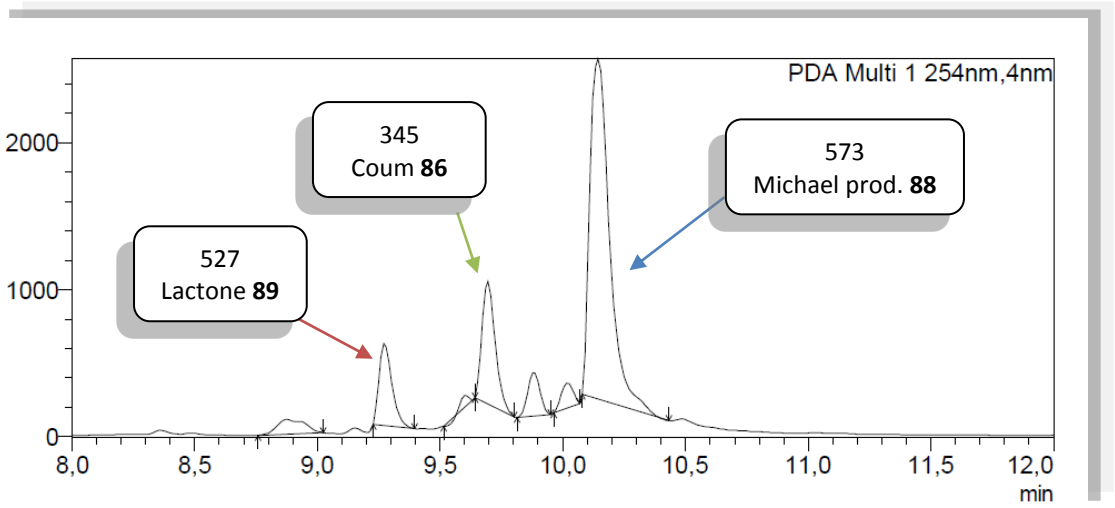
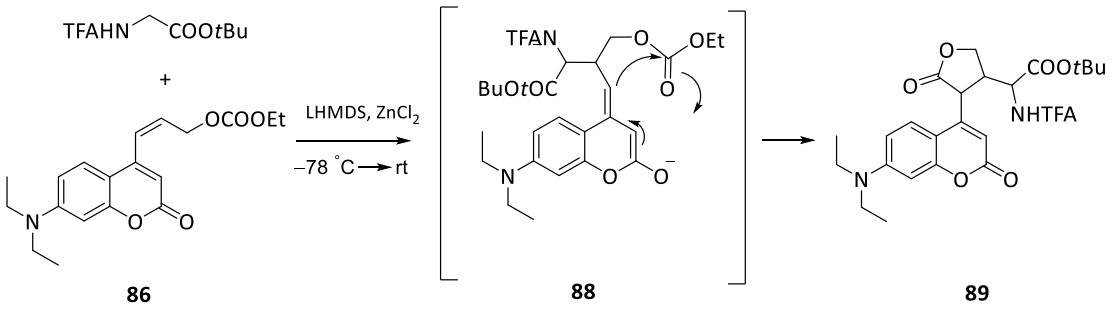


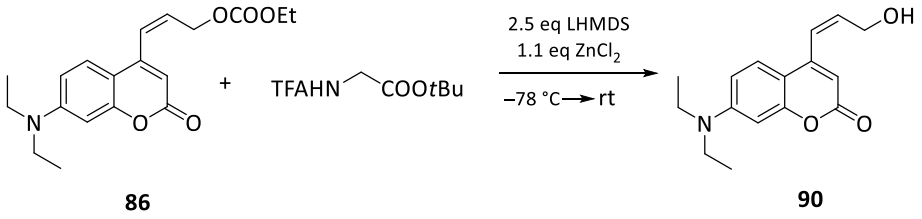
Figure 3.6: LC-MS chromatogram of Michael addition reaction with fluorescent dye **86**.

If the reaction mixture was allowed to warm overnight to ambient temperature without the isolation of the intermediate, only lactone **89** was isolated in 65% yield. The proposed mechanism of this reaction is depicted in scheme 3.34.



Scheme 3.34: Proposed mechanism of Michael-addition/Lactonisation

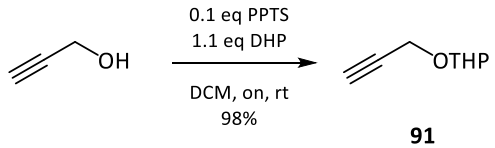
The Michael acceptor **86** was subjected to standard Michael addition conditions, [19] i.e. without Pd-catalyst. In this case only cleavage of carbonate group was observed and alcohol **90** was isolated in 60% yield (Scheme 3.35).

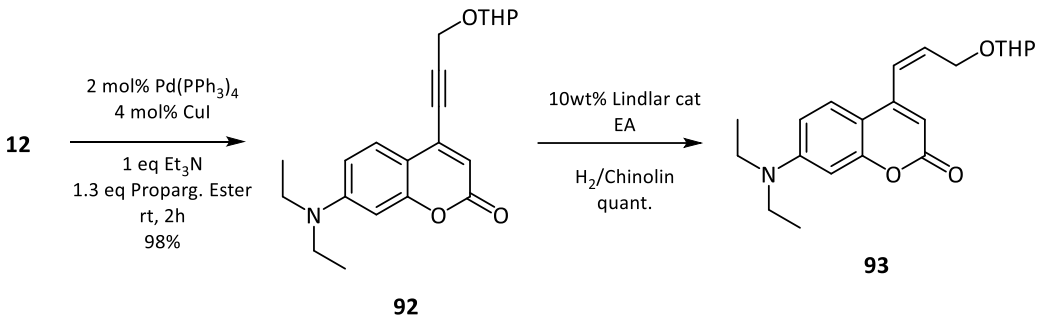


Scheme 3.35: Michael-addition without Palladium-catalyst

Since the Michael-acceptor **86** with a carbonat leaving group was giving two different products **88** and **89** further Michael acceptors were developed in order to avoid the formation of a lactone.

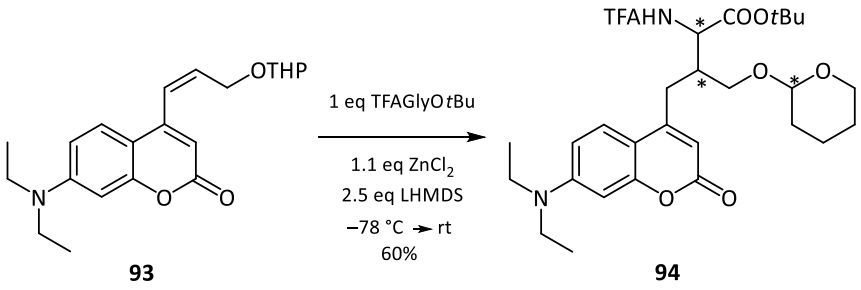
The synthesis of the propargylic ether **91** with a tetrahydropyranyl ether protecting group proceeded in almost quantative yield. Following Pd-catalyzed Sonogashira coupling reaction resulted in the formation of alkine **92** in 98% yield, which was subsequently subjected to Lindlar-hydrogenation (Scheme 3.36).





Scheme 3.36: Synthesis of the Michael-acceptor **93**.

The Michael addition with fluorescent dye **93** provided the addition compound **94** (TLC and LC-MS control, the change of fluorescence colour from yellow to purple, Figure 3.7), though the stereo center in a tetrahydropyranyl group resulted in the formation of a mixture of diastereomeres (yield 60%), the separation of which was not possible through chromatography.



Scheme 3.37: Michael-addition with fluorescent label **93.**

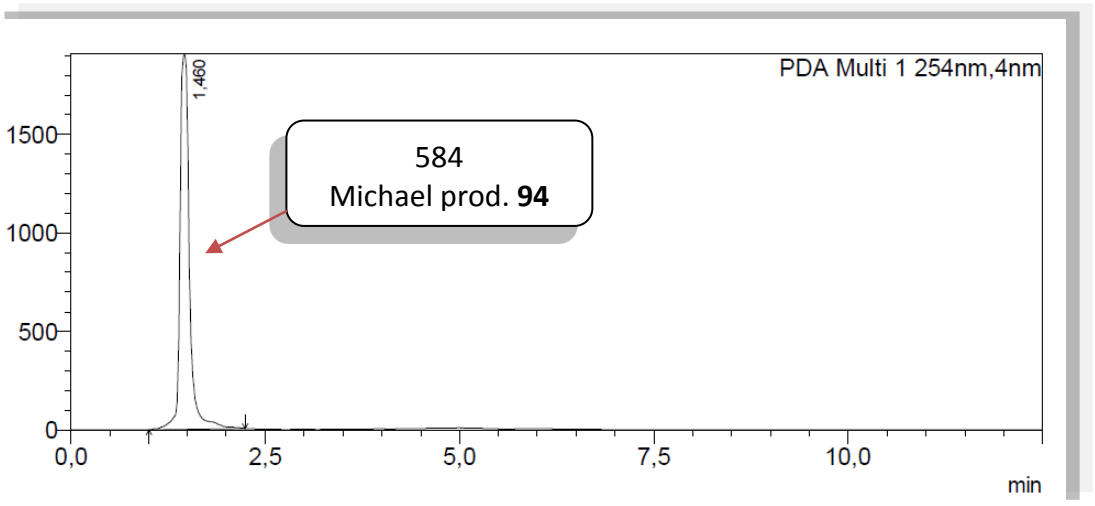
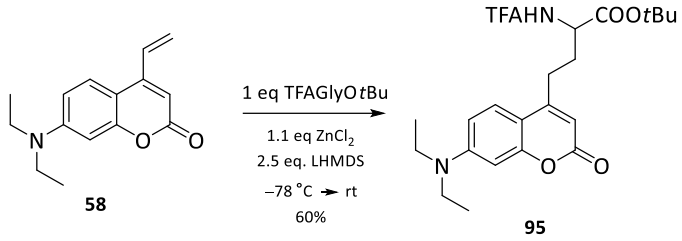


Figure 3.7: LC-MS chromatogram of Michael addition reaction with fluorescent dye **94**.

Synthesis of a Michael-acceptor through Stille coupling reaction

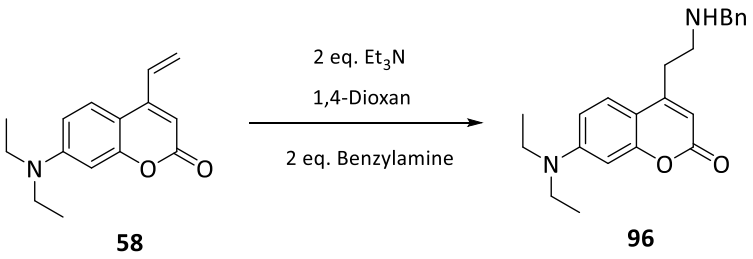
In chapter 3.5 the synthesis of vinyl coumarin **58** *via* Stille-coupling was described. Compound **58** was also screened as a potential Michael-acceptor. The Michael-addition proceeded in 60% yield (Scheme 3.38).



Scheme 3.38: Michael-addition with vinyl coumarin fluorescent label.

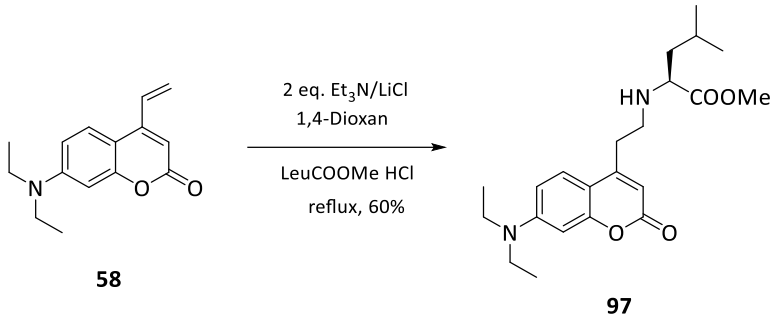
The Michael acceptor **58** was used for labelling of further nucleophiles. It was shown in chapter 2.6.1 that biomolecules with an *N*-terminus, as well as thiols, are good nucleophiles for Michael addition reaction. The fluorescent labelling of several amines was performed. The reaction with benzylamine at room temperature did not give any changes (table 3.11, entry 1), even after refluxing the reaction mixture for 2h (entry 2). After addition of LiCl traces of a purple fluorescenting product were observed already after 2 h of refluxing (entry 3). Refluxing of the reaction mixture overnight in presence of LiCl furnished the desired product **93** in 83% yield (entry 4). The Li-salt as a Lewis acid propably increased the electrophility of the coumarin through coordination to the carbonyl group.

Table 3.11 Michael-addition reaction with benzylamine:



	Conditons	Yield
1	Room temperature, 2h	No reaction
2	2h, reflux	No reaction
3	+LiCl, 2h, reflux	Traces of the product
4	+LiCl, reflux, overnight	83%

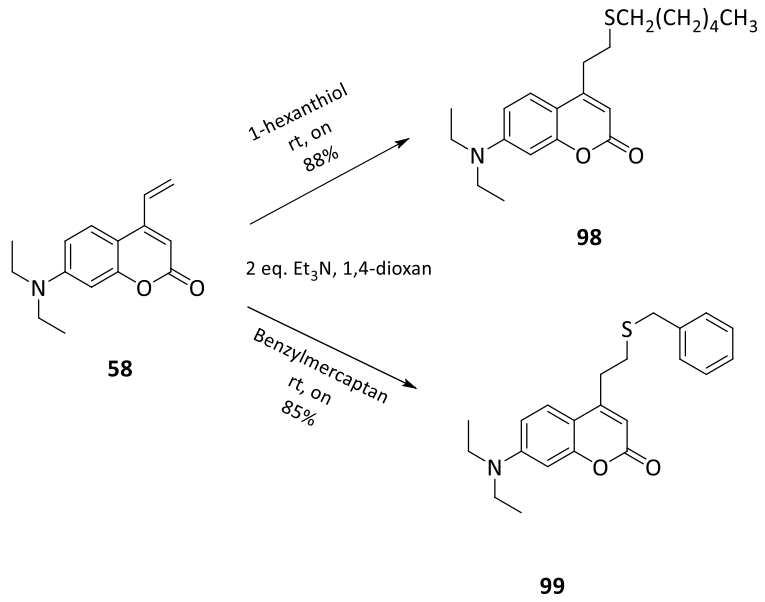
The further *N*-nucleophile Leucine methylester·HCl furnished the addition compound **97** in 60% yield.



58 **97**

Scheme 3.39: Michael-addition reaction with BocLeucine methylester.

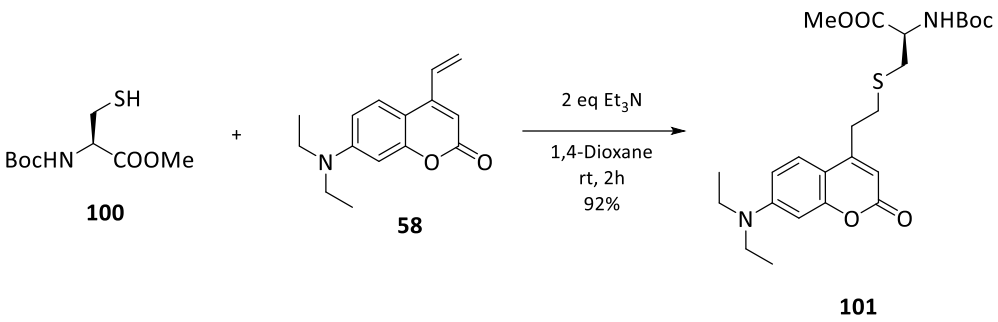
The thiol nucleophiles which were successfully used in En-Click-Chemistry[165] were employed as possible nucleophiles in Michael-addition with fluorescent dye **58**. 1-hexanthiol and benzylmercaptan furnished the addition compounds **98** and **99** in 88% and 85% yield, correspondingly (Scheme 3.40).



58 **98** **99**

Scheme 3.40: Michael-addition reaction with 1-hexanthiol and benzylmercaptan.

Cystein is one of the most known targets for fluorescent labelling (Chap.2.6.1). Therefore, BocCysOMe[165] was chosen as a target nucleophile for Michael-addition, which proceeded in a very good yield (Scheme 3.41).



Scheme 3.41: Fluorescent labelling of Boc-Cystein methylester.

3.8 Spectral characteristics of aminocoumarins

Absorption, excitation, emission spectra and life times of all synthesized 7-aminocoumarin derivatives were measured at Saarland University in the group of Prof. G. Jung by Andreas Grüter. The measurements were done in quartz cuvettes with 1 cm diameter in spectroscopically pure DMSO. The absorption spectra were measured with *V-650* spectrometer of *Jasco* Company, while excitation and emission spectra were done using *FP-6500* spectrometer of *Jasco* Company. The obtained data were processed with the Software Origin 6.1. Absorption, excitation and emmistion maxima are given in table 3.12.

Table 3.12 Absorption, excitation and emmision maxima of all the coumarins:

	Compound	$\lambda_{max,abs}$ [nm]	$\lambda_{max,ex}$ [nm]	$\lambda_{max,\ em}$ [nm]
1	**85**	405	410	502
2	**92**	397	404	481
3	**21**	399	404	479
4	**71**	393		484
5	**75**	396		478
6	**26**	401	409	498
7	**29**	400	407	483
8	**31**	398	407	485
9	**27**	400	409	488
10	**36**	398	411	490
11	**37**	400	411	489
12	**38**	404	411	486
13	**39**	396	410	536
14	**44**	386	388	520

15	**40**	386	387	522
16	**41**	386	388	520
17	**42**	384	389	524
18	**45**	384	388	517
19	**46**	381	390	528
20	**47**	383	385	513
21	**48**	388	391	531
22	**20**	388	389	500
23	**49**	356	358	412
24	**50**	345	345	390
25	**51**	346	350	405
26	**53**	352	416	417
27	**54**	356	359	418
28	**52**	355	356	409
29	**55**	351	361	410
30	**96**	364	370	420
31	**97**	363	368	421
32	**56**	441	369	432

Absorption maxima for all the coumarin lay in the range between 345-414 nm, while emission maxima are in the range between 384-528 nm (in the range between violet and yellow). Coumarins synthesized through Sonogashira coupling reactions (Chap. 3.4.2, Table 3.12, entries 1-12) exhibit (due to π-system expansion) absorption maxima at the range around 400 nm, emission occurs in the range between 479-502 nm (Figure 3.8 and 3.9).

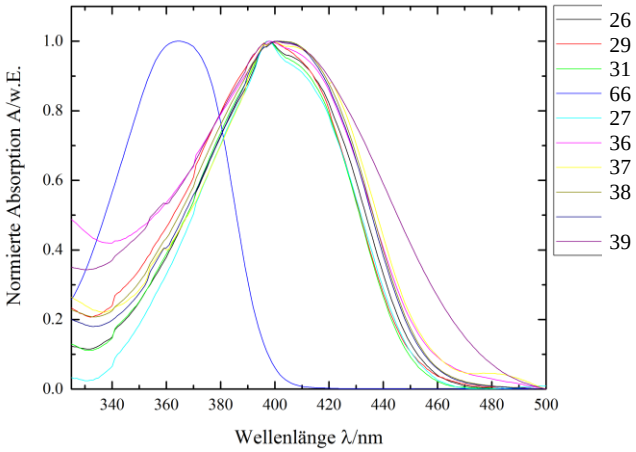


Figure 3.8: Absorption spectra of coumarins obtained through Sonogashira coupling.

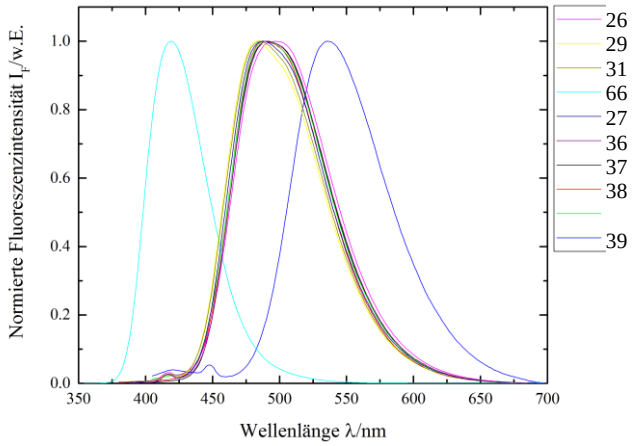


Figure 3.9: Emmision spectra of coumarins obtained through Sonogashira coupling.

An interesting observation was made for the coumarins obtained through Pd-catalyzed cross-coupling with CO-insertion (Chap. 3.4.3, entries 13-22). For these amides absorption occurs at the range between 381-388 nm, while the emission occurs at over 500 nm (Table 3.12, Figure 3.10-3.11).

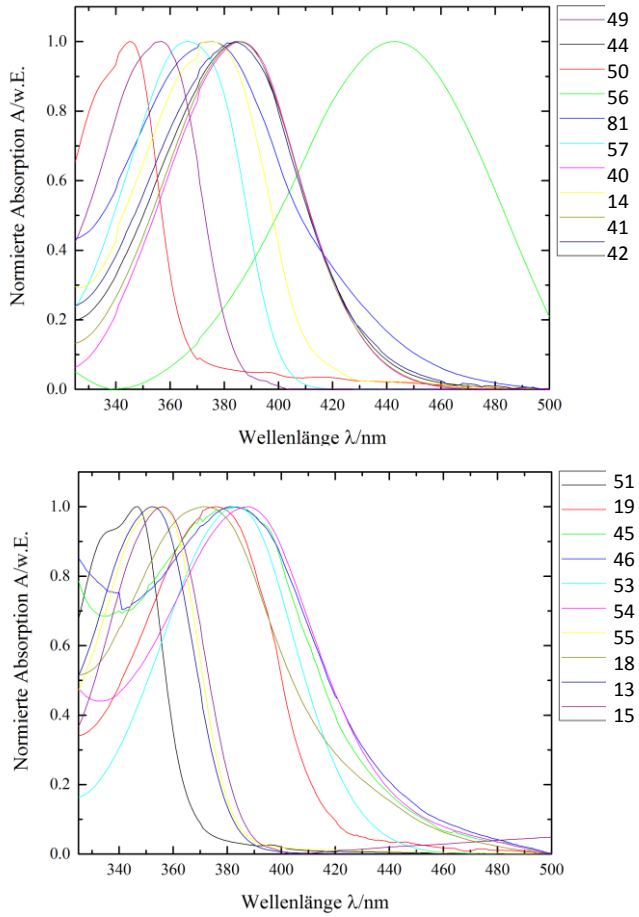


Figure 3.10: Absorption spectra of coumarins obtained through cross-coupling with CO-insertion and through direct substitution.

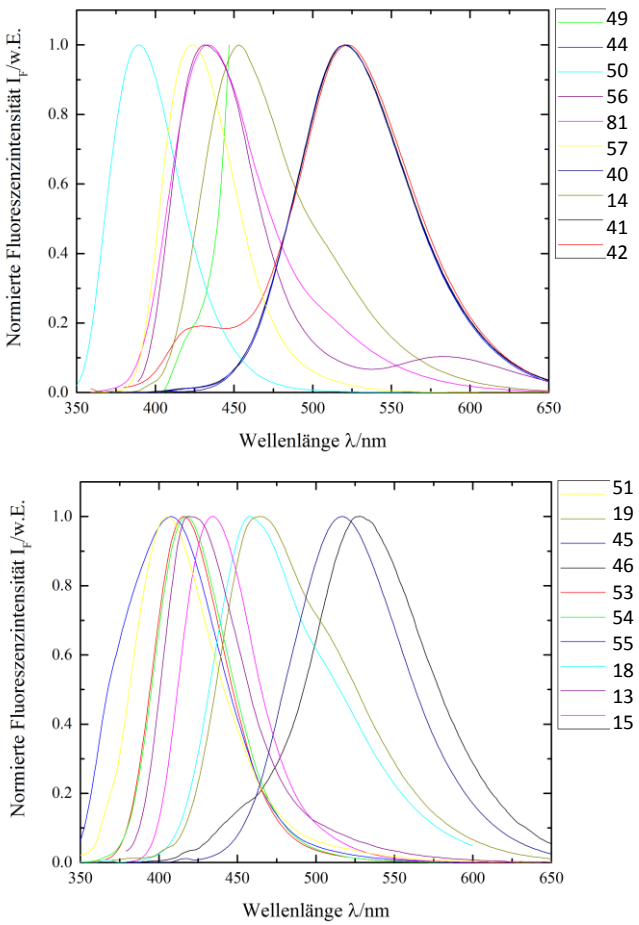


Figure 3.11: Emission spectra of coumarins obtained through cross-coupling with CO-insertion and through direct substitution.

Coumarins obtained through direct substitutions (Chap. 3.4.3, table 3.12, entries 23-32) do not emit at longer wavelength, in this case absorption was observed in the range 345-366 nm, and emission at 396-423 nm. Fluorescenting aldehyde (Chap. 3.5, table 3.12, entry 42) reveals anti-Stokes shift, while absorption occurs at 441 nm and emmisiton maxima is at 432 nm (Figure 3.10 and 3.11).

The fluorescent data of all other coumarins are given in the experimental section.

4

Experimental Section

4.1 General information

All reactions were carried out in oven-dried glassware (100 °C) under nitrogen atmosphere unless otherwise stated. For drying of organic phases water-free sodium sulphate was used.

^{1}H-NMR spectra were recorded on a 400 MHz nuclear magnetic resonance spectrometer (model *Bruker AVII-400*). $CDCl_3$ was used as solvent. The solvent peak was calibrated according the used solvent ($CDCl_3$: δ=7.26 ppm). The analysis of spectra was done with *PC-software MestRe-C*. Multiplicities are reported as (br) broad, (s) singlet, (d) doublet, (dd) doublet of doublets, (t) triplet, (q) quartet and (m) multiplet. Chemical shifts are δ-values and reported in ppm.

^{13}C-NMR-spectra were recorded on a frequency of 100 MHz on a nuclear magnetic resonance spectrometer (*Bruker AV-400*). $CDCl_3$ was used as solvent. The solvent peak was calibrated at 77.0 ppm. The analysis of spectra was done with *PC-software MestRe-C*. Multiplicities are reported as: (s) singlet (quarterly C- atom), (d) doublet (CH-group), (t) triplet (CH_2-group), (q) quartet (CH_3-group). Chemical shifts are δ-values and reported in ppm. Two-dimensional measuring methods as **H,H-COSY** and **HSQC** were used for the correlation of ^{1}H- and ^{13}C-signals.

The products were purified by **flash chromatography** on silica gel columns (*Macherey-Nagel* 60, 0.063-0.2 mm).

Melting points were measured in open glass capillaries on apparatus *MEL-TEMP II* purchased from *Laboratory Devices* and are uncorrected.

Thin-layer chromatography was done by using commercially available precoated Polygram® SIL-G/UV 254 plates purchased from *Fluka.* The detection of spots was done under UV-light, Ninhydrin or $KMnO_4$ solution.

Elemental analysis was performed at the Institute for Organic Chemistry, University of Saarland by Ms Susanne Harling on the instrument *Leco* (model *CHN900*).

High resolution mass spectrometry (HRMS) was performed at the Institute for Organic Chemistry, University of Saarland by Mr. Rudi Thomas on the instrument *MAT 95Q* purchased from the company *Finnigan*. The fragmentation was carried out through chemical ionization (CI) or electron ionization (EI).

Dried **solvents** were distilled before use: THF was dried over sodium with benzophenone as an indicator, dichloromethane (DCM) was dried over powdered CaH_2. Commercial grade solvents like ethyl acetate, hexane, diethylether were distilled prior to use.

Absorption, excitation, emission spectra and life times were measured at Saarland University in the group of Prof. G. Jung by Andreas Grüter. The measurements were done in quartz cuvettes with 1 cm diameter in spectroscopically pure DMSO. The absorption spectra were measured with *V-650* spectrometer of *Jasco* Company, while excitation and emission spectra were done using *FP-6500* spectrometer of *Jasco* Company.

4.2 General Experimental Procedures (GPs)

GP 1: Suzuki-Miyaura coupling [93]

To the solution of $Pd(PPh_3)_4$ (2 mol%), K_2CO_3 (2 eq.) and enol triflate **12** (1eq.) in toluene (5 mL/mmol) under nitrogen atmosphere arylboronic acid (1.2 eq.) was added at rt and the reaction mixture was refluxed until complete conversion of the coumarin was observed. Afterwards the reaction was quenched with 1M NH_4Cl and extracted with EA. The combined organic phase was dried over anhydrous Na_2SO_4, concentrated and chromatographed on silica gel column to afford the desired product.

GP 2: Sonogashira coupling reaction [137]

To the solution of $Pd(PPh_3)_4$ (2 mol%), CuI (4 mol%) and enol triflate **12** (1eq.) in abs. THF (5 mL/mmol) under nitrogen atmosphere Et_3N (1 eq.) and propargylic substrate (2 eq.) were added at rt; the reaction mixture was stirred at rt until complete conversion of coumarin was observed. Afterwards the reaction was quenched with 1M NH_4Cl, extracted with DCM. The combined organic phase was dried over anhydrous Na_2SO_4, concentrated, and chromatographed on silica gel column to afford the desired product.

GP 3: Ugi reaction

To the solution of benzyl amine (1 eq.) in dry DCM (2 ml/mmol) aldehyde **32** (1 eq.) in 0.5 ml dry DCM was added at 0 °C. The mixture was stirred for 30 min at this temperature before amino acid (1 eq.) in 0.5 ml dry DCM was added. The mixture was stirred for another 30 minutes. Solution of isocyanide (1 eq. 1 mmol) in 0.5 ml dry DCM was added slowly and the reaction mixture was warmed up to rt overnight. The reaction mixture was dissolved with DCM, washed 2x with conc. $NaHCO_3$ and 1M $KHSO_4$ solutions. The combined organic phase was dried over anhydrous Na_2SO_4, concentrated, and chromatographed on silica gel column to afford the desired product.

GP 4: Palladium-catalyzed carbonylation of enol triflates [151]

To the solution of enol triflate **12** (1 eq.) and amine (1.2 eq.) in dry toluene (10 ml /mmol) $Pd(PPh_3)_2Cl_2$ (10 mol%) and Et_3N (2 eq.) were added. The reaction mixture was stirred under CO atmosphere (15 bar) overnight before it was diluted with DCM and quenched with 1M NH_4Cl-solution. The aqueous layer was extracted three times

with DCM and combined organic layers were dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product purified by column chromatography.

GP 5: Nucleophilic substitution

To the solution of enol triflate **12** (1 eq.) and amine (1.2 eq.) in dry toluene (10 ml /mmol) Et_3N (2 eq.) was added. The reaction mixture was refluxed overnight. Afterwards it was diluted with DCM and quenched with 1M NH_4Cl-solution. The aqueous layer was extracted three times with DCM and combined organic layers were dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product purified by column chromatography.

GP 6: Dess-Martin oxidation

To the solution of the corresponding alcohol (1 eq) in DCM (20 mL/mmol) Dess-Martin periodinane (1.2 eq.) was added at rt and the resulting solution was stirred until the complete conversion of alcohol at rt. Afterwards the reaction mixture was washed with sat. solution of $Na_2S_2O_3$ and NaOH. The aqueous layer was extracted three times with DCM and combined organic layers were dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product purified by column chromatography.

GP 7: Grignard Reaction

To the solution of the corresponding aldehyde (1 eq) in abs. THF (1.2 ml/mmol) 1M Vinylmagnesiumbromide-solution in THF (1.3 eq) was added slowly at –20 °C. The cooling bath was removed and the mixture was stirred until complete conversion was observed. Afterwards the reaction mixture was diluted with DCM and hydrolyzed at 0 °C with 1M NH_4Cl-solution. The aqueous layer was extracted three times with DCM and the combined organic layers were dried over Na_2SO_4. The solvent was evaporated in vacuo and the crude product purified by column chromatography.

GP 8: Synthesis of carbonates [152]

To the solution of an allylalcohol (1 eq.) in dry pyridine (1ml/mmol) ethyl chloroformate (2 eq.) was added dropwise at 0 °C. The reaction mixture was allowed to warm to rt overnight before it was diluted with DCM and the organic layer was washed with 1M copper(II)solution until complete removal of the pyridine. The combined organic layers were dried over Na_2SO_4, the solvent evaporated in vacuo and the crude product purified by column chromatography.

GP 9: Pd-catalyzed allylic alkylation of amino acids [152,117]

In a Schlenk flask HMDS (2.8 eq) was dissolved in abs. THF (2 ml), *n*-Buli (1.6M in hexane, 2.5 eq.) was added dropwise at –78 °C. The cooling bath was removed and the colourless mixture was stirred for 10 min at room temperature. After cooling again to –78 °C, TFA-glycin-*tert*-butylester (1 eq.), dissolved in abs. THF (5 ml/mmol) was added slowly. The mixture was stirred for another 15 minutes. In the second Schlenk flask $ZnCl_2$ (1.1 eq) was heated carefully in vacuo, and after cooling down to room temperature it was dissolved in 1 ml abs. THF. The $ZnCl_2$-solution was added dropwise at –78 °C to the Li-enolate and the resulting mixture was stirred for additional 45 minutes to complete transmetallation.

Meanwhile, allylpalldium(II) chloride dimer (1 mol%) and triphenylphosphine (4.5 mol%) were dissolved in THF (2 ml) and stirred for 10 minutes at rt before the allylcarbonate (0.7 eq.) was added.

The catalyst-substrate solution was added dropwise to the Zink-enolate solution at –78 °C. The reaction mixture was warmed up overnight to rt, after monitoring by TLC, the reaction mixture was diluted with EA and quenched with 1N $KHSO_4$-solution. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography.

GP 10: Michael addition of Thiols/amines [153]

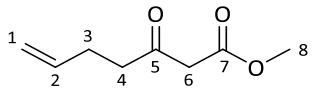
To the solution of the corresponding thiol/amine (1.2 eq) in 1.4-dioxane (5 mL/mmol) Et_3N (1.0 eq.) and vinyl coumarin (1 eq.) solution in 1.4-Dioxane were added at rt. The resulting solution was stirred until complete conversion of the coumarin at rt. Afterwards the reaction mixture was diluted with DCM and quenched with 1M NH_4Cl-solution. The aqueous layer was extracted three times with DCM and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by column chromatography.

4.3 Synthesis of compounds

Methyl 3-oxohept-6-enoate (1)[120]

A suspension of 2.40 g (25.3 mmol, 1.04 eq.) magnesiumchloride and 5.70 g (36.5 mmol, 1.50 eq.) methyl-potassiummalonate in 35 ml abs. THF was mixed under nitrogen atmosphere at 50 °C for 4 h. Meanwhile in another Schlenk flask 4.73 g (29.2 mmol, 1.20 eq.) 1,1'-carbonyldiimidazole was added in portions to the solution of 2.43 g (24.3 mmol, 1.00 eq.) 4-Pentenoic acid in 50 ml abs. THF at 5 °C and the mixture was stirred for one h at rt. The imidazolid solution of the second flask was added to methylmagnesiummalonatsolution at rt and was stirred overnight at rt. The solvent was removed in vacuo and the crude product was suspended in ethylacetate. The suspension was washed with 1 M $KHSO_4$-solution, sat. $NaHCO_3$-solution and sat. NaCl-solution. The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (silica gel, Hex:EA 8:2) to furnish 2.93 g (18.7 mmol, 87%) of **1** a slightly yellow oil.

R_f(1) = 0.32 (Hex:EA 8:2)



1

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 2.34 (m, 2 H, 3-H), 2.64 (t, $^3J_{4,3}$ = 7.3 Hz, 2 H, 4-H), 3.45 (s, 2 H, 6-H), 3.73 (s, 3 H, 8-H), 5.04 (m, 2 H, 1-H), 5.79 (ddt, $^3J_{2,1cis}$ = 16.8 Hz, $^3J_{2,1trans}$ = 10.2 Hz, $^3J_{2,3}$ = 6.5 Hz, 1 H, 2-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

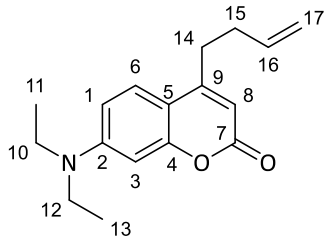
δ = 27.4 (t, C-3), 42.0 (t, C-4), 49.0 (t, C-6), 52.3 (q, C-8), 115.6 (t, C-1), 136.5 (d, C-2), 167.5 (s, C-7), 201.8 (s, C-5).

4-(But-3-enyl)-7-(diethylamino)-2H-chromen-2-one (2) [19]

1.65 g (10.0 mmol, 1.00 eq.) 3-diethylaminophenol and 1.5 g (10.0 mmol, 1.00 eq.) β-Ketoester **1** were suspended in 30 ml abs. toluene. Afterwards 20 ml (20.0 mmol, 2.00 eq.) 1 M chlorotri-*iso*-propoxytitan solution in hexane was added. The reaction mixture was refluxed overnight. After cooling down to room temperature the mixture was diluted with dichlomethane, poured into the sat. Na-/K-Tatrat-solution and stirred until phases separation. The phases were separated in the separation funnel and the water phase was extracted three times with DCM. The combined organic layers were dried over Na_2SO_4 and the solvent was

evaporated in vacuo. The crude product was purified by column chromatography (silica gel, PE:EA 7:3) to furnish 810 mg (3 mmol, 30%) of **2** as a yellow oil.

R_f(2) = 0.11 (DCM:EA 95:5)



2

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 13-H, 11-H), 2.42 (td, $^3J_{15,14}$ = 7.7 Hz, $^3J_{15,16}$ = 6.5 Hz, 2 H, 15-H), 2.74–2.78 (sh, 2 H, 17-H), 3.41 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.07 (m, 2 H, 14-H), 5.87 (ddt, $^3J_{16,17cis}$ = 16.8 Hz, $^3J_{16,17trans}$ = 10.2 Hz, $^3J_{16,15}$ = 6.5 Hz, 1 H, 16-H), 5.94 (s, 1 H, 8-H), 6.50 (d, $^4J_{3,1}$ = 2.6, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.40 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H)

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C-13), 31.0 (t, C-15), 32.4 (t, C-14), 44.7 (t, C-10/C-12), 97.9 (d, C-3), 107.8 (d, C-1), 108.2 (s, C-5), 108.4 (d, C-8), 115.9 (t, C-17), 125.2 (d, C-6), 136.8 (d, C-16), 150.4 (s, C-2), 155.9 (s, C-4), 156.3 (s, C-9), 162.4 (s, C-7).

Elemental Analysis:

$C_{17}H_{21}NO_2$	Calculated	C 74.68	H 7.44	N 5.44
271.3600	Found	C 74.94	H 7.44	N 5.73

HRMS (CI):	Calculated	Found
$C_{17}H_{21}NO_2[M+H]^+$:	272.1645	272.1649

Absorption max.: 369 nm

Exitation max.: 368 nm

Emission max.: 423 nm

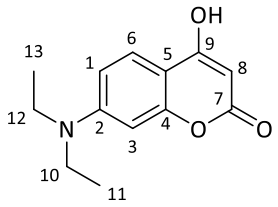
Lifetime: 3.11 ns

7-(Diethylamino)-4-hydroxy-2H-chromen-2-one (4) [154]

10.7 g (65.0 mmol, 1 eq) 3-diethylaminophenol and 30 g (65.0 mmol, 1 eq.) bis(2,4,6-trichlorphenyl)malonate **6** were dissolved in anhydrous toluene (195 ml) and heated to reflux overnight. Cooling to room temperature afforded a precipitate

that was filtered off. The crude product was washed with toluene to furnish 11 g (47 mmol, 72%) of **4** as a light-green solid compound.

R_f(4) = 0.14 (DCM:MeOH 95:5)



4

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.25 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.48 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.51 (s, 1 H, 8-H), 6.52 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.72 (dd, $^3J_{1,6}$ = 9.2 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.87 (d, $^3J_{6,1}$ = 9.2 Hz, 1 H, 6-H), 10.99 (s, 1 H, OH)

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.3 (q, C-11/C13), 44.0 (t, C-12/C-10), 86.1 (d, C-8), 96.4 (d, C-3), 103.5 (s, C-5), 108.2 (d, C-1), 124.2 (d, C-6), 150.9 (s, C-2), 156.1 (s, C-4), 163.0 (s, C-7), 166.6 (s, C-9).

Elemental Analysis:

$C_{13}H_{15}NO_3$	Calculated	C 66.94	H 6.48	N 6.00
(233.2665)	Found	C 66.67	H 6.52	N 5.97

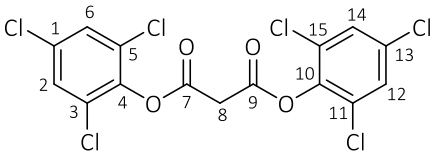
HRMS (CI):	Calculated	Found
$C_{13}H_{15}NO_3[M]^+$:	233.1046	233.1047
Absorption max.:	350 nm	
Exitation max.:	349 nm	
Emission max.:	387 nm	
Lifetime:	2.61/1.31 ns	
Melting point:	234-236 °C (lit 238 °C)	

Bis(2,4,6-trichlorphenyl)malonate (6) [154]

15.8 g (80.0 mmol, 1 eq) 2,4,6-trichlorphenol and 4.16 g (40.0 mmol, 0.5 eq.) malonic acid were refluxed in 10 ml (107 mmol, 1.2 eq) phosphorus (V) oxychloride for 3 h. After cooling to rt, the reaction mixture was quenched with ice water (50 ml), and the resulting precipitate was collected by filtration. The crude product was suspended in water (20 ml), saturated sodium hydrogen carbonate solution (5 ml) was added, and the suspension was stirred for 15 min. The undissolved material was

filtered off and recrystallized from ethyl acetate to furnish **6** 15.2 g (33 mmol, 83%) as colorless cubes.

R_f(6) = 0.44 (PE:EA 7:3)



6

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 4.04 (s, 2 H, 8-H), 7.38 (s, 4 H, 2-H, 6-H, 12-H, 14-H).

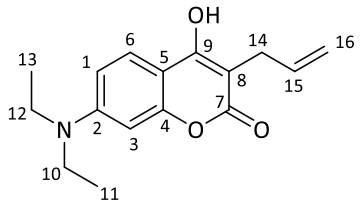
^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 39.7 (t, C-8), 128.7 (d, C-12/C-14/C-2/C-6), 129.5 (d, C-12/C-14/C-2/C-6), 132.7 (s, C-3/C5/C-11/C-15/C-13/C-1), 142.4 (s, C-10/C4), 161.4 (s, C-7/C-9).

3-Allyl-7-(diethylamino)-4-hydroxy-2H-chromen-2-one (7)

58 mg (0.25 mmol, 1 eq.) 4-hydroxycoumarin **4**, 36 mg (0.28 mmol, 1.1 eq.) allyl ethyl carbonate, 1 mg (2.5 µmol, 2 mol%) allylpalladium(II) chloride dimer, 3 mg (11 µmol, 4.5 mol%) PPh_3 and 45 mg (0.33 mmol, 1.3 eq.) K_2CO_3 were dissolved in 1 ml DMF. The mixture was refluxed overnight. After cooling down to room temperature the mixture was filtered through celite, the filtrate was concentrated in vacuo and the crude product purified by column chromatography (silica gel, DCM:MeOH 98:2) to furnish 57.6 mg (0.21 mmol, 84%) of **7** as a yellow oil.

R_f(7) = 0.21 (DCM:MeOH 98:2)



7

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.44 (td, $^3J_{14,15}$ = 6.4 Hz, $^4J_{14,16cis}$ = $^4J_{14,16trans}$ = 1.6 Hz, 2 H, 14 H), 5.21 (ddd, $^3J_{16trans,15}$ = 10.0 Hz, $^2J_{16trans,16cis}$ = $^4J_{16trans14}$ = 1.4 Hz, 1 H, 16_{trans}-H), 5.33 (ddd, $^3J_{16cis,15}$ = 17.2 Hz, $^2J_{16cis,16trans}$ = $^4J_{16cis14}$ = 1.6 Hz, 1 H, 16_{cis}-H), 6.02 (ddd, $^3J_{15,16cis}$ = 16.4 Hz, $^3J_{15,16trans}$ = 10.0 Hz, $^3J_{15,14}$ = 6.4 Hz, 1 H, 15-H), 6.46 (d, $^4J_{3,1}$ = 2.3 Hz, 1 H,

3-H), 6.58 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.60 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 28.4 (t, C-14) 44.7 (t, C-12/C-10), 96.6 (d, C-3), 97.0 (s, C-8) 103.4 (s, C-5), 108.4 (d, C-1), 117.0 (t, C-16) 123.7 (d, C-6), 135.8 (d, C-15), 150.7 (s, C-2), 155.0 (s, C-4), 162.4 (s, C-9), 164.7 (s, C-7).

Elemental Analysis:

$C_{16}H_{19}NO_3$	Calculated	C 70.31	H 7.01	N 5.12
(273.3270)	Found	C 70.24	H 7.06	N 5.15

HRMS (CI):	Calculated	Found
$C_{16}H_{20}NO_3$ $[M+H]^+$:	274.1438	274.1438
Absorption max.:	348 nm	
Exitation max.:	352 nm	
Emission max.:	396 nm	
Lifetime:	2.44/0.87 ns	

4-(Allyloxy)-7-(diethylamino)-2H-chromen-2-one (8) [155]

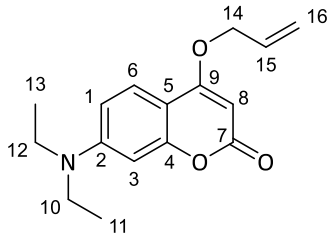
Method A

233 mg (1 mmol, 1 eq.) 4-hydroxycoumarin **4**, 0.09 ml (1 mmol, 1 eq.) allylbromide and 180 mg (1.3 mmol, 1.3 eq.) K_2CO_3 were dissolved in 1.3 ml acetone. The mixture was refluxed overnight. After cooling down to room temperature the mixture was filtered through a celite plug, the filtrate was concentrated in vacuo and the crude product was purified by column chromatography (silica gel, DCM:MeOH 98:2) to furnish 93 mg (0.34 mmol, 34%) of **8** as a yellow oil.

Method B (Mitsunobu reaction) [156]

1.57 g (6 mmol, 1.5 eq) PPh_3 was dissolved in 8 ml abs. THF, and the solution was cooled to –20 °C. 1.2 g (1.17 ml, 40 wt% solution in toluene, 6 mmol, 1.5 eq.) DIAD, 0.3 ml (4.8 mmol, 1.2 eq) allyl alcohol and 933 mg (4 mmol, 1.0 eq.) 4-hydroxycoumarin **4** were added successively. The reaction was allowed to warm to room temperature over 2 h and stirred at room temperature until complete converstion of 4-hydroxycoumarin **4**. 80 ml hexane was added to the reaction mixture to precipitate the triphenylphosphine oxide by-product. After filtration through celite, the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (silica gel, DCM:MeOH 98:2) to furnish 967 mg (3.54 mmol, 89%) of **8** as a yellow oil.

R_f(8) = 0.33 (DCM:MeOH 98:2)



8

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.63 (dt, $^3J_{14,15}$ = 5.3 Hz, $^4J_{14,16cis}$ = $^4J_{14,16trans}$ = 1.4 Hz, 2 H, 14-H), 5.37 (ddd, $^3J_{16trans15}$ = 10.6 Hz, $^2J_{16trans,16cis}$ = 2.4 Hz, $^4J_{16trans14}$ = 1.2 Hz, 2 H, 16_{trans}-H), 5.41 (s, 1 H, 8-H), 5.47 (ddd, $^3J_{16cis,15}$ = 17.3 Hz, $^2J_{16cis,16trans}$ = 2.8 Hz, $^4J_{16cis,14}$ = 1.5 Hz, 1 H, 16_{cis}-H), 6.06 (ddt, $^3J_{15,16cis}$ = 17.2 Hz, $^3J_{15,16trans}$ = 10.6 Hz, $^3J_{15,14}$ = 5.4 Hz, 1 H, 15-H), 6.46 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.59 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H)

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 44.7 (t, C-12/C-10), 69.4 (t, C-14), 86.0 (d, C-8), 97.1 (d, C-3), 103.8 (s, C-5), 108.2 (d, C-1), 118.9 (t, C-16) 123.9 (d, C-6), 131.2 (d, C-15), 151.0 (s, C-2), 155.8 (s, C-4), 164.2 (s, C-7), 166.2 (s, C-9).

Elemental Analysis:

$C_{16}H_{19}NO_3$	Calculated	C 70.31	H 7.01	N 5.12
(273.3270)	Found	C 70.25	H 7.12	N 5.09

HRMS (CI):	Calculated	Found
$C_{16}H_{20}NO_3$ $[M+H]^+$:	274.1438	274.1444

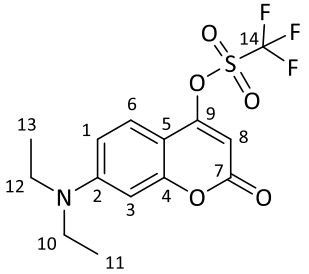
Absorption max.:	350 nm
Exitation max.:	350 nm
Emission max.:	398 nm
Lifetime:	2.44/0.87 ns

7-(Diethylamino)-2-oxo-2H-chromen-4-yl trifluoromethanesulfonate (12) [129]

Under nitrogen atmosphere 3.2 g (13.8 mmol, 1 eq.) 4-hydroxycoumarin **4** was dissolved in dry DCM (69 mL) and 3.8 ml (27.6 mmol, 2 eq.) Et_3N was added. The solution was cooled to –20 °C, and treated with dropwise addition of 5 ml (5 g, 18 mmol, 1.3 eq.) fresh triflic anhydride. The resulting mixture was stirred for additional 2 h. After complete conversion of **4**, the mixture was passed through a pad of silica gel with 1:30 EA/hexane washings, until no more aryl triflate was eluted out. The filtrate was concentrated under reduced pressure. The crude product was purified by

column chromatography (silica gel, DCM) to furnish 4.8 g (13.2 mmol, 96%) of **12** as a yellow solid compound.

R_f(12) = 0.15 (DCM:EA, 98:2)



12

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.22 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.43 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.05 (s, 1 H, 8-H), 6.51 (d, $^4J_{3,1}$ = 2.4 Hz, 1 H, 3-H), 6.64 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.42 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.3 (q, C-11/C13), 45.0 (t, C-12/C-10), 97.4 (d, C-3), 98.5 (s, C-8) 102.0 (s, C-5), 109.4 (d, C-1), 116.9 (s, C-14) 123.5 (d, C-6), 152.1 (s, C-2), 156.3 (s, C-4), 158.1 (s, C-7), 161.3 (s, C-9).

Elemental Analysis:

$C_{14}H_{14}F_3NO_5S$	Calculated	C 46.03	H 3.86	N 3.83
(365.3249)	Found	C 46.30	H 3.69	N 3.88

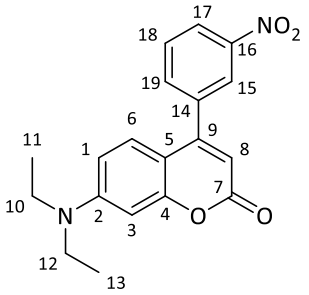
HRMS (CI):	Calculated	Found
$C_{14}H_{15}F_3NO_5S$ $[M]^+$:	365.0539	365.0531
Absorption max.:	351 nm	
Exitation max.:	383 nm	
Emission max.:	384 nm	
Lifetime:	2.93/0.22 ns	
Melting point:	77-79 °C	

7-(Diethylamino)-4-(3-nitrophenyl)-2H-chromen-2-one (13)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 40 mg (0.24 mmol, 1.2 eq.) 3-nitrophenylboronic acid was added. The reaction mixture was refluxed for 1 h. After work-up and column chromatography

(silica gel, DCM:EA 95:5) 60 mg (0.18 mmol, 89%) of **13** was isolated as an orange solid compound.

R_f(13) = 0.38 (DCM:EA 9:1)



13

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.22 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.43 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.02 (s, 1 H, 8-H), 6.53 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.58 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 7.10 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 7.68-7.78, 8.32-8.36 (2m, 4 H, Phe).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.9 (t, C-12/C-10), 98.0 (d, C-3), 107.1 (d, C-1/C-8), 108.9 (s, C-5), 123.4 (d, C-15/C-17), 124.1 (d, C-15/C-17), 127.1 (d, C-6), 130.0 (s, C-14), 134.3 (d, C-19), 137.9 (s, C-9), 148.4 (s, C-16), 151.0 (s, C-2), 156.9 (s, C-4), 161.5 (s, C-7).

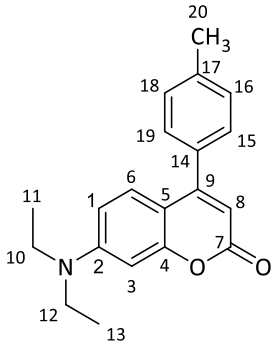
Elemental Analysis:

$C_{19}H_{18}N_2O_4$	Calculated	C 67.45	H 5.36	N 8.28
(338.3630)	Found	C 67.30	H 5.61	N 8.12
HRMS (CI):	Calculated		Found	
$C_{19}H_{18}N_2O_4$ $[M]^+$:	338.1261		338.1263	
Absorption max.:	382 nm			
Exitation max.:	367 nm			
Emission max.:	420 nm			
Melting point:	69-75 °C			

7-(Diethylamino)-4-(p-tolyl)-2H-chromen-2-one (14)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 33 mg (0.24 mmol, 1.2 eq.) p-tolylboronic acid was added. The reaction mixture was refluxed for 1 h. After work-up and column chromatography (silica gel, PE:EA 7:3) 60.1 mg (0.19 mmol, 95%) of **14** was isolated as a yellow solid compound.

R_f(14) = 0.22 (DCM:EA 98:2)



14

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.23 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 2.46 (s, 3 H, 20-H), 3.43 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.02 (s, 1 H, 8-H), 6.53 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.59 (d, $^4J_{3,1}$ = 2.4 Hz, 1 H, 3-H), 7.32 (m, 5 H, 6-H, Phe).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 21.3 (q, C-20), 44.8 (t, C-12/C-10), 97.9 (d, C-3), 108.0 (d, C-1/C-8), 108.4 (s, C-5), 126.9 (d, C-6), 128.4-129.2 (4d, C-Phe), 133.4 (s, C-14), 139.3 (s, C-17), 150.6 (s, C-2), 156.2 (s, C-4), 156.8 (s, C-9), 162.2 (s, C-7).

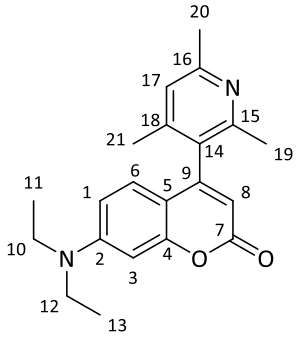
Elemental Analysis:

$C_{20}H_{21}NO_2$	Calculated	C 78.15	H 6.89	N 4.56
(307.3920)	Found	C 77.82	H 6.94	N 4.50
HRMS (CI):	Calculated		Found	
$C_{20}H_{21}NO_2$ $[M]^+$:	307.1567		307.1578	
Absorption max.:	373 nm			
Exitation max.:	375 nm			
Emission max.:	454 nm			
Melting point:	121-123 °C			

7-(Diethylamino)-4-(2,4,6-trimethylpyridin-3-yl)-2H-chromen-2-one (15)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 40 mg (0.24 mmol, 1.2 eq (2,4,6-trimethylpyridin-3-yl)boronic acid was added. The reaction mixture was refluxed for 1 h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 26.7 mg (0.079 mmol, 40%) of **15** was isolated as a yellow solid compound.

R_f(15) = 0.17 (DCM:EA 9:1)



15

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.50 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 2.37 (s, 3 H, 20-H), 2.61 (s, 3 H, 19-H), 2.85 (s, 3 H, 21-H), 3.70 (q$^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.19 (s, 1 H, 8-H), 6.73 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.86 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 7.00 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 7.26 (s, 1 H, 17-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 19.4 (q, C-21), 22.8 (q, C-19), 24.2 (q, C-20), 44.8 (t, C-12/C-10), 97.7 (d, C-3), 107.8 (d, C-1/C-8), 108.8 (d, C-1/C-8), 109.1 (s, C-5), 122.2 (d, C-17), 126.8 (s, C-14), 127.1 (d, C-6), 145.3 (s, C-2/C-18), 151.0 (s, C-4), 154.1 (s, C-9), 156.7 (s, C15/C-16), 157.6 (s, C-15/C-16), 162.1 (s, C-7).

Elemental Analysis:

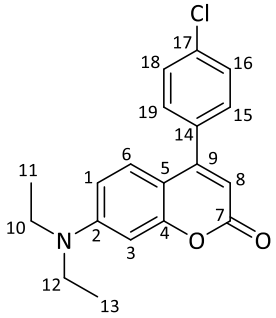
$C_{21}H_{24}N_2O_2$	Calculated	C 74.97	H 7.19	N 8.33
(336.4350)	Found	C 74.07	H 7.21	N 7.76
HRMS (CI):	Calculated		Found	
$C_{21}H_{24}N_2O_2$ $[M]^+$:	336.1838		336.1852	
Absorption max.:	376 nm			
Exitation max.:	378 nm			
Emission max.:	434 nm			
Melting point:	126-130 °C			

4-(4-Chlorophenyl)-7-(diethylamino)-2H-chromen-2-one (16)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 38 mg (0.24 mmol, 1.2 eq) 4-chlorophenylboronic acid was added. The reaction mixture was refluxed for 2 h. After work-up and column chromatography

(silica gel, DCM:EA 95:5) 61.8 mg (0.19 mmol, 94%) of **16** was isolated as a yellow solid compound.

R_f(16) = 0.37 (DCM:EA 9:1)



16

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.98 (s, 1 H, 8-H), 6.51 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.56 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 7.19 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 7.39 (dt, $^3J_{19,18}$ = $^3J_{15,16}$ = 8.5 Hz, $^4J_{19,15}$ = $^4J_{15,19}$ = 2.2 Hz, 2 H, 19-H, 15-H), 7.48 (d, $^3J_{16,15}$ = $^3J_{18,19}$ = 8.5 Hz, $^4J_{16,18}$ = $^4J_{18,16}$ = 2.2 Hz, 2 H, 16-H, 18-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.9 (d, C-3), 107.6 (d, C-1/C-8), 108.4 (d, C-1/C-8), 108.6 (s, C-5), 127.6 (d, C-6), 129.0 (d, C-19/C-15), 129.7 (d, C-16/C-18), 134.7 (s, C-17), 135.4 (s, C-14), 150.8 (s, C-2), 154.8 (s, C-4), 156.8 (s, C-9), 161.9 (s, C-7).

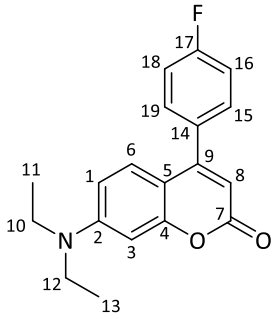
Elemental Analysis:

$C_{19}H_{18}ClNO_2$	Calculated	C 69.62	H 5.53	N 4.27
(327.8080)	Found	C 69.50	H 5.75	N 4.39
HRMS (CI):	Calculated		Found	
$C_{19}H_{18}ClNO_2$ $[M]^+$:	327.1021		327.1021	
Absorption max.:	380 nm			
Exitation max.:	383 nm			
Emission max.:	477 nm			
Lifetime:	4.11 ns			
Melting point:	126-130 °C			

7-(Diethylamino)-4-(4-fluorophenyl)-2H-chromen-2-one (17)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 33.6 mg (0.24 mmol, 1.2 eq) 4-fluorophenylboronic acid was added. The reaction mixture was refluxed for 2 h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 46.3 mg (0.15 mmol, 85%) of **17** was isolated as a yellow solid compound.

R_f(17) = 0.18 (DCM:EA 9:1)



17

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.42 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.98 (s, 1 H, 8-H), 6.52 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.57 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 7.19 (m, 3 H, 6-H, 19-H, 15-H), 7.42 (m, 2 H, 16-H, 18-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.9 (d, C-3), 106.7 (d, C-1/C-8), 107.8 (d, C-1/C-8), 108.56 (s, C-5), 115.9 (d, C-18/C-16), 127.7 (d, C-6), 130.3 (d, C-15/C-19), 132.3 (s, C-14), 150.7 (s, C-2/C-4), 155.0 (s, C-9), 162.0 (s, C-7), 164.5 (s, C-17).

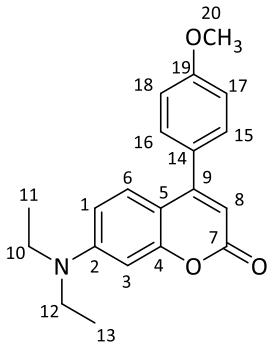
Elemental Analysis:

$C_{19}H_{18}FNO_2$	Calculated	C 73.30	H 5.83	N 4.50
(311.3564)	Found	C 72.12	H 4.84	N 4.36
HRMS (CI):	Calculated		Found	
$C_{19}H_{18}FNO_2$ $[M]^+$:	311.1316		311.1319	
Absorption max.:	375 nm			
Exitation max.:	381 nm			
Emission max.:	460 nm			
Lifetime:	3.62 ns			
Melting point:	125-128 °C			

7-(Diethylamino)-4-(4-methoxyphenyl)-2H-chromen-2-one (18)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 36.4 mg (0.24 mmol, 1.2 eq.) 4-methoxyphenylboronic acid was added. The reaction mixture was refluxed for 1 h. After work-up and column chromatography (silica gel, PE:EA 7:3) 60.7 mg (0.19 mmol, 95%) of **18** was isolated as a yellow solid compound.

R_f(18) = 0.59 (DCM:EA 95:5)



18

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.88 (s, 3 H, 21-H), 5.99 (s, 1 H, 8-H), 6.52 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.56 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 7.01 (dt, $^3J_{18,16}$ = $^3J_{17,15}$ = 8.8 Hz, $^4J_{18,17}$ = $^4J_{17,18}$ = 2.8 Hz, 2 H, 18-H, 17-H), 7.31 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H), 7.39 (dt, $^3J_{16,18}$ = $^3J_{15,17}$ = 8.8 Hz, $^4J_{16,15}$ = $^4J_{15,16}$ = 2.8 Hz, 2 H, 15-H, 16-H)

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 55.4 (q, C-20), 97.9 (d, C-3), 108.0 (d, C-1/C-8), 108.4 (s, C-5), 114.1 (d, C-18/C-17), 127.9 (s, C-14), 128.6 (d, C-6), 129.8 (d, C-16/C-15), 150.5 (s, C-2), 155.8 (s, C-4), 156.8 (s, C-9), 160.5 (s, C-19), 162.3 (s, C-7).

Elemental Analysis:

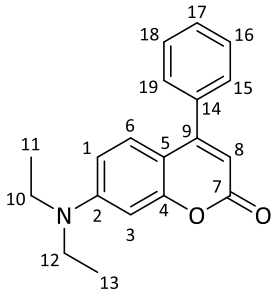
$C_{20}H_{21}NO_3$	Calculated	C 74.28	H 6.55	N 4.33
(323.3920)	Found	C 73.80	H 6.91	N 4.00
HRMS (CI):	Calculated		Found	
$C_{20}H_{21}NO_3$ $[M]^+$:	323.1516		323.1488	
Absorption max.:	376 nm			
Exitation max.:	379 nm			
Emission max.:	456 nm			

Melting point: 121-123 °C

7-(Diethylamino)-4-phenyl-2H-chromen-2-one (19)

According to the **GP-1** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 4.6 mg (4 µmol, 2 mol%.) $Pd(PPh_3)_4$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 1 ml dry toluone 29 mg (0.24 mmol, 1.2 eq.) phenylboronic acid was added. The reaction mixture was refluxed for 1 h. After work-up and column chromatography (silica gel, PE:EA 7:3) 61.6 mg (0.2 mmol, quant) of **19** was isolated as a yellow solid compound.

R_f(19) = 0.25 (PE:EA 7:3)



19

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.01 (s, 1 H, 8-H), 6.51 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.4 Hz, 1 H, 1-H), 6.57 (d, $^4J_{3,1}$ = 2.4 Hz, 1 H, 3-H), 7.25 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H), 7.45 (m, 5 H, Phe).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.8 (d, C-3), 108.0 (d, C-1/C-8), 108.5 (s, C-5), 127.9 (s, C-14), 128.4-129.2 (5d, C-Phe), 136.8 (s, C-14), 150.6 (s, C-2), 156.1 (s, C-4), 156.8 (s, C-9), 162.2 (s, C-7).

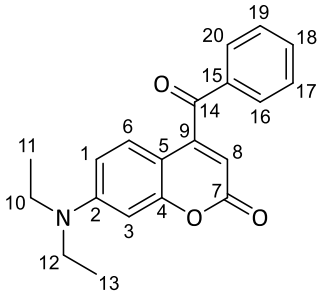
Elemental Analysis:

$C_{19}H_{19}NO_2$	Calculated	C 77.79	H 6.53	N 4.77
(293.3660)	Found	C 77.76	H 6.71	N 4.62
HRMS (CI):	Calculated		Found	
$C_{19}H_{19}NO_2$ $[M]^+$:	293.1410		293.1410	
Absorption max.:	376 nm			
Exitation max.:	379 nm			
Emission max.:	464 nm			
Melting point:	119-120 °C			

4-Benzoyl-7-(diethylamino)-2H-chromen-2-one (20) [157]

To the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12**, 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 33 mg (0.24 mmol, 2 eq.) K_2CO_3 in 2 ml anisole 29 mg (0.24 mmol, 1.2 eq) phenylboronic acid was added. The reaction mixture was refluxed under CO atmosphere (15 bar) overnight Afterwards the reaction was quenched with 1M NH_4Cl, extracted with EA. The combined organic phase was dried over anhydrous Na_2SO_4, concentrated, and purified by column chromatography (silica gel, DCM, DCM:EA 95:5) to furnish 10.2 mg (0.032 mmol, 16%) of **20** as a yellow oil.

R_f(20) = 0.35 (DCM:EA 9:1)



20

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.03 (s, 1 H, 8-H), 6.50 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.55 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 7.17 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 7.50 (m, 2 H, 17-H, 18-H), 7.66 (m, 1 H, 19 H), 7.96 (m, 2 H, 20-H, 16-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.3 (d, C-3), 107.9 (d, C-1/C-8), 108.4 (d, C-1/C-8), 108.5 (s, C-5), 127.7 (d, C-6), 128.4 (d, C-16/C-20), 128.6 (d, C-19/C-17), 134.5 (d, C-18), 137.9 (s, C-15), 150.6 (s, C-2/C-4), 156.1 (s, C-9), 159.3 (s, C-7), 197.5 (s, C-14)

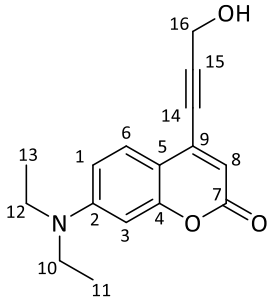
Elemental Analysis:

$C_{20}H_{19}NO_3$	Calculated	C 74.75	H 5.96	N 4.36
(321.3760)	Found	C 74.50	H 6.24	N 4.16
HRMS (CI):	Calculated		Found	
$C_{20}H_{19}NO_3$ $[M]^+$:	321.1365		321.1362	
Absorption max.:	388 nm			
Exitation max.:	389 nm			
Emission max.:	500 nm			
Lifetime:	2.99 ns			

7-(Diethylamino)-4-(3-hydroxyprop-1-yn-1-yl)-2H-chromen-2-one (21)

According to the **GP-2** to the solution of 23 mg (20 µmol, 2 mol%) $Pd(PPh_3)_4$, 4.5 mg (23 µmol, 4 mol%) CuI and 365 mg (1 mmol, 1 eq.) enol triflate **12** in 5 ml abs. THF under nitrogen atmosphere 0.15 ml (1 mmol, 1 eq) Et_3N and 0.1 ml (2 mmol, 2 eq.) propargylic alcohol were added at rt. The reaction mixture was stirred for 2 h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 218 mg (0.8 mmol, 80%) of **21** was isolated as a yellow solid compound.

R_f(21) = 0.24 (DCM:EA 9:1)



21

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 2.15 (sh, 1 H, OH), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.60 (s, 2 H, 16-H), 6.17 (s, 1 H, 8-H), 6.45 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.57 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.57 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 51.5 (t, C-16), 80.0 (s, C-14), 97.4 (s, C-15), 98.6 (d, C-3), 107.8 (d, C-1), 108.8 (s, C-5), 111.7 (d, C-8) 127.5 (d, C-6), 136.8 (s, C-9), 151.1 (s, C-2), 156.1 (s, C-4), 161.7 (s, C-7).

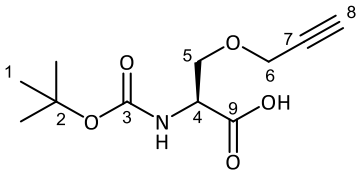
Elemental Analysis:

$C_{16}H_{17}NO_3$	Calculated	C 70.83	H 6.32	N 5.16
(271.32)	Found	C 70.95	H 6.41	N 4.87
HRMS (CI):	Calculated		Found	
$C_{16}H_{18}NO_3$ $[M]^+$:	271.1203		271.1209	
Absorption max.:	399 nm			
Exitation max.:	404 nm			
Emission max.:	479 nm			
Lifetime:	5.417 ns			
Melting point:	144-145 °C			

N-(tert-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine (23) [158]

To 25 mg (1.03 mmol, 1.03 eq.) NaH suspension in 1 ml abs. DMF the solution of 219 mg (1 mmol, 1 eq.) N-Boc-L-serine in 1 ml abs. DMF was added at 0 °C. The reaction mixture was stirred for 1 h at 0 °C before 91 µl (121.73 mg, 1.03 mmol, 1.03 eq.) propargylic bromide was added. The reaction mixture was stirred overnight. The solvent was evaporated in vacuo and the crude product was dissolved in dest. H_2O and extracted 3x with diethylether; the water phase acidified with conc. HCl-solution to pH 2 and extracted again with EA. The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (silica gel, PE:EA:HOAc 1:1:0.01) to furnish 170 mg (0.7 mmol, 70%) of the desired product **23** as a light yellow oil.

R_f(23) = 0.54 (PE:EA 1:1)



23

^{1}H-NMR (400 MHz, *$CDCl_3$*)**:**

δ = 1.46 (s, 9 H, 1-H), 2.50 (t, $^3J_{8,6}$ = 2.5 Hz, 1 H, 8-H), 3.90-4.02 (m, 2 H, 5-H), 4.43 (s, 1 H), 4.42 (sh, 1 H, 4-H), 4.78 (d, $^3J_{6,8}$ = 2.5 Hz, 1 H, 6-H), 5.42 (sh, 1 H, NH).

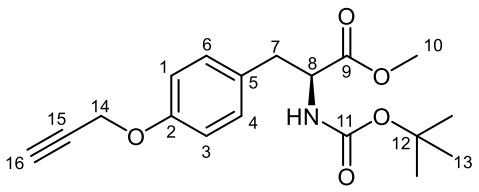
^{13}C-NMR (100 MHz, *$CDCl_3$*)**:**

δ = 28.3 (q, C-1), 53.7 (d, C-4), 58.7 (t, C-6), 69.5 (t, C-5), 75.3 (d, C-8), 78.7 (s, C-2), 80.4 (s, C-7), 155.7 (s, C-3), 175.1 (s, C-9).

L-tert-Butyloxycarbonyl-O-propargyl-L-tyrosine methyl ester (25)

563 mg (2 mmol, 1 eq.) Boc-tyrosine was dissolved in 4 ml abs. DMF, 331.7 mg (2.4 mmol, 1.2 eq.) K_2CO_3 and 0.18 ml (243 mg, 2.06 mmol, 1.03 eq.) propargylic bromide were added at 0 °C. The reaction mixture was stirred overnight at rt before it was washed with sat. NaCl-solution. The aqueous layer was extracted 3x with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, PE:EA 7:3) to furnish 628 mg (1.97 mmol, 98.5 %) of **25** as a colourless oil.

R_f(25) = 0.32 (PE:EA 7:3)



25

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.41 (s, 9 H, 13-H), 2.51 (t, $^{4}J_{16,14}$ = 2.4 Hz, 1 H, 16-H), 3.02 (dq, $^{3}J_{7,8}$ = 5.9 Hz, $^{2}J_{7,7'}$ = $^{2}J_{7',7}$ = 14.0 Hz, 2 H, 7-H, 7'-H), 3.70 (s, 3 H, 10-H), 4.54 (m, 1 H, 8-H), 4.7 (d, $^{4}J_{14,16}$ = 2.4 Hz, 2 H, 14-H), 4.96 (d, $^{3}J_{NH,8}$ = 7.9 Hz, 1H, NH), 6.90 (d, $^{3}J_{3,4}$ = $^{3}J_{1,6}$ = 8.7 Hz, 2 H, 1-H, 3-H), 7.05 (d, $^{3}J_{4,3}$ = $^{3}J_{6,1}$ = 8.5 Hz, 2 H, 6-H, 4-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 28.3 (q, C-13), 37.5 (t, C-7), 52.2 (q, C-10), 54.5 (t, C-14), 55.8 (d, C-8), 75.2 (d, C-16), 78.6 (s, C-15/C-12), 79.9 (s, C-15/C-12), 115.0 (d, C-1/C-3) 129.0 (s, C-5), 130.3 (d, C-4/C-6), 155.1 (s, C-2/C-11), 156.7 (s, C-2/C-11), 172.4 (s, C-9).

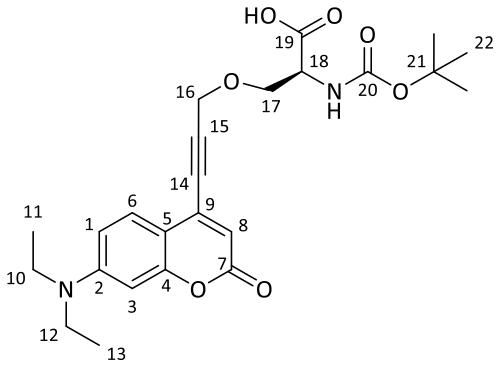
Elemental Analysis:

$C_{18}H_{23}NO_5$	Calculated	C 64.85	H 6.95	N 4.20
(333.3840)	Found	C 64.57	H 6.84	N 4.71
HRMS (CI):	Calculated		Found	
$C_{18}H_{24}NO_5$ $[M+H]^+$:	334.1649		334.1636	

N-(tert-Butoxycarbonyl)-O-(3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-argyl-L-serine (26)

According to the **GP-2** to the solution of 4.6 mg (4 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.5 mg (8 µmol, 4 mol%) CuI and 72 mg (0.2 mmol, 1 eq.) enol triflate **12** in 1 ml abs. THF under nitrogen atmosphere 27 µl (20 mg, 0.2 mmol, 1 eq) Et_3N and 48 mg (0.2 mmol, 1 eq.) N-(*tert*-butoxycarbonyl)-O-(prop-2-yn-1-yl)-L-serine **23** were added at rt. The reaction mixture was stirred for 1 h. After work-up and column chromatography (silica gel, DCM:MeOH 98:2) 81.5 mg (0.178 mmol, 90%) of **26** was isolated as a yellow solid compound.

R_f(26) = 0.15 (DCM:MeOH 95:5)



26

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.43 (s, 9 H, 22-H), 3.09 (sh, 1 H, OH), 3.38 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.93 (d, $^{2}J_{17,17'}$ = 11.0 Hz, 1 H, 17-H), 4.07-4.12 (sh, 1 H, 17'-H), 4.44 (sh, 1 H, 18-H), 5.05 (s, 2 H, 16-H), 5.62 (d, $^{3}J_{NH,18}$ = 7.4 Hz, 1 H, NH), 6.08 (s, 1 H, 8-H), 6.40 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.56 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.49 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 14.1 (q, C-11/C13), 28.3 (q, C-22), 44.8 (t, C-12/C-10), 53.2 (d, C-18), 55.8 (t, C-16), 63.2 (t, C-17), 80.3 (s, C-14/C-21), 81.1 (s, C-14/C-21), 93.5 (s, C-15), 97.3 (d, C-3), 107.6 (d, C-1), 109.0 (s, C-5), 111.6 (d, C-8) 127.5 (d, C-6), 136.3 (s, C-9), 151.1 (s, C-2), 151.1 (s, C-4), 156.1 (s, C-20), 161.6 (s, C-7), 170 (s, C-19).

Elemental Analysis:

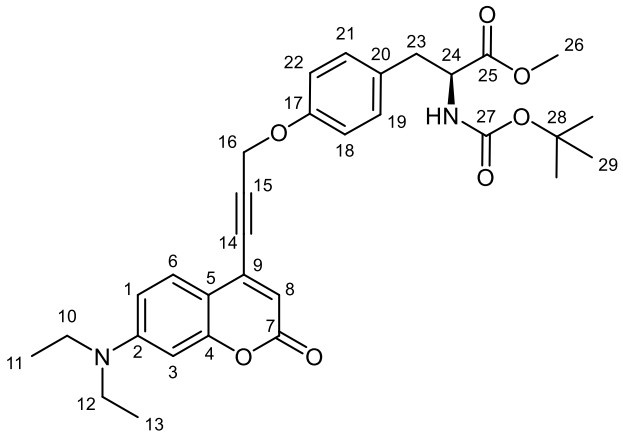
$C_{24}H_{30}N_2O_7$	Calculated	C 62.87	H 6.59	N 6.11
(458.5110)	Found	C 63.13	H 6.13	N 5.85
HRMS (CI):	Calculated		Found	
$C_{24}H_{30}N_2O_7$ $[M]^+$:	458.2048		458.2033	
Absorption max.:	401 nm			
Exitation max.:	409 nm			
Emission max.:	498 nm			
Lifetime:	5.90 ns			
Melting point:	88-90 °C			

L-tert-Butyloxycarbonyl-(3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-O-propargyl-L-tyrosine methyl ester (27)

According to the **GP-2** to the solution of 6 mg (5 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.9 mg (10 µmol, 4 mol%) CuI and 91 mg (0.25 mmol, 1.3 eq.) enol triflate **12** in 1.25 ml abs.

THF under nitrogen atmosphere 40 µl (25 mg, 0.25 mmol, 1.3 eq) Et_3N and 61 mg (0.19 mmol, 1 eq.) L-*tert*-butyloxycarbonyl-O-propargyl-L-tyrosine methyl ester **25** were added at rt. The reaction mixture was stirred for 2 h. After work-up and column chromatography (silica gel, PE:EA 7:3) 97.5 mg (0.178 mmol, 94%) of **27** was isolated as a yellow solid compound.

R_f(27) = 0.30 (DCM:EA 95:5)



27

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.41 (s, 9 H, 29-H), 3.05 (sh, 2 H, 23-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.70 (s, 3 H, 26-H), 4.56 (m, 1 H, 24-H), 4.98 (s, 2 H, 16-H), 6.15 (s, 1 H, 8-H), 6.45 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.97 (d, $^3J_{22,21}$ = $^3J_{18,19}$ = 8.6 Hz, 2 H, 22-H, 18-H), 7.09 (d, $^3J_{21,22}$ = $^3J_{19,18}$ = 8.6 Hz, 2 H, 21-H, 19-H), 7.43 (d, $^3J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 28.3 (q, C-29), 37.6 (t, C-23), 44.8 (t, C-12/C-10), 52.2 (q, C-26), 54.5 (t, C-16), 56.5 (d, C-24), 80.1 (s, C-28), 81.6 (s, C-14), 94.0 (s, C-15), 97.4 (d, C-3), 107.7 (d, C-1), 108.9 (s, C-5), 115.4 (d, C-18/C-22) 127.6 (d, C-6), 129.3 (d, C-20), 130.5 (d, C-19/C-21), 146.5 (s, C-2/C-9), 151.1 (s, C-4), 155.1 (s, C-17), 161.4 (s, C-7), 172.3 (s, C-25).

Elemental Analysis:

$C_{31}H_{36}N_2O_7$	Calculated	C 67.87	H 6.61	N 5.11
(548.6360)	Found	C 66.46	H 6.67	N 4.85

HRMS (CI):	Calculated	Found
$C_{31}H_{36}N_2O_7$ $[M]^+$:	548.2523	548.2523

Absorption max.: 400 nm

Exitation max.: 409 nm

Emission max.: 488 nm

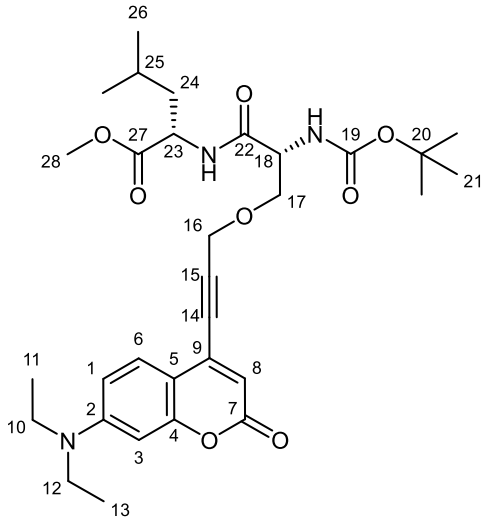
Lifetime: 5.81 ns

Melting point: 89-91 °C

N-(tert-Butoxycarbonyl)-O-(3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)-L-seryl-L-leucine methyl ester (29)

According to the **GP-2** to the solution of 6 mg (5 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.9 mg (10 µmol, 4 mol%) CuI and 91 mg (0.25 mmol, 1.3 eq.) enol triflate **12** in 1.25 ml abs. THF under nitrogen atmosphere 40 µl (25 mg, 0.25 mmol, 1.3 eq) Et_3N and 70 mg (0.19 mmol, 1 eq.) dipeptide **28** were added at rt. The reaction mixture was stirred for 2 h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 105.4 mg (0.18 mmol, 95%) of **29** was isolated as a yellow solid compound.

R_f(29) = 0.22 (DCM:EA 95:5)



29

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.91 (dd, $^3J_{26,25}$ = 5.9 Hz, 6 H, 26-H), 1.20 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.46 (s, 9 H, 21-H), 1.60-1.69 (sh, 3 H, 25-H, 24-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.69-3.71 (m, 4 H, 17'-H, 28-H), 4.06 (dd, $^3J_{17,17'}$ = 9.05 Hz, $^3J_{17,18}$ = 3.8 Hz, 1 H, 17-H), 4.35 (bs, 1 H, 18-H), 4.52 (s, 2 H, 16-H), 4.64 (m, 1 H, 23-H), 5.42 (bs, 1 H, NH-Ser), 6.17 (s, 1 H, 8-H), 6.45 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.61 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.87 (d, $^3J_{NH\text{-}Ile,23}$ = 6.2 Hz, 1 H, NH-Ile), 7.56 (d, $^3J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 22.9 (q, C-26), 24.8 (d, C-25), 28.3 (q, C-21), 41.6 (t, C-24), 44.8 (t, C-12/C-10), 50.9 (q, C-28), 51.6 (d, C-23/C-18), 52.3 (d, C-23/C-18), 59.3 (t, C-16), 69.8 (t, C-17), C-20 under $CDCl_3$, 81.3 (s, C-14), 97.4 (d, C-3), 107.7 (d, C-1), 108.9 (s, C-5), 111.9 (d, C-8) 127.6 (d, C-6),

136.5 (s, C-9), 151.1 (s, C-2), 156.4 (s, C-4), 161.4 (s, C-19), 169.8 (s, C-7), 173.0 (s, C-22/C-27), 173.4 (s, C-22/C-27).

Elemental Analysis:

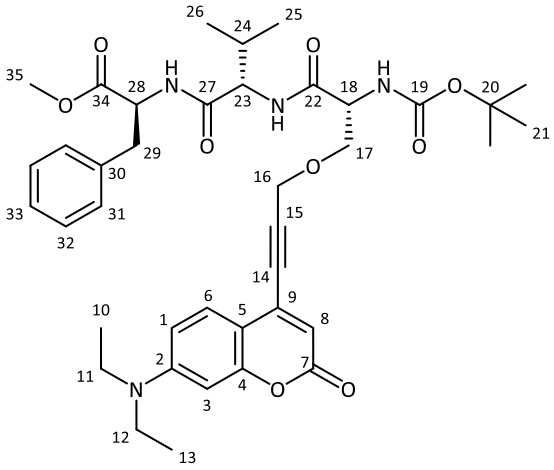
$C_{31}H_{43}N_3O_8$	Calculated	C 63.57	H 7.40	N 7.17
(585.6980)	Found	C 62.69	H 7.59	N 6.41

HRMS (CI):	Calculated	Found
$C_{31}H_{43}N_3O_8$ $[M]^+$:	585.3045	585.3055
Absorption max.:	400 nm	
Exitation max.:	407 nm	
Emission max.:	483 nm	
Lifetime:	5.69 ns	
Melting point:	98-100 °C	

N-(tert-Butoxycarbonyl)-O-(3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)-L-seryl-L-valyl-L-phenylalanine methyl ester (31)

According to the **GP-2** to the solution of 6 mg (5 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.9 mg (10 µmol, 4 mol%) CuI and 91 mg (0.25 mmol, 1.3 eq.) enol triflate **12** in 1.25 ml abs. THF under nitrogen atmosphere 40 µl (25 mg, 0.25 mmol, 1.3 eq) Et_3N and 96 mg (0.19 mmol, 1 eq.) tripeptide **30** were added at rt. The reaction mixture was stirred for 2 h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 105.4 mg (0.18 mmol, 95%) of **31** was isolated as a yellow solid compound.

R_f(31) = 0.13 (DCM:EA 9:1)



31

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.86 and 0.92 (2d, $^{3}J_{26/25,24}$ = 6.8 Hz, 6 H, 26-H, 25-H), 1.20 ($^{3}J_{11.10}$ = $^{3}J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.46 (s, 9 H, 21-H), 2.17 (dt, $^{3}J_{24,26/25}$ = 6.7 Hz, $^{3}J_{24,23}$ = 13.1 Hz, 1 H, 24-H), 3.09 (m, 2H, 29-H, 29'-H), 3.41 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.67-3.72 (sh, 4 H, 17'-H, 35-H), 4.05 (dd, $^{3}J_{17,17'}$ = 9.0 Hz, $^{3}J_{17,18}$ = 4.0 Hz, 1 H, 17-H), 4.27 (dd, $^{3}J_{23,24}$ = 8.7 Hz, $^{3}J_{23,NH}$ = 5.9 Hz, 1 H, 23-H), 4.35 (bs, 1 H, 18-H), 4.52 (s, 2 H, 16-H), 4.64 (m, 1 H, 23-H), 5.42 (bs, 1 H, NH-Ser), 6.17 (s, 1 H, 8-H), 6.45 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.61 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.87 (d, $^{3}J_{NH\text{-}Ile,23}$ = 6.2 Hz, 1 H, NH-Ile), 7.56 (d, $^{3}J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 22.9 (q, C-26), 24.8 (d, C-25), 28.3 (q, C-21), 41.6 (t, C-24), 44.8 (t, C-12/C-10), 50.9 (q, C-28), 51.6 (d, C-23/C-18), 52.3 (d, C-23/C-18), 59.3 (t, C-16), 69.8 (t, C-17), C-20 under $CDCl_3$, 81.3 (s, C-14), 97.4 (d, C-3), 107.7 (d, C-1), 108.9 (s, C-5), 111.9 (d, C-8) 127.6 (d, C-6), 136.5 (s, C-9), 151.1 (s, C-2), 156.4 (s, C-4), 161.4 (s, C-19), 169.8 (s, C-7), 173.0 (s, C-22/C-27), 173.4 (s, C-22/C-27).

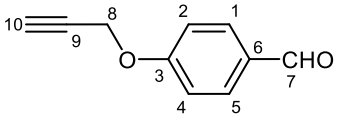
Elemental Analysis:

$C_{39}H_{50}N_4O_9$	Calculated	C 65.16	H 7.01	N 7.79
(718.8480)	Found	C 64.40	H 7.10	N 7.55
HRMS (CI):	Calculated		Found	
$C_{39}H_{50}N_4O_9$ $[M]^+$:	718.3572		718.3586	
Absorption max.:	398 nm			
Exitation max.:	407 nm			
Emission max.:	485 nm			
Lifetime:	5.72 ns			
Melting point:	101-104 °C			

4-O-proparg-benzaldehyde (32) [139]

To the solution of 1.22 g (10 mmol, 1 eq.) 4-hydroxybenzaldehyde in 50 ml acetone 1.94 g (14 mmol, 1.4 eq) K_2CO_3 was added and the mixture was refluxed for 30 minute. 2.22 ml (20 mmol, 2 eq.) propargylbromide was added and the mixture was refluxed for another 2 h before the solvent was evaporated in vacuo. The crude product was dissolved in water and extracted 3x with EA. The combined organic layers were washed with water and brine, dried over Na_2SO_4. The solvent was removed in vacuo. The crude product was purified by column chromatography (silica gel, PE:EA 7:3) to furnish 1.5 g (9.5 mmol, 95%) of the desired product **32** as a white solid compound.

R_f(32) = 0.29 (PE:EA 7:3)



32

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 2.57 (t, $^{2}J_{10,8}$ = 2.4 Hz, 1 H, 10-H), 4.78 (d, $^{2}J_{8,10}$ = 2.4 Hz, 2 H, 8-H), 7.09 (dt, $^{3}J_{2,1}$ = $^{3}J_{4,5}$ = 8.8 Hz, $^{4}J_{2,4}$ = $^{4}J_{4,2}$ = 2.6 Hz, 2 H, 2-H, 4-H), 7.86 (dt, $^{3}J_{1,2}$ = $^{3}J_{5,4}$ = 8.8 Hz, $^{4}J_{1,5}$ = $^{4}J_{5,1}$ = 2.6 Hz, 2 H, 1-H, 5-H), 9.90 (s, 1 H, 7-H).

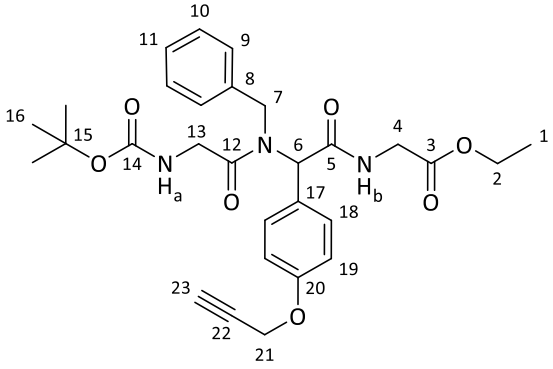
^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 56.0 (t, C-8), 76.4 (d, C-10), 77.5 (s, C-9), 115.2 (d, C-2/C-4), 130.6 (s, C-6), 131.9 (d, C1/C-5), 162.4 (s, C-3), 190.8 (s, C-7).

2-(N-Benzyl-2-((tert-butoxycarbonyl)amino)acetamido)-2-(4-(prop-2-yn-1-yl-oxy)phenyl)acetyl)glycine ester (33)

According to the **GP 3** 0.1 ml (107 mg, 1 mmol, 1 eq.) benzylamine, 160 mg (1 mmol, 1 eq.) aldehyde **32**, and 175 mg (1 mmol, 1 eq.) Boc-glycine reacted with 113 mg (1 mmol, 1 eq.) isocyanide. The reaction mixture was stirred for 24 h. Work-up and column purification (silica gel, DCM:EA 98:2, 9:1) furnished 323 mg (0.6 mmol, 60%) of the product **33** as a white solid compound.

R_f(33) = 0.32 (DCM:EA 8:2)



33

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.26 (t, $^{3}J_{1,2}$ = 7.1 Hz, 3 H, 1-H), 1.41 (s, 9 H, 16-H), 2.50 (t, $^{4}J_{23,21}$ = 2.4 Hz, 1 H, 23-H), 3.78 (dd, $^{3}J_{13,NHa}$ = 17.3 Hz, $^{2}J_{13,13'}$ = 3.9 Hz, 1 H, 13-H), 4.04 (m, 3 H, 13'-H, 4-H), 4.18 (q, $^{3}J_{2,1}$ = 7.1 Hz, 2 H, 2-H), 4.48 (d, $^{2}J_{17,17'}$ = 17.5 Hz, 17-H), 4.63 (d, $^{4}J_{21,23}$ = 2.4 Hz, 2 H, 21-H), 4.63-4,65 (sh, 1 H, 17'-H), 5.44 (bs, 1 H, NH_a), 5.81 (bs, 1 H, 6-H), 6.28 (bs, 1 H, NH_b), 6.85-7.32 (m, 9 H, H_{Ar}).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 14.1 (q, C-1), 28.3 (q, C-16), 41.5 (t, C-13), 43.0 (t, C-4), 55.8 (t, C-7), 61.5 (t, C-2), 63.1 (t, C-21), 75.8 (d, C-23), 78.1 (s, C-22/C-15), 115.2 (d, C-19), 126.2-128.6 (d, C-9/C-10/C-11/C-17/C-18), 131.4 (s, C-8), 157.9 (s, C-20/C-14), 169.5 (s, C-5/C-3).

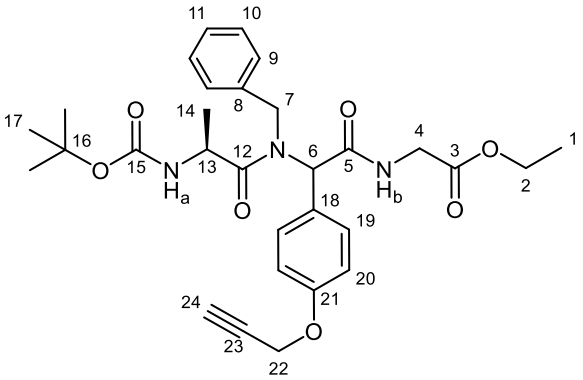
Elemental Analysis:

$C_{29}H_{35}N_3O_7$	Calculated	C 64.79	H 6.56	N 7.82
(537.6130)	Found	C 63.93	H 6.53	N 7.78
HRMS (CI):	Calculated		Found	
$C_{29}H_{36}N_3O_7$ $[M+H]^+$:	538.2548		538.2552	
Melting point:	97-99 °C			

2-((S)-N-Benzyl-2-((tert-butoxycarbonyl)amino)propanamido)-2-(4-(prop-2-yn-1-yloxy)phenyl)acetyl)glycine ester (34)

According to the **GP 3** 0.1 ml (107 mg, 1 mmol, 1 eq.) benzylamine, 160 mg (1 mmol, 1 eq.) aldehyde **32**, and 189 mg (1 mmol, 1 eq.) Boc-alanine reacted with 113 mg (1 mmol, 1 eq.) isocyanide. The reaction mixture was stirred for 24 h. Work-up and column chromatography (silica gel, DCM:EA 98:2, 9:1) furnished 331 mg (0.6 mmol, 60%) of the product **34** as a white solid compound (dr = 58:42).

R_f(34) = 0.13 (DCM:EA 9:1)



34

Mixture of diastereomers

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ ppm 1.23-1.29 (t, $^3J_{1,2}$ = 7.1 Hz, 3 H, 1-H), 1.49-1.45 (s, 9 H, 17-H), 2.50 (t, $^4J_{24,22}$ = 2.4 Hz, 1 H, 24-H), 3.78-4.27 (m, 4 H, 2-H, 4-H), 4.33 (m, 1 H, 13-H), 4.50-4.74 (m, 4 H, 22-H, 7-H), 5.77 (bs, 1 H, NH_a), 6.03 (bs, 1 H, 6-H), 6.30 (bs, 1 H, NH_b), 6.79-7.37 (m, 9 H, H_{Ar}).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 14.1 (q, C-1), 17.3 (q, C-14), 28.3 (q, C-17), 41.6 (t, C-4), 47.1 (t, C-7), 50.1 (d, C-13), 55.8 (t, C-22), 61.5 (t, C-2), 63.2 (t, C-6), 75.7 (d, C-24), 78.3 (s, C-23), 80.2 (s, C-16), 115.2 (d, C-19), 126.6-128.6 (d, C-9/C-10/C-11/C-18/C-20), 131.6 (s, C-8), 156.1 (s, C-21), 157.7 (s, C-15), 169.5 (s, C-5/C-3/C-15).

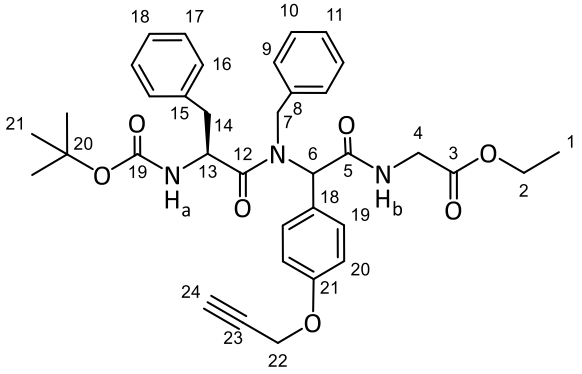
Elemental Analysis:

$C_{30}H_{37}N_3O_7$	Calculated	C 65.32	H 6.76	N 7.62
(551.6307)	Found	C 64.87	H 6.74	N 7.50
HRMS (CI):	Calculated		Found	
$C_{30}H_{38}N_3O_7$ $[M+H]^+$:	552.2704		552.2723	
Melting point:	60-64 °C			

2-((S)-N-Benzyl-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-2-(4-(prop-2-yn-1-yloxy)phenyl)acetyl)glycine ester (35)

According to the **GP 3** 0.1 ml (107 mg, 1 mmol, 1 eq.) benzylamine, 160 mg (1 mmol, 1 eq.) aldehyde **32**, and 265 mg (1 mmol, 1 eq.) Boc-phenylalanine reacted with 113 mg (1 mmol, 1 eq.) isocyanide. The reaction mixture was stirred for 24 h. Work-up and column purification (silica gel, PE:EA 7:3, 6:4) furnished 365 mg (0.63 mmol, 63%) of the product **35** as a white solid compound.

R_f(35) = 0.13 (PE:EA 7:3)



35

Mixture of diastereomers (dr = 53:47)

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.25-1.31 (m, 3 H, 1-H), 1.35-1.37 (s, 9 H, 21-H), 2.49 (t, $^4J_{24,22}$ = 2.2 Hz, 1 H, 24-H), 2.57-2.69 (m, 2 H, 14-H), 3.80-4.29 (m, 4 H, 2-H, 4-H), 4.37-4.43 (m, 1 H, 13-H), 4.48-4.74 (m, 4 H, 22-H, 7-H), 5.57 (bs, 1 H, NH_a), 6.18 (s, 1 H, 6-H), 6.65-7.37 (m, 15 H, $H_{Ar,}$ NH_b).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 14.1 (q, C-1), 28.3 (q, C-21), 38.0 (t, C-14), 41.6 (t, C-4), 50.1 (t, C-7), 55.8 (t, C-22/d, C-13), 61.4 (t, C-2), 63.02 (t, C-6), 75.7 (d, C-24), 78.3 (s, C-23), 79.6 (s, C-20), 115.2 (d, C-20), 126.5-129.6 (d, C-9/C-10/C-11/C-13/C-17/C-18/C-19), 136.3 (s, C-8/C-15), 137.4 (s, C-8/C-15), 156.1 (s, C-21/C-19), 157.7 (s, C-21/C-19), 169.5 (s, C-5/C-3/C-12).

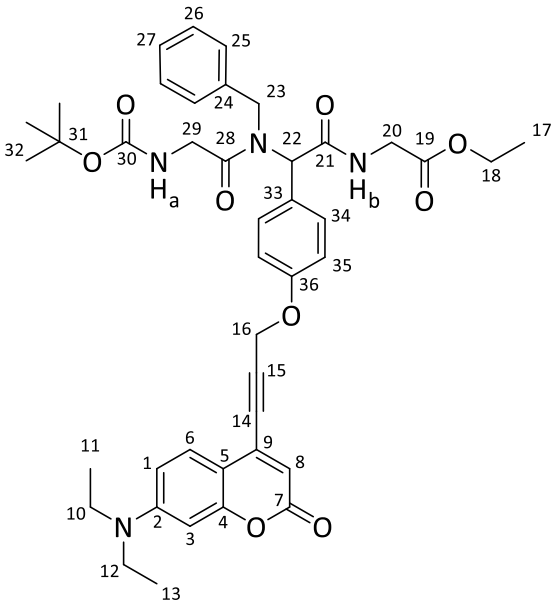
Elemental Analysis:

$C_{36}H_{41}N_3O_7$	Calculated	C 68.88	H 6.58	N 6.69
(627.7266)	Found	C 68.52	H 6.52	N 6.65
HRMS (CI):	Calculated		Found	
$C_{36}H_{42}N_3O_7$ $[M+H]^+$:	628.3017		628.3029	
Melting point:	60-64 °C			

2-(N-Benzyl-2-((tert-butoxycarbonyl)amino)acetamido)-2-(4-((3-(7-(diethyl-amino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)oxy)phenyl)acetyl)glycine ester (36)

According to the **GP 2** to the solution of 6 mg (5 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.9 mg (10 µmol, 4 mol%) CuI and 91 mg (0.25 mmol, 1.3 eq.) enol triflate **12** in 1.25 ml abs. THF 45 µl (32.9 mg, 0.325 mmol, 1.3 eq.) Et_3N and 102 mg (0.19 mmol, 1 eq.) tripeptide **33** were added at rt and the reaction mixture was stirred at rt for 2 h. Work-up and column chromatography (silica gel, DCM:MeOH 98:2) furnished 140.78 mg (0.187 mmol, 98%) of the product **36** as a yellow solid compound.

R_f(36) = 0.34 (DCM:MeOH 95:5)



36

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.16 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.21 (t, $^{3}J_{17,18}$ = 7.1 Hz, 3 H, 17-H), 1.37 (s, 9 H, 32-H), 3.37 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.72 (dd, $^{3}J_{29,NHa}$ = 2.8 Hz, 1 H, 29-H), 3.99 (m, 3 H, 29'-H, 20-H), 4.18 (q, $^{3}J_{18,17}$ = 7.1 Hz, 2 H, 18-H), 4.47 (d, $^{2}J_{23,23'}$ = 17.7 Hz, 1 H, 23-H), 4.65 (d, $^{2}J_{23',23}$ = 17.5 Hz, 1 H, 23'-H), 4.90 (s, 2 H, 16-H), 5.47 (bs, 1 H, NH_a), 5.93 (bs, 1 H, 22-H), 6.05 (s, 1 H, 8-H), 6.40 (d, $^{4}J_{3,1}$ = 2.3 Hz, 1 H, 3-H), 6.53 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.2 Hz, 1 H, 1-H), 6.63 (bs, 1 H, NH_b), 6.87-7.33 (m, 9 H, H_{Ar}), 7.42 (d, $^{3}J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.2 (q, C-17), 28.3 (q, C-32), 41.5 (t, C-20), 43.0 (t, C-29), 44.8 (t, C-12/C-10), 49.5 (t, C-23), 56.4 (t, C-16), 61.5 (t, C-18), C-22 not seen, 79.6 (s, C-31), 81.7 (s, C-14), 94.4 (s, C-15), 97.4 (d, C-3), 107.6 (d, C-1), 108.8 (s, C-5), 112.0 (d, C-8), 115.3 (d, C-35), 126.2-128.9 (d, C-6/C-25/C-26/C-27/C-33/C-34), 136.2 (s, C-24/C-9), 151.1 (s, C-2), 156.2 (s, C-4/C-36), 157.8 (s, C-30), 161.3 (s, C-28/C-7), 169.5 (s, C-21/C-19), 170.7 (s, C-21/C-19).

Elemental Analysis:

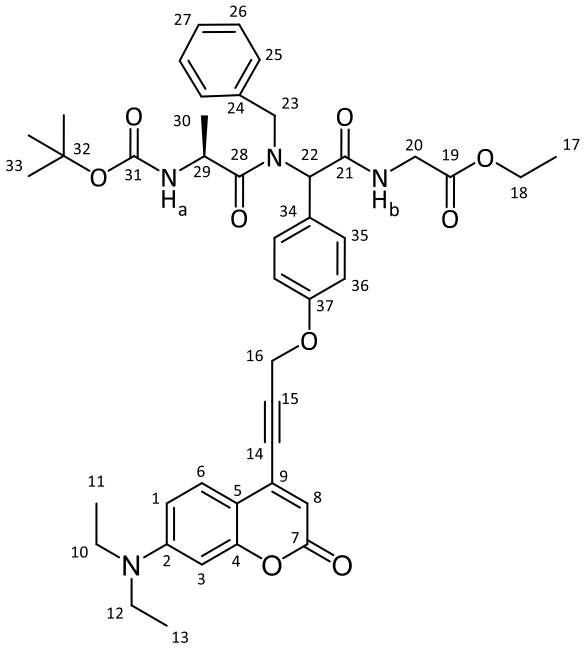
$C_{42}H_{48}N_4O_9$	Calculated	C 67.01	H 6.43	N 7.44
(752.8650)	Found	C 66.76	H 6.66	N 7.31

HRMS (CI):	Calculated	Found
$C_{42}H_{49}N_4O_9$ $[M+H]^+$:	753.3494	753.3475
Absorption max.:	398 nm	
Exitation max.:	411 nm	
Emission max.:	490 nm	
Lifetime:	5.85 ns	
Melting point:	99-101 °C	

2-((S)-N-Benzyl-2-((tert-butoxycarbonyl)amino)propanamido)-2-(4-((3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)oxy)phenyl)acetyl)glycine ester (37)

According to the **GP 2** to the solution of 6 mg (5 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.9 mg (10 µmol, 4 mol%) CuI and 91 mg (0.25 mmol, 1.3 eq.) enol triflate **12** in 1.25 ml abs. THF 45 µl (32.9 mg, 0.325 mmol, 1.3 eq.) Et_3N and 105 mg (0.19 mmol, 1 eq.) tripeptide **34** were added at rt and the reaction mixture was stirred at rt for 2 h. Work-up and column chromatography (silica gel, DCM:MeOH 98:2) furnished 136.9 mg (0.179 mmol, 94%) of the product **37** as a yellow solid compound.

R_f(37) = 0.11 (DCM:EA 95:5)



37

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.89 (d, $^{3}J_{30,29}$ = 6.6 Hz, 3 H, 30-H), 1.19 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.23-1.29 (t, $^{3}J_{1,2}$ = 7.1 Hz, 3 H, 17-H), 1.39-1.46 (s, 9 H, 33-H), 3.40 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.0 Hz, 4 H, 10-H, 12-H), 3.92-4.24 (m, 4 H, 20-H, 18-H), 4.33 (m, 1 H, 29-H), 4.52-4.72 (sh, 1 H, 23-H), 4.93-5.11 (m, 3 H, 16-H, 23'-H), 5.77 (bs, 1 H, NH_a), 6.13-6.15 (m, 2 H, 6-H, 8-H), 6.32 (bs, 1 H, NH_b), 6.42-6.44 (m, 1 H, 3-H), 6.56-6.59 (m, 1 H, 1-H), 6.86-7.43 (m, 9 H, H_{Ar}), 7.46 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.2 (q, C-17), 17.1 (q, C-30), 28.3 (q, C-33), 41.6 (t, C-20), 44.8 (t, C-12/C-10), 50.1 (t, C-23), 50.8 (d, C-29), 56.5 (t, C-16), 61.5 (t, C-18), 80.3 (s, C-32), 81.7 (s, C-14), 94.6 (s, C-15), 97.4 (d, C-3), 107.6 (d, C-1), 108.9 (s, C-5), 112.0 (d, C-8), 115.3 (d, C-36), 126.5-128.5 (d, C-6/C-25/C-26/C-27/C-34/C-35), 136.3 (s, C-24/C-9), 151.1 (s, C-2), 156.2 (s, C-4/C-37), 157.8 (s, C-31), 161.3 (s, C-28/C-7), 169.5 (s, C-21/C-19).

Elemental Analysis:

$C_{43}H_{50}N_4O_9$	Calculated	C 67.35	H 6.57	N 7.31
(766.8785)	Found	C 67.15	H 6.46	N 6.52

HRMS (CI):	Calculated	Found
$C_{43}H_{51}N_4O_9$ $[M+H]^+$:	767.3651	767.3660
Absorption max.:	400 nm	
Exitation max.:	411 nm	
Emission max.:	489 nm	

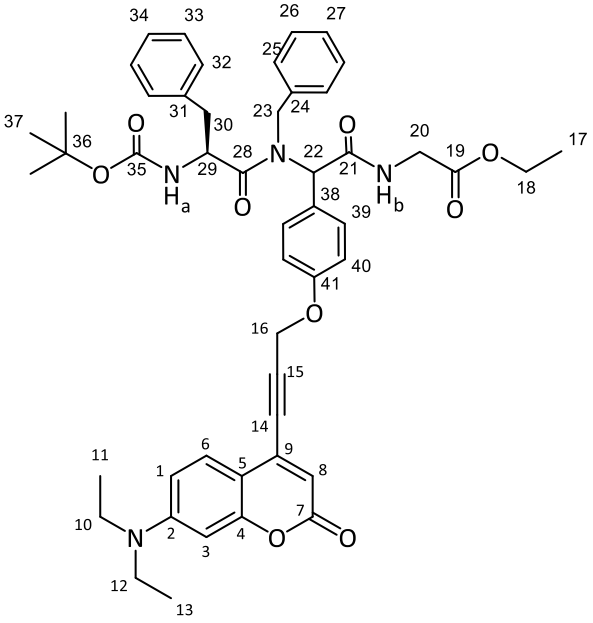
Lifetime: 5.85 ns

Melting point: 95-100 °C

2-((S)-N-Benzyl-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)-2-(4-((3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)oxy)phenyl)acetyl)glycine ester (38)

According to the **GP 2** to the solution of 6 mg (5 µmol, 2 mol%) $Pd(PPh_3)_4$, 1.9 mg (10 µmol, 4 mol%) CuI and 91 mg (0.25 mmol, 1.3 eq.) enol triflate **12** in 1.25 ml abs. THF 45 µl (32.9 mg, 0.325 mmol, 1.3 eq.) Et_3N and 119 mg (0.19 mmol, 1 eq.) tripeptide **35** were added at rt and the reaction mixture was stirred at rt for 2 h. Work-up and column chromatography (silica gel, DCM:MeOH 98:2) furnished 161.1 mg (0.19 mmol, quant.) of the compound **38** as a yellow solid compound.

R_f(38) = 0.15 (DCM:EA 95:5)



38

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.25-1.31 (m, 3 H, 17-H), 1.35-1.36 (s, 9 H, 33-H), 2.54-2.67 (m, 2 H, 30-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.0 Hz, 4 H, 10-H, 12-H), 3.79-4.30 (m, 4 H, 20-H, 18-H), 4.34-4.77 (m, 2 H, 29-H, 23-H), 4.93-5.27 (m, 3 H, 16-H, 23'-H), 5.58 (bs, 1 H, NH_a), 6.12-6.16 (m, 2 H, 6-H, 8-H), 6.27 (bs, 1 H, NH_b), 6.42-6.44 (m, 1 H, 3-H), 6.55-6.62 (m, 1 H, 1-H), 6.88-7.47 (m, 15 H, H_{Ar}, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.2 (q, C-17), 28.2 (q, C-37), 37.8 (q, C-30), 41.5 (t, C-20), 44.8 (t, C-12/C-10), 50.0 (t, C-23), 56.4 (d, C-29), 57.1 (t, C-16), 61.4 (t, C-18), 79.7 (s, C-36), 81.7 (s, C-14), 94.6 (s, C-15), 97.4 (d, C-3),

107.6 (d, C-1), 108.9 (s, C-5), 111.9 (d, C-8), 115.4 (d, C-40), 126.5-128.9 (d, C-6/C-25/C-26/C-27/C-38/C-39/C-32/C-33/C-34), 136.3 (s, C-24/C-9/C-31), 151.1 (s, C-2), 156.2 (s, C-4/C-41), 157.8 (s, C-35), 161.4 (s, C-7), 169.5 (s, C-21/C-19).

Elemental Analysis:

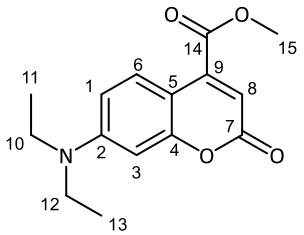
$C_{49}H_{54}N_4O_9$	Calculated	C 69.82	H 6.46	N 6.65
(842.9745)	Found	C 68.67	H 6.41	N 6.15

HRMS (CI):	Calculated	Found
$C_{49}H_{55}N_4O_9$ $[M+H]^+$:	843.3964	843.3980
Absorption max.:	404 nm	
Exitation max.:	411 nm	
Emission max.:	486 nm	
Lifetime:	5.78 ns	
Melting point:	86-88 °C	

Methyl 7-(diethylamino)-2-oxo-2H-chromene-4-carboxylate (39)

To the solution of 44 mg (0.12 mmol, 1 eq.) enol triflate **12** in 1.6 ml dry toluone and 0.2 ml MeOH 8.4 mg (12 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 33 µl (24.3 mg, 0.24 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. Afterwards the reaction was quenched with 1N $KHSO_4$-solution, extracted 3x with EA. The combined organic phase was filtered through a celite and dried over anhydrous Na_2SO_4, The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM) to furnish 31.7 mg (0.115 mmol, 97%) of the product **39** as a yellow solid compound.

R_f(39) = 0.33 (PE:EA 7:3)



39

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.42 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.96 (s, 3 H, 15-H), 6.51 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.52 (s, 1 H, 8-H), 6.60 (dd, $^3J_{1,6}$ = 9.2 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 8.00 (d, $^3J_{6,1}$ = 9.2 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.6 (q, C-11/C13), 44.7 (t, C-12/C-10), 52.8 (q, C-15), 97.6 (d, C-3), 104.1 (d, C-1), 109.1 (s, C-5), 111.3 (d, C-8) 127.7 (d, C-6), 150.8 (s, C-4/C-2), 157.2 (s, C-9), 165.1 (s, C-7), 168.2 (s, C-14).

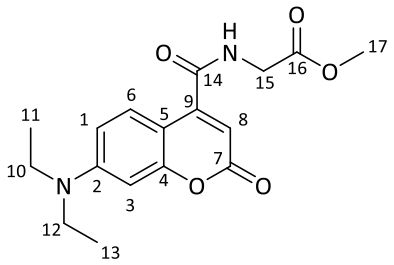
Elemental Analysis:

$C_{15}H_{17}NO_4$	Calculated	C 65.44	H 6.22	N 5.09
(275.2998)	Found	C 65.49	H 6.11	N 4.92
HRMS (CI):	Calculated		Found	
$C_{15}H_{17}NO_4$ $[M]^+$:	275.1158		275.1171	
Absorption max.:	396 nm			
Exitation max.:	410 nm			
Emission max.:	536 nm			
Lifetime:	5.89 ns			

7-(Diethylamino)-2-oxo-2H-chromene-4-carbonyl)glycine methyl ester (40)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 30 mg (0.24 mmol, 1.2 eq.) glycine methyl ester hydrochloride in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM:EA 95:5, 9:1) 57.1 mg (0.17 mmol, 86%) of **40** was isolated as a yellow solid compound.

R_f(40) = 0.19 (DCM:EA 8:2)



40

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.81 (s, 3 H, 17-H), 4.24 (d, $^3J_{15,NH}$ = 5.5 Hz, 2 H, 15-H), 6.13 (s, 1 H, 8-H), 6.47 (d, $^4J_{3,1}$ = 2.3 Hz, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.69 (sh, 1 H, NH), 7.64 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 41.4 (t, C-15), 44.8 (t, C-12/C-10), 52.6 (q, C-17), 97.7 (d, C-3), 104.9 (d, C-1), 106.7 (s, C-5), 109.2 (d, C-8) 127.5 (d, C-6), 148.7 (s, C-2), 151.2 (s, C-4), 157.1 (s, C-9), 161.7 (s, C-7), 165.6 (s, C-16), 169.7 (s, C-14).

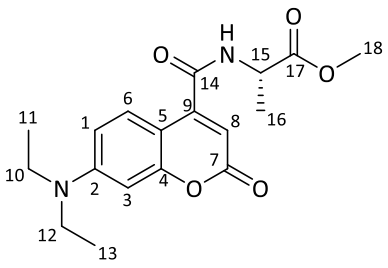
Elemental Analysis:

$C_{17}H_{20}N_2O_5$	Calculated	C 61.44	H 6.07	N 8.43
(332.3560)	Found	C 61.72	H 6.32	N 8.12
HRMS (CI):	Calculated		Found	
$C_{17}H_{20}N_2O_5$ $[M]^+$:	332.1367		332.1361	
Absorption max.:	386 nm			
Exitation max.:	387 nm			
Emission max.:	425 nm			
Lifetime:	3.03 ns			
Melting point:	141 °C			

7-(Diethylamino)-2-oxo-2H-chromene-4-carbonyl)-L-alanine methyl ester (41)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 33.5 mg (0.24 mmol, 1.2 eq.) L-alanine methyl ester hydrochloride in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM:EA 95:5) 48.9 mg (0.14 mmol, 71%) of **41** was isolated as a yellow solid compound.

R_f(41) = 0.19 (DCM:EA 9:1)



41

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.53 (d, $^3J_{16,15}$ = 7.2 Hz, 3 H, 16-H), 3.38 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.78 (s, 3 H, 18-H), 4.74 (q, $^3J_{15,16}$ = 7.2 Hz, 1 H, 15-H), 6.09 (s, 1 H, 8-H), 6.43 (d,

$^{4}J_{3,1}$ = 2.3 Hz, 1 H, 3-H), 6.55 (dd, $^{3}J_{1,6}$ = 9.1 Hz, $^{4}J_{1,3}$ = 2.4 Hz, 1 H, 1-H), 6.93 (d, $^{3}J_{NH,15}$ = 7.2 Hz, 1 H, NH), 7.59 (d, $^{3}J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 18.0 (q, C-16), 44.8 (t, C-12/C-10), 48.5 (q, C-18), 52.7 (d, C-15), 97.6 (d, C-3), 105.0 (d, C-1), 106.6 (s, C-5), 109.2 (d, C-8) 127.5 (d, C-6), 149.0 (s, C-2), 151.1 (s, C-4), 157.0 (s, C-9), 161.8 (s, C-7), 165.1 (s, C-14), 172.8 (s, C-17).

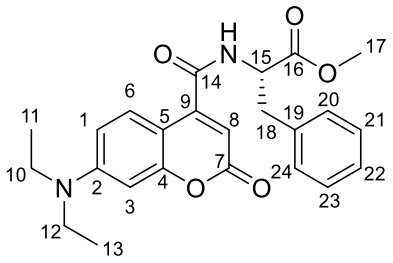
Elemental Analysis:

$C_{18}H_{22}N_2O_5$	Calculated	C 62.42	H 6.40	N 8.09
(346.3820)	Found	C 62.22	H 6.39	N 7.57
HRMS (CI):	Calculated		Found	
$C_{18}H_{22}N_2O_5$ $[M]^+$:	346.1523		346.1525	
Absorption max.:	386 nm			
Exitation max.:	388 nm			
Emission max.:	520 nm			
Lifetime:	6.57 ns			
Melting point:	144-146 °C			

7-(Diethylamino)-2-oxo-2H-chromene-4-carbonyl-L-phenylalanine methyl ester (42)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 51.8 mg (0.24 mmol, 1.2 eq.) L-phenylalanine methyl ester hydrochloride in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM:EA 95:5) 63.3 mg (0.15 mmol, 75%) of **42** was isolated as a yellow solid compound.

R_f(42) = 0.27 (DCM:EA 95:5)



42

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.15 (dd, $^{2}J_{18,18'}$ = 14.0 Hz, $^{3}J_{18,16}$ = 6.9 Hz, 1 H, 18-H), 3.33 (dd, $^{2}J_{18',18}$ = 14.0 Hz, $^{3}J_{18',16}$ = 5.4 Hz, 1 H, 18'-H), 3.39 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.80 (s, 3 H, 17-H), 5.06 (m, 1 H, 15-H), 5.96 (s, 1 H, 8-H), 6.44 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.51 (td, $^{3}J_{1,6}$ = 9.1 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.58 (d, $^{3}J_{NH,15}$ = 8.0 Hz, 1 H, NH), 7.15 (d, $^{3}J_{20,21}$ = $^{3}J_{24,23}$ = 6.6 Hz, 2 H, 20-H, 24-H), 7.29 (m, 3 H, 23-H, 22-H, 21-H), 7.40 (d, $^{3}J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 37.7 (t, C-18), 44.8 (t, C-12/C-10), 52.7 (d, C-15), 53.4 (q, C-17), 97.6 (d, C-3), 104.9 (d, C-1), 106.6 (s, C-5), 109.1 (d, C-8) 127.4 (d, C-6/C-22), 128.8 (d, C-24/C-20), 129.2 (d, C-21/C-23), 135.5 (s, C-19), 148.7 (s, C-2), 151.1 (s, C-4), 157.0 (s, C-9), 161.6 (s, C-7), 165.0 (s, C-16), 171.4 (s, C-14).

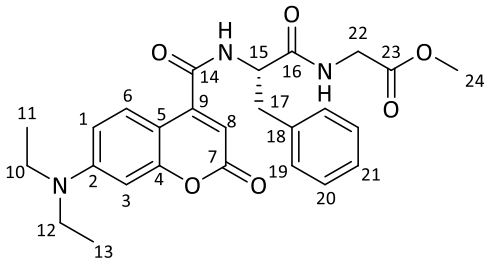
Elemental Analysis:

$C_{24}H_{26}N_2O_5$	Calculated	C 68.23	H 6.20	N 6.63
(422.4810)	Found	C 67.74	H 6.28	N 6.18
HRMS (CI):	Calculated		Found	
$C_{24}H_{26}N_2O_5$ $[M]^+$:	422.1836		422.1842	
Absorption max.:	384 nm			
Exitation max.:	389 nm			
Emission max.:	524 nm			
Lifetime:	6.49 ns			
Melting point:	74-78 °C			

7-(Diethylamino)-2-oxo-2H-chromene-4-carbonyl-L-phenylalanylglycine methyl ester (43)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 66 mg (0.24 mmol, 1.2 eq.) dipeptide in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM:EA 95:5) 67.3 mg (0.14 mmol, 70%) of **43** was isolated as a yellow solid compound.

R_f(43) = 0.15 (DCM:MeOH 95:5)



43

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.13 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.13 (m, 2 H, 17 H), 3.33 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.68 (s, 3 H, 14-H), 3.96 (dq, $^{3}J_{22,NH}$ = 5.3 Hz, 2 H, 22-H), 4.86 (q, $^{3}J_{15,17}$ = 7.3 Hz, 1 H, 15-H), 5.91 (s, 1 H, 8-H), 6.32 (t, $^{3}J_{NH,22}$ = 4.9 Hz, 1 H, NH_{Gly}), 6.39 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.42 (dd, $^{3}J_{1,6}$ = 9.1 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.67 (d, $^{3}J_{NH,15}$ = 7.8 Hz, 1 H, NH_{Phe}), 7.19-7.27 (sh, 5 H, Phe).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

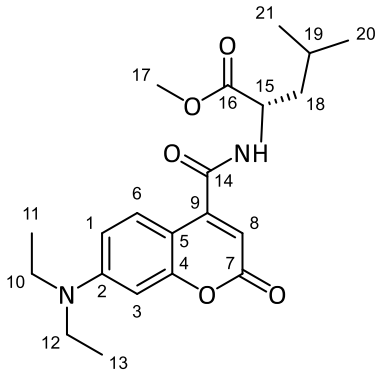
δ = 12.4 (q, C-11/C13), 38.2 (t, C-17), 41.3 (t, C-22), 44.8 (t, C-12/C-10), 52.5 (q, C-24), 54.5 (d, C-15), 97.7 (d, C-3), 104.8 (d, C-1), 106.8 (s, C-5), 109.1 (d, C-8), 127.7 (d, C-22), 127.3 (d, C-Ar), 129.2 (d, C-Ar), 136.6 (s, C-18), 161.5 (s, C-7), 165.3 (s, C-23), 169.7 (s, C-14).

HRMS (CI):	Calculated	Found
$C_{26}H_{30}N_3O_6$ $[M+H]^+$:	480.2129	480.2147
Melting point:	139-141 °C	

7-(Diethylamino)-2-oxo-2H-chromene-4-carbonyl)-L-leucine methyl ester (44)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 43.6 mg (0.24 mmol, 1.2 eq.) leucine methyl ester hydrochloride in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM:EA 95:5) 52.5 mg (0.13 mmol, 65%) of **44** was isolated as a yellow solid compound.

R_f(44) = 0.14 (DCM:EA 9:1)



44

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.98 (2d, $^{3}J_{21,19}$ = $^{3}J_{20,19}$ = 6.2 Hz, 6 H, 20-H, 21-H), 1.20 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.71 (m, 3 H, 19-H, 18-H), 3.41 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.79 (s, 3 H, 17-H), 4.81 (m, 1 H, 15-H), 6.11 (s, 1 H, 8-H), 6.47 (d, $^{4}J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.52 (d, $^{3}J_{NH,15}$ = 8.4 Hz, 1 H, NH), 6.58 (dd, $^{3}J_{1,6}$ = 9.1 Hz, $^{4}J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.60 (d, $^{3}J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 21.9 (q, C-21/C-20), 22.9 (q, C-21/C-20), 25.1 (d, C-19), 41.3 (t, 18-C), 44.8 (t, C-12/C-10), 51.1 (d, C-15), 52.6 (q, C-17), 97.7 (d, C-3), 105.0 (d, C-1), 106.6 (s, C-5), 109.2 (d, C-8) 127.4 (d, C-6), 149.0 (s, C-2), 151.2 (s, C-9), 157. (s, C-4), 161.7 (s, C-7), 165.3 (s, C-14), 172.8 (s, C-16).

Elemental Analysis:

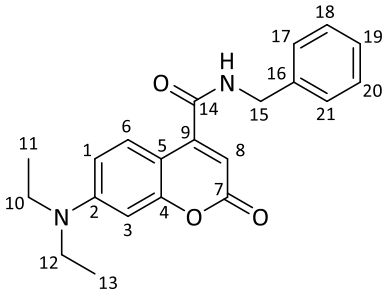
$C_{21}H_{28}N_2O_5$	Calculated	C 64.93	H 7.27	N 7.21
(388.4574)	Found	C 67.23	H 7.41	N 9.52
HRMS (CI):	Calculated		Found	
$C_{21}H_{28}N_2O_5$ $[M]^+$:	388.1993		388.1984	
Absorption max.:	386 nm			
Exitation max.:	388 nm			
Emission max.:	520 nm			
Lifetime:	6.38/3.43 ns			
Melting point:	146-148 °C			

N-Benzyl-7-(diethylamino)-2-oxo-2H-chromene-4-carboxamide (45)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 26 µl (25.7 mg, 0.24 mmol, 1.2 eq.) benzylamine in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The

reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM, DCM:EA 95:5) 67.3 mg (0.17 mmol, 83%) of **45** was isolated as a yellow solid compound.

R_f(45) = 0.45 (DCM:EA 95:5)



45

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.61 (d, $^3J_{15,NH}$ = 5.9 Hz, 2 H, 15-H), 6.04 (s, 1 H, 8-H), 6.41 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.54 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.83 (t, $^3J_{NH,15}$ = 5.4 Hz, 1 H, NH), 7.30-7.37 (sh, 5 H, Phe-H), 7.59 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 43.8 (t, C-15), 44.8 (t, C-12/C-10), 97.6 (d, C-3), 105.1 (d, C-1), 106.4 (s, C-5), 109.2 (d, C-8) 127.5-128.7 (d, C-17/C-18/C-21/C-20), 137.5 (s, C-16), 149.4 (s, C-2), 151.1 (s, C-4), 157.0 (s, C-9), 161.9 (s, C-7), 165.3 (s, C-14).

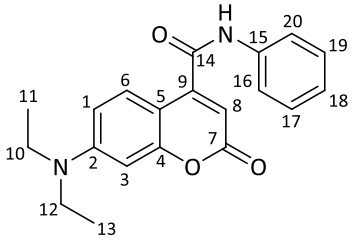
Elemental Analysis:

$C_{21}H_{22}N_2O_3$	Calculated	C 71.98	H 6.33	N 7.99
(350.4180)	Found	C 72.01	H 6.41	N 7.89
HRMS (CI):	Calculated		Found	
$C_{21}H_{22}N_2O_3$ $[M]^+$:	350.1625		350.1631	
Absorption max.:	384 nm			
Exitation max.:	388 nm			
Emission max.:	517 nm			
Lifetime:	6.64 ns			
Melting point:	169-170 °C			

7-(Diethylamino)-2-oxo-N-phenyl-2H-chromene-4-carboxamide (46)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 22 µl (22.4 mg, 0.24 mmol, 1.2 eq.) aniline in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM, DCM:EA 95:5) 52.4 mg (0.16 mmol, 78%) of **46** was isolated as a yellow solid compound.

R_f(46) = 0.16 (DCM:EA 95:5)



46

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.37 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.14 (s, 1 H, 8-H), 6.35 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 9.2 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.19 (t, $^3J_{18,17}$ = $^3J_{18,19}$ = 7.4 Hz, 1 H, 18-H), 7.39 (dd, $^3J_{17,18}$ = $^3J_{17,16}$ = $^3J_{19,18}$ = $^3J_{19,20}$ = 7.9 Hz, 2 H, 17 H, 19-H), 7.66 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H), 7.78 (d, $^3J_{16,17}$ = $^3J_{20,19}$ = 7.7 Hz, 2 H, 16-H, 20-H), 8.80 (s, 1 H, NH).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.5 (d, C-3), 105.1 (d, C-1), 106.0 (s, C-5), 109.4 (d, C-8), 120.3 (d, C-16/C-20), 125.1 (d, C-18/C-6), 127.7 (d, C-18/C-6), 137.7 (s, C-15), 149.9 (s, C-2), 151.2 (s, C-4), 157.0 (s, C-9), 162.5 (s, C-7), 163.5 (s, C-14).

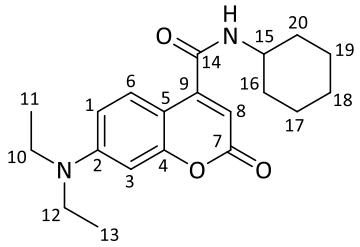
Elemental Analysis:

$C_{20}H_{20}N_2O_3$	Calculated	C 71.41	H 5.99	N 8.33
(336.3910)	Found	C 69.97	H 5.94	N 7.95
HRMS (CI):	Calculated		Found	
$C_{20}H_{20}N_2O_3$ $[M]^+$:	336.1468		336.1473	
Absorption max.:	381 nm			
Exitation max.:	390 nm			
Emission max.:	528 nm			
Lifetime:	6.16 ns			
Melting point:	157-160 °C			

N-Cyclohexyl-7-(diethylamino)-2-oxo-2H-chromene-4-carboxamide (47)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 27.5 µl (23.8 mg, 0.24 mmol, 1.2 eq.) cyclohexylamine in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM, DCM:EA 95:5) 63.3 mg (0.18 mmol, 92%) of **47** was isolated as a yellow solid compound.

R_f(47) = 0.18 (DCM:EA 95:5)



47

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.24-1.45 (sh, 5 H, 17-H, 19-H, 18'-H), 1.62-1.65 (sh, 1 H, 18-H), 1.73-2.08 (sh, 4 H, 16-H, 20-H), 3.37 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.94 (m, 1 H, 15-H), 5.98 (s, 1 H, 8-H), 6.39 (d, $^4J_{3,1}$ = 2.3 Hz, 1 H, 3-H), 6.43 (d, $^3J_{NH,15}$ = 8.0 Hz, 1 H, NH), 6.54 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.4 Hz, 1 H, 1-H), 7.56 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 24.8 (t, C-17/C-19), 25.4 (t, C-18), 32.9 (t, C-16/C-20), 44.8 (t, C-12/C-10), 48.9 (d, C-15), 97.5 (d, C-3), 105.2 (d, C-1), 106.1 (s, C-5), 109.1 (d, C-8), 127.5 (d, C-6), 150.1 (s, C-2), 151.0 (s, C-4), 157.0 6(s, C-9), 162.0 (s, C-7), 164. (s, C-14).

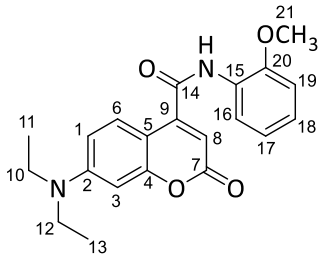
Elemental Analysis:

$C_{20}H_{26}N_2O_3$	Calculated	C 70.15	H 7.65	N 8.18
(342.4390)	Found	C 69.91	H 7.95	N 7.99
HRMS (CI):	Calculated		Found	
$C_{20}H_{26}N_2O_3$ $[M]^+$:	342.1938		342.1942	
Absorption max.:	383 nm			
Exitation max.:	385 nm			
Emission max.:	513 nm			
Lifetime:	6.50 ns			
Melting point:	158-161 °C			

7-(Diethylamino)-N-(2-methoxyphenyl)-2-oxo-2H-chromene-4-carboxamide (48)

According to the **GP-4** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 27.0 µl (29.6 mg, 0.24 mmol, 1.2 eq.) o-anisidine in 2 ml dry toluone 14 mg (20 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ and 55 µl (0.4 mmol, 2 eq.) Et_3N were added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. After work-up and column chromatography (silica gel, DCM, DCM:EA 95:5) 74.4 mg (0.2 mmol, quant) of **48** was isolated as a yellow solid compound.

R_f(48) = 0.2 (DCM:EA 95:5)



48

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.37 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.14 (s, 1 H, 8-H), 6.35 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 9.2 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.19 (t, $^3J_{18,17}$ = $^3J_{18,19}$ = 7.4 Hz, 1 H, 18-H), 7.39 (dd, $^3J_{17,18}$ = $^3J_{17,16}$ = $^3J_{19,18}$ = $^3J_{19,20}$ = 7.9 Hz, 2 H, 17 H, 19-H), 7.66 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H), 7.78 (d, $^3J_{16,17}$ = $^3J_{20,19}$ = 7.7 Hz, 2 H, 16-H, 20-H), 8.80 (s, 1 H, NH).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.5 (d, C-3), 105.1 (d, C-1), 106.0 (s, C-5), 109.4 (d, C-8), 120.3 (d, C-16/C-20), 125.1 (d, C-18/C-6), 127.7 (d, C-18/C-6), 137.7 (s, C-15), 149.9 (s, C-2), 151.2 (s, C-4), 157.0 (s, C-9), 162.5 (s, C-7), 163.5 (s, C-14).

Elemental Analysis:

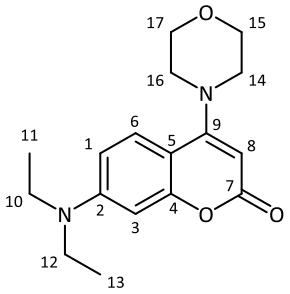
$C_{21}H_{22}N_2O_4$	Calculated	C 68.84	H 6.05	N 7.65
(366.4170)	Found	C 68.01	H 6.00	N 8.00
HRMS (CI):	Calculated		Found	
$C_{21}H_{22}N_2O_4$ $[M]^+$:	366.1574		366.1579	
Absorption max.:	388 nm			
Exitation max.:	391 nm			
Emission max.:	531 nm			
Lifetime:	6.50 ns			

Melting point: 155-156 °C

7-(Diethylamino)-4-morpholino-2H-chromen-2-one (49)

According to the **GP-4** 44 mg (0.12 mmol, 1 eq.) enol triflate **12** reacted with 12 µl (15.8 mg, 0.18 mmol, 1.5 eq.) morpholine. After work-up and column chromatography (silica gel, DCM:EA 95:5) 34.4 mg (0.114 mmol, 95%) of **49** was isolated as a yellow solid compound.

R_f(49) = 0.32 (DCM:EA 9:1)



49

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.22 (sh, 4 H, 17-H, 15-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.89 (sh, 4 H, 16-H, 14-H), 5.45 (s, 1 H, 8-H), 6.48 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.54 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.36 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.6 (t, C-12/C-10), 51.5 (t, C-16/C-14), 66.5 (t, C-17/C-15), C-8 under $CDCl_3$, 98.0 (d, C-3), 104.3 (d, C-1), 107.9 (s, C-1), 125.6 (d, C-6), 150.3 (s, C-4/C-2), 156.7 (s, C-9), 162.0 (s, C-7).

Elemental Analysis:

$C_{17}H_{22}N_2O_3$	Calculated	C 67.53	H 7.33	N 9.26
(302.3682)	Found	C 67.23	H 7.41	N 9.52

HRMS (CI):	Calculated	Found
$C_{17}H_{22}N_2O_3$ $[M]^+$:	302.1625	302.1626

Absorption max.: 356 nm

Exitation max.: 358 nm

Emission max.: 412 nm

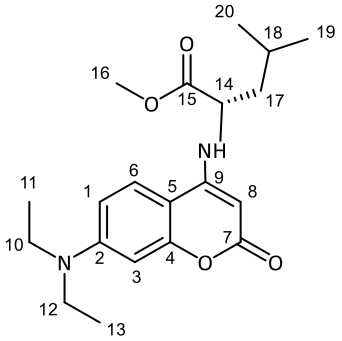
Lifetime: 2.46 ns

Melting point: 132-133 °C

7-(Diethylamino)-2-oxo-2H-chromen-4-yl-L-leucine methyl ester (50)

According to the **GP-5** 73 mg (0. 2 mmol, 1 eq.) enol triflate **12** reacted with 43.6 mg (0.24 mmol, 1.2 eq.) leucine methyl ester hydrochloride. After work-up and column chromatography (silica gel, DCM:EA 95:5) 45 mg (0.12 mmol, 62%) of **50** was isolated as a yellow solid compound.

R_f(50) = 0.51 (DCM:EA 9:1)



50

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.94 (2d, $^3J_{20,18}$ = $^3J_{19,18}$ = 6.2 Hz, 6 H, 20-H, 19-H), 1.17 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.69-1.82 (sh, 3-H, 17-H, 18-H), 3.31-3.41(sh, 4 H, 10-H, 12-H), 3.79 (s, 3 H, 16-H), 4.18 (dd, $^3J_{14,17}$ = 13.8 Hz, $^3J_{14,NH}$ = 7.7 Hz, 1 H, 14-H), 4.98 (s, 1 H, 8-H), 5.82 (d, $^3J_{NH,14}$ = 7.9 Hz, 1 H, NH), 6.29 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.42 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.23 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 21.9 (q, C-21/C-20), 22.7 (q, C-20/C-19), 25.0 (d, C-18), 41.0 (t, C-17), 44.6 (t, C-12/C-10), 52.7 (q, C-16), 53.8 (d, C-14), 80.9 (d, C-8), 97.8 (d, C-3), 102.2 (s, C-5), 107.8 (d, C-1), 121.5 (d, C-6), 150.5 (s, C-2), 153.3 (s, C-4), 155.6 (s, C-9), 164.1 (s, C-7), 174.2 (s, C-15).

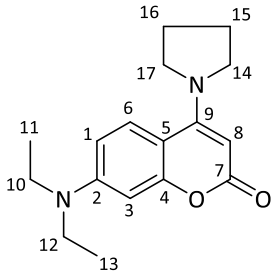
Elemental Analysis:

$C_{20}H_{28}N_2O_4$	Calculated	C 66.64	H 7.83	N 7.77
(360.4473)	Found	C 66.54	H 8.18	N 7.47
HRMS (CI):	Calculated		Found	
$C_{20}H_{29}N_2O_4$ $[M+H]^+$:	361.2122		361.2120	
Absorption max.:	345 nm			
Exitation max.:	345 nm			
Emission max.:	390 nm			
Melting point:	198-200 °C			

7-(Diethylamino)-4-(pyrrolidin-1-yl)-2H-chromen-2-one (51)

According to the **GP-5** to the solution of 91 mg (0.25 mmol, 1 eq.) enol triflate **12** and 41 µl (35.6 mg, 0.5 mmol, 2 eq.) pyrrolidine in 3 ml dry toluene 68.8 µl (0.5 mmol, 2 eq) Et_3N was added. The reaction mixture was refluxed overnight. After work-up and column chromatography (silica gel, DCM:EA 95:5) 69.8 mg (0.24 mmol, 97%) of **51** was isolated as a brown solid compound.

R_f(51) = 0.21 (DCM:EA 9:1)



51

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ ppm 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 2.00 (sh, 4 H, 15-H, 16-H), 3.38 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.64 (sh, 4 H, 14-H, 17-H), 5.05 (s, 1 H, 8-H), 6.48 (m, 2 H, 1 H, 3-H), 7.73 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 25.8 (t, C-15/C-16), 44.5 (t, C-12/C-10), 51.6 (t, C-14/C-17), 82.9 (d, C-8), 98.6 (d, C-3), 104.6 (d, C-1), 107.0 (s, C-5), 126.7 (d, C-6), 149.7 (s, C-2), 156.0 (s, C-4), 156.8 (s, C-9), 163.8 (s, C-7).

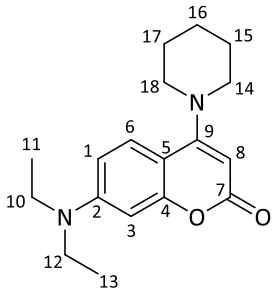
Elemental Analysis:

$C_{17}H_{22}N_2O_2$	Calculated	C 71.30	H 7.74	N 9.78
(286.3750)	Found	C 71.03	H 7.69	N 9.24
HRMS (CI):	Calculated		Found	
$C_{17}H_{23}N_2O_2$ $[M+H]^+$:	287.1754		287.1746	
Absorption max.:	346 nm			
Exitation max.:	350 nm			
Emission max.:	405 nm			
Melting point:	119-122 °C			

7-(Diethylamino)-4-(piperidin-1-yl)-2H-chromen-2-one (52)

According to the **GP-5** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 23.7 µl (20.4 mg, 0.24 mmol, 2 eq.) piperidine in 2 ml dry toluene 55 µl (0.4 mmol, 2 eq) Et_3N was added. The reaction mixture was refluxed overnight. After work-up and column chromatography (silica gel, DCM:EA 98:2, 95:5, 9:1) 59.1 mg (0.197 mmol, 98%) of **52** was isolated as a yellow solid compound.

R_f(52) = 0.19 (DCM:EA 9:1)



52

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.12 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.61-1.71 (sh, 6 H, 15-H, 16-H, 17-H), 3.11-3.13 (sh, 4 H, 14-H, 18-H), 3.32 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.35 (s, 1 H, 8-H), 6.40 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.47 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.30 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 24.5 (t, C-16), 25.8 (t, C-15/C-17), 44.7 (t, C-12/C-10), 52.3 (t, C-14/C-18), 91.9 (d, C-8), 98.0 (d, C-3), 105.0 (s, C-5), 107.7 (d, C-1), 126.0 (d, C-6), 150.1 (s, C-2), 156.6 (s, C-4), 162.8 (s, C-9), 164.0 (s, C-7).

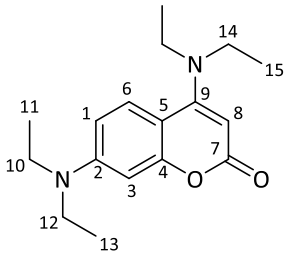
Elemental Analysis:

$C_{18}H_{24}N_2O_2$	Calculated	C 71.97	H 8.05	N 9.33
(300.4020)	Found	C 71.99	H 8.27	N 9.04
HRMS (CI):	Calculated		Found	
$C_{18}H_{24}N_2O_2$ $[M]^+$:	300.1832		300.1838	
Absorption max.:	355 nm			
Exitation max.:	356 nm			
Emission max.:	409 nm			
Melting point:	100-101 °C			

4,7-Bis(diethylamino)-2H-chromen-2-one (53)

According to the **GP-5** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 25 µl (17.6 mg, 0.24 mmol, 2 eq.) diethylamine in 2 ml dry toluene 55 µl (0.4 mmol, 2 eq) Et_3N was added. The reaction mixture was refluxed overnight. After work-up and column chromatography (silica gel, DCM:EA 9:1) 39.5 mg (0.14 mmol, 68%) of **53** was isolated as a yellow oil.

R_f(53) = 0.19 (DCM:EA 9:1)



53

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.22 (dt, $^3J_{11,10}$ = $^3J_{13,12}$ = $^3J_{15,14}$ = 7.1 Hz, 12 H, 13-H, 11-H, 15-H), 3.38 (dq, $^3J_{10,11}$ = $^3J_{12,13}$ = $^3J_{14,15}$ = 7.1 Hz, 8 H, 12-H, 10-H, 14-H), 5.34 (s, 1 H, 8-H), 6.45 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.50 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.7 Hz, 1 H, 1-H), 7.40 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.3 (q, C-15), 12.5 (q, C-11/C13), 44.6 (t, C-12/C-10), 45.4 (t, C-14), 90.7 (d, C-8), 98.2 (d, C-3), 105.1 (s, C-5), 107.7 (d, C-1), 126.1 (d, C-6), 149.9 (s, C-2), 156.7 (s, C-4), 160.1 (s, C-9), 163.9 (s, C-7).

Elemental Analysis:

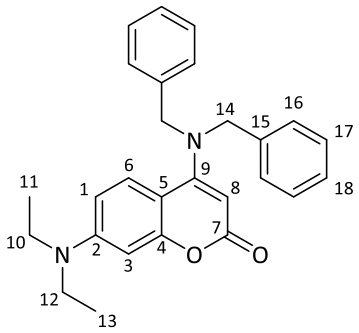
$C_{17}H_{24}N_2O_2$	Calculated	C 70.80	H 8.39	N 9.71
(288.3910)	Found	C 70.94	H 7.64	N 9.47
HRMS (CI):	Calculated		Found	
$C_{17}H_{24}N_2O_2$ $[M]^+$:	288.1832		288.1841	
Absorption max.:	352 nm			
Exitation max.:	416 nm			
Emission max.:	417 nm			

4-(Dibenzylamino)-7-(diethylamino)-2H-chromen-2-one (54)

According to the **GP-5** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 46 µl (47 mg, 0.24 mmol, 2 eq.) dibenzylamine in 2 ml dry toluene 55 µl (0.4 mmol, 2 eq) Et_3N was added. The reaction mixture was refluxed overnight. After

work-up and column chromatography (silica gel, DCM:EA 9:1) 50 mg (0.12 mmol, 60%) of **54** was isolated as a beige solid compound.

R_f(54) = 0.1 (DCM:EA 9:1)



54

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.11 (t, $^3J_{11,10}$ = $^3J_{13,}$12 = 7.1 Hz, 6 H, 13-H, 11-H), 3.31 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.42 (s, 4 H, 14-H), 5.32 (s, 1 H, 8-H), 6.43 (m, 2 H, 1-H, 3-H), 7.22 (m, 10 H, 2Phe), 7.51 (d, $^3J_{6,1}$ = 8.8 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.6 (t, C-12/C-10), 55.3 (t, C-14), 93.2 (d, C-8), 98.3 (d, C-3), 104.7 (s, C-5), 107.9 (d, C-1), 125.8 (d, C-6), 127.5-128.8 (d, C-16, C-17, C-18), 136.4 (s, C-15), 150.2 (s, C-2), 156.8 (s, C-4), 160.6 (s, C-9), 163.5 (s, C-7).

Elemental Analysis:

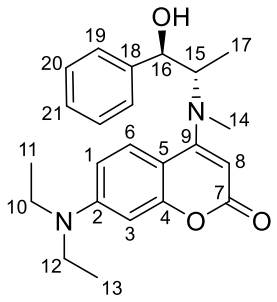
$C_{27}H_{28}N_2O_2$	Calculated	C 78.61	H 6.84	N 6.79
(412.5330)	Found	C 78.99	H 6.96	N 6.69
HRMS (CI):	Calculated		Found	
$C_{27}H_{28}N_2O_2$ $[M]^+$:	412.2145		412.2161	
Absorption max.:	356 nm			
Exitation max.:	359 nm			
Emission max.:	418 nm			

7-(Diethylamino)-4-[1R,2S)-1-hydroxy-1-phenylpropan-2-yl)(methyl)amino]-2H-chromen-2-one (55)

According to the **GP-5** to the solution of 73 mg (0.2 mmol, 1 eq.) enol triflate **12** and 40 mg (0.24 mmol, 2 eq.) ephedrine in 2 ml dry toluene 55 µl (0.4 mmol, 2 eq) Et_3N was added. The reaction mixture was refluxed overnight. After work-up and

column chromatography (silica gel, DCM:EA 9:1) 42.3 mg (0.11 mmol, 56%) of **55** was isolated as a beige solid compound.

R_f(55) = 0.1 (DCM:MeOH 95:5)



55

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.51 (d, $^3J_{17,15}$ = 6.7 Hz, 3 H, 17-H), 2.44 (bs, 1 H, OH), 2.82 (s, 3 H, 14-H), 3.38 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.26 (dt, $^3J_{15,17}$ = $^3J_{15,16}$ = 6.7 Hz, 1 H, 15-H), 4.75 (d, $^3J_{16,15}$ = 6.9 Hz, 1 H, 16-H), 4.91 (s, 1 H, 8-H), 6.40 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.44 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.68 Hz, 1 H, 1-H), 7.18 (m, 6 H, 6-H, Phe).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 13.5 (q, C-17), 33.4 (q, C-14), 44.6 (t, C-12/C-10), 62.0 (d, C-15), 76.5 (d, C-16), 90.5 (d, C-8), 98.1 (d, C-3), 104.6 (s, C-5), 107.4 (d, C-1), 126.1 (d, C-21), 126.4 (d, C-19), 128.5 (d, C-20), 141.8 (s, C-18), 149.8 (s, C-2), 156.4 (s, C-4), 161.5 (s, C-9), 163.8 (s, C-7).

Elemental Analysis:

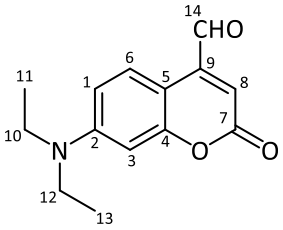
$C_{23}H_{28}N_2O_3$	Calculated	C 72.60	H 7.42	N 7.36
(380.4880)	Found	C 72.02	H 7.78	N 6.85
HRMS (CI):	Calculated		Found	
$C_{23}H_{29}N_2O_3$ $[M+H]^+$:	381.2173		381.2146	
Absorption max.:	351 nm			
Exitation max.:	361nm			
Emission max.:	410 nm			
Melting point:	100-102 °C			

7-(Diethylamino)-2-oxo-2H-chromene-4-carbaldehyde (56) [161]

To the solution of 300 mg (1.2 mmol, 1 eq.) fluorescenting alken **58** solution in 1.5 ml water and 1.6 ml of dioxane 6 µl (2.5 wt% solution in n-hexan, 1.68 mg, 6.6 µmol,

0.55 mol%) OsO_4 was added. The reaction mixture was stirred for 5 minutes during which time the mixture became dark orange (due to osmate ester formation). While the temperature of the stirred mixture was maintained at 24-26 °C, a total of 513 mg (2.4 mmol, 2 eq.) of finely powdered sodium metaperiodate was added in portions over a period of 30 minutes. The tan-coloured slurry then was stirred for an additional 1.5 h. The mixture (now pale yellow) was extracted thoroughly with diethylether and the combined organic layers were filtered through celite, dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (silica gel, PE:EA 7:3) to furnish 190 mg (0.78 mmol, 65%) of product **56** as a red solid compound.

R_f(56) = 0.20 (PE:EA 6:4)



56

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.22 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.43 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.45 (s, 1 H, 8-H), 6.52 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.63 (dd, $^3J_{1,6}$ = 9.2 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 8.30 (d, $^3J_{6,1}$ = 9.2 Hz, 1 H, 6-H), 10.03 (s, 1 H, 14-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

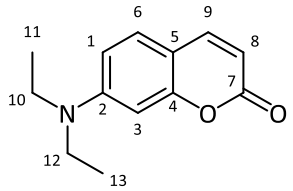
δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.6 (d, C-3), 109.5 (d, C-1), 117.3 (s, C-5), 127.0 (d, C-6/C-8), 143.9 (d, C-2), 151.0 (s, C-4), 157.4 (s, C-7), 161.9 (s, C-9), 192.5 (s, C-14).

HRMS (CI):	Calculated	Found
$C_{14}H_{15}NO_3$ $[M]^+$:	245.1046	245.1050
Absorption max.:	441 nm	
Exitation max.:	369 nm	
Emission max.:	432 nm	
Lifetime:	3.53 ns	
Melting point:	78-80 °C	

7-(Diethylamino)-2H-chromen-2-one (57) [159]

To the solution of 44 mg (0.12 mmol, 1 eq.) enol triflate **12** and 35 µl (38 mg, 0.132 mmol, 1.1 eq.) Bu_3SnH in 2 ml dry toluone 8.4 mg (12 µmol, 10 mol%.) $Pd(PPh_3)_2Cl_2$ was added. The reaction mixture was stirred under CO atmosphere (15 bar) at rt overnight. Afterwards it was diluted with EA and sat. KF-solution was added. The solution was stirred at rt for another 2 h. The aqueous layer was extracted three times with DCM and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by column chromatography (silica gel, PE:EA 7:3) to furnish 24.8 mg (0.114 mmol, 95%) of coumarin **57** as a yellow solid compound.

R_f(57) = 0.25 (PE:EA 6:4)



57

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 6.01 (d, $^3J_{8,9}$ = 9.3 Hz, 1 H, 8-H), 6.47 (d, $^4J_{3,1}$ = 2.4 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 8.8 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.23 (d, $^3J_{6,1}$ = 8.8 Hz, 1 H, 6-H), 7.52 (d, $^3J_{9,8}$ = 9.3 Hz, 1 H, 9-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.5 (d, C-3), 108.3 (d, C-1/C8), 108.7 (d, C-1/C-8), 109.2 (s, C-5), 128.8 (d, C-6), 143.7 (d, C-9), 150.7 (s, C-2), 156.7 (s, C-4), 162.3 (s, C-7).

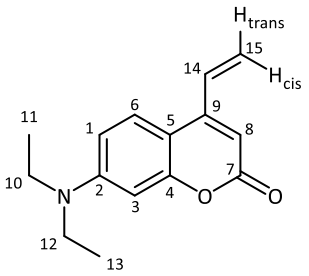
Elemental Analysis:

$C_{13}H_{15}NO_2$	Calculated	C 71.87	H 6.96	N 6.45
(217.2680)	Found	C 71.68	H 6.99	N 6.34
HRMS (CI):	Calculated		Found	
$C_{13}H_{15}NO_2$ $[M]^+$:	217.1097		217.1101	
Absorption max.:	365 nm			
Exitation max.:	373 nm			
Emission max.:	425 nm			
Lifetime:	3.03 ns			
Melting point:	84-85 °C			

7-(Diethylamino)-4-vinyl-2H-chromen-2-one (58) [160]

5.4 mg (15 µmol, 0.5 mol%) $[AllylPdCl]_2$ and 7.9 mg (30 µmol, 1 mol%) were dissolved in 10 ml abs. THF and stirred for 15 minutes at rt. This mixture was added to solution of Bu_3SnH 0.9 ml (813 mg, 2.5 mmol, 1 eq.) and 1 g (3 mmol, 1.2 eq.) enol triflate **12** dissolved in 20 ml abs. THF at 60 °C. The reaction mixture was refluxed for 2 h, cooled down to rt, diluted with EA. After addition of sat. KF-solution the mixture was stirred overnight at rt. The aqueous layer was extracted with DCM, the organic layer was washed with H_2O and dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by column chromatography (silica gel, DCM:Et_2O 95:5) to furnish 579 mg (2.38 mmol, 95%) of **58** as a yellow solid compound.

R_f(58) = 0.33 (DCM:Et_2O 95:5)



58

The compound is sensitive to light.

^{1}H-NMR (400 MHz, *$CDCl_3$*)**:**

δ = 1.21 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.63 (dd, $^3J_{15trans,14}$ = 11.0 Hz, $^2J_{15trans,15cis}$ = 1.0 Hz, 1 H, 15_{trans}-H), 5.94 (dd, $^3J_{15cis,14}$ = 17.3 Hz, $^2J_{15cis,15trans}$ = 1.0 Hz, 1 H, 15_{cis}-H), 6.12 (s, 1 H, 8-H), 6.52 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.93 (dd, $^3J_{14,15cis}$ = 17.4 Hz, $^3J_{14,15trans}$ = 11.0 Hz, 2 H, 14-H), 7.45 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 44.8 (t, C-12/C-10), 97.2 (d, C-3), 104.7 (d, C-8), 108.5 (d, C-1), 110.0 (s, C-5), 115.8 (t, C-15), 130.8 (d, C-6), 150.6 (d, C-14), 151.1 (s, C-2), 153.2 (s, C-4), 156.5 (s, C-7), 162.5 (s, C-9).

Elemental Analysis:

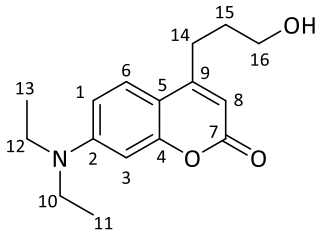
$C_{15}H_{17}NO_2$	Calculated	C 74.05	H 7.04	N 5.76
(243.3060)	Found	C 73.50	H 6.99	N 5.68
HRMS (CI):	Calculated		Found	
$C_{15}H_{17}NO_2$ $[M]^+$:	243.1254		243.1263	
Absorption max.:	372 nm			

Exitation max.:	372 nm
Emission max.:	422 nm
Lifetime:	4.19/2.39 ns
Melting point:	50-53 °C

7-(Diethylamino)-4-(3-hydroxypropyl)-2H-chromen-2-one (59)

3.8 g (14 mmol, 1 eq.) of fluorescenting alcohol **21** was dissolved in MeOH (40 ml), and 380 mg (10 wt%) Pd/C was added. The mixture was stirred under H_2 atmosphere overnight (until complete conversion of the starting material) and filtered through celite. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:MeOH 98:2) to furnish 3.7 g (13.5 mmol, 96%) of the product **59** as a yellow oil.

R_f(59) = 0.25 (DCM:MeOH 8:2)



59

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.88-1.95 (sh, 2 H, 14-H), 2.77-2.81 (sh, 2 H, 15-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.75 (t, $^3J_{16,15}$ = 6.1 Hz, 2 H, 16-H), 5.95 (s, 1 H, 8-H), 6.48 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.56 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.44 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 28.0 (t, C-13/C-12), 31.4 (t, C-13/C-12), 44.7 (t, C-12/C-10), 61.8 (t, C-16), 97.8 (d, C-3), 107.6 (d, C-1), 108.2 (d, C-8), 108.5 (s, C-6) 125.4 (s, C-6), 150.5 (s, C-2), 156.3 (s, C-4/C9), 156.7 (s, C-4/C-9) 162.5 (s, C-7).

Elemental Analysis:

$C_{16}H_{21}NO_3$	Calculated	C 69.79	H 7.69	N. 5.09
(275.3480)	Found	C 70.33	H 7.10	N 4.96

HRMS (CI):	Calculated	Found
$C_{16}H_{22}NO_3$ $[M]^+$:	275.1516	275.1542

Absorption max.:	363 nm
Exitation max.:	377 nm

Emission max.: 418 nm

Lifetime: 5.47/2.78 ns

3-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)propionaldehyde (60)

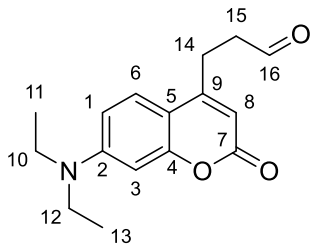
Lemieux-Johnson Oxidation [161]

A mixture of 7 ml ml water, 8 ml of dioxane, 1.63 g (6 mmol, 1 eq.) of fluorescenting alken **2** and 8.25 mg (33µmol, 0.55 mol%, 0.3 ml 2.5 wt% solution in n-hexan) OsO_4 was stirred for 5 minutes during which time the mixture became dark orange (due to osmate ester formation). While the temperature of the stirred mixture was maintained at 24-26 °C, a total of 2.6 g (12 mmol, 2 eq.) of finely powdered sodium metaperiodate was added in portions over a period of 30 minutes. The tan-coloured slurry then was stirred for an additional 1.5 h. The mixture (now pale yellow) was extracted thoroughly with diethylether and the combined organic layers were filtered through celite, dried over Na_2SO_4 and the solvent was evaporated in vacuo. The crude product was purified by column chromatography (silica gel, DCM:EA 98:5) to furnish 950 mg (3.5 mmol, 56%) **60** as a yellow oil.

Dess-Martin

According to the **GP 6** to 1.3 g (4.7 mmol, 1 eq) fluorescenting alcohol **59** in 94 ml abs. DCM 2.4 g (5.64 mmo, 1.2 eq.) Dess-Martin periodinane was added at rt. The reaction mixture was stirred for 2 h. After work-up and column purification (silica gel, DCM:EA 95:5) 1.079 g (3.72 mmol, 79 %) of **60** was isolated as a yellow oil.

R_f(60) = 0.29 (DCM:EA 95:5)



60

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.0 Hz, 6 H, 11-H, 13-H), 2.86 (t, $^3J_{15,14}$ = 7.6 Hz, 2 H, 15-H), 3.01 (t, $^3J_{14,15}$ = 7.4 Hz, 2 H, 14-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 10-H, 12-H), 5.92 (s, 1 H, 8-H), 6.49 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.58 (dd, $^4J_{1,3}$ = 2.6, $^3J_{1,6}$ = 9.0 Hz, 1 H, 1-H), 7.37 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 9.87 (s, 1 H, 16-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C-13), 23.5 (t, C-14), 42.0 (t, C-15), 44.7 (t, C-10/C-12), 97.8 (d, C-3), 107.5 (d, C-1), 107.7 (s, C-5), 108.6 (d, C-8), 124.9 (d, C-6), 150.6 (s, C-2), 154.7 (s, C-4), 156.2 (s, C-9), 162.0 (s, C-7), 199.8 (d, C-16).

Elemental Analysis:

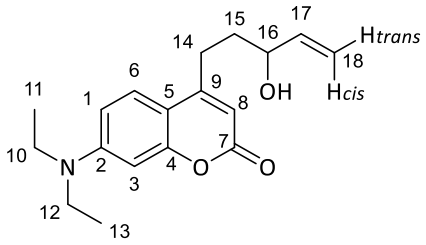
$C_{16}H_{19}NO_3$	Calculated	C 70.31	H 7.01	N 5.12
(273.33)	Found	C 69.01	H 7.04	N 4.83

HRMS (CI):	Calculated	Found
$C_{16}H_{19}NO_3$[M+]:	273.1359	273.1353
Absorption max.:	364 nm	
Exitation max.:	370 nm	
Emission max.:	419 nm	
Lifetime:	3.05 ns	

7-(Diethylamino)-4-(3-hydroxypent-4-en-1-yl)-2H-chromen-2-one (61)

According to the **GP-7** 2.5 g (9.4 mmol, 1 eq) fluorescenting aldehyde **60** was dissolved in 113 ml abs. THF. 12.2 ml (12.2 mmol, 1.3 eq) 1M Vinylmagnesiumbromide-solution in THF was added slowly at –20 °C. The reaction mixture was stirred for 3 h. After column chromatography 2.029 mg (6.7 mmol, 71%) of the alcohol **61** was isolated as yellow oil.

R_f(61) = 0.29 (DCM:EA 95:5)



61

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.88 (m, 2 H, 15-H), 2.80 (m, 2 H, 14-H), 3.41 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.23 (m, 1 H, 16-H), 5.19 (ddd, $^3J_{18cis,17}$ = 17.2 Hz, $^2J_{18cis,\ 18trans}$ = $^4J_{18cis,16}$ = 1.3 Hz, 1 H, 18_{cis}-H), 5.30 (ddd, $^3J_{18trans,17}$ = 10.4 Hz, $^2J_{18trans,18cis}$ = $^4J_{18trans,16}$ = 1.2 Hz, 1 H, 18_{trans}-H), 5.91 (m, 1 H, 17-H), 5.96 (s, 1 H, 8-H), 6.50 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.45 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C-13), 25.4 (t, C-14), 38.0 (t, C-15), 44.7 (t, C-10/C-12), 58.1 (d, C-16), 97.6 (d, C-3), 104.3 (d, C-1), 107.8 (s, C-5), 108.4 (d, C-8),

122.0 (t, C-18), 125.4 (d, C-6), 130.7 (d, C-17), 150.5 (s, C-2), 154.5 (s, C-4), 156.2 (s, C-9), 162.2 (s, C-7).

Elemental Analysis:

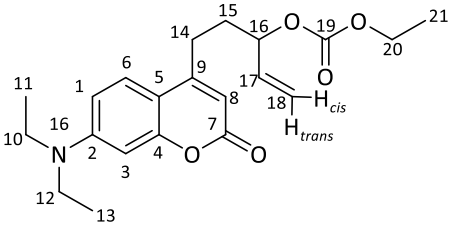
$C_{18}H_{23}NO_3$	Calculated	C 71.71	H 7.69	N 4.65
(301.39)	Found	C 71.81	H 7.71	N 4.42

HRMS (CI):	Calculated	Found
$C_{18}H_{24}NO_3$ $[M+H]^+$:	302.1751	302.1754
Absorption max.:	370 nm	
Exitation max.:	368 nm	
Emission max.:	423 nm	
Lifetime:	3.13 ns	

5-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)pent-1-en-3-yl ethyl carbonate (62)

According to the **GP-8** 253 mg (0.8 mmol, 1 eq.) allylalcohol **61** was dissolved in 0.8 ml dry pyridine. 0.2 ml (174 mg, 1.6 mmol, 2 eq.) ethyl chloroformate was added at 0 °C. The reaction mixture was allowed to warm to rt overnight. After work-up and column chromatography (silica gel, PE:EA 7:3) 238 mg (0.62 mmol, 78%) of allylcarbonate **62** was isolated as a yellow oil.

R_f(62) = 0.36 (PE:EA 7:3)



62

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.33 (t, $^3J_{21,20}$ = 7.1 Hz, 3 H, 21-H), 1.96-2.1 (sh, 2 H, 15-H, 15'-H), 2.71-2.77 (sh, 2 H, 14-H), 3.41 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.21 (q, $^3J_{20,21}$ = 7.2 Hz, 2 H, 20-H), 5.17 (dd $^3J_{16,15}$ = 12.7 Hz, $^3J_{16,17}$ = 6.5 Hz, 1 H, 16-H), 5.29 (ddd, $^3J_{18trans,17}$ = 10.5 Hz, $^2J_{18trans,cis}$ = $^4J_{18trans,16}$ = 1.0 Hz, 1 H, 18_{trans}-H), 5.38 (ddd, $^3J_{18cis,17}$ = 17.2 Hz, $^2J_{18cis,18trans}$ = $^4J_{18cis,16}$ = 1.1 Hz, 1 H, 18_{cis}-H) 5.85 (ddd, $^3J_{17,18cis}$ = 17.2 Hz, $^3J_{17,18trans}$ = 10.5 Hz, $^3J_{17,16}$ = 6.6 Hz, 1 H, 17-H), 5.93 (s, 1 H, 8-H), 6.50 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.37 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11, C-13), 14.2 (q, C-21) 27.1 (t, C-14), 33.0 (t, C-15), 44.7 (t, C-10, C-12), 60.4 (t, C-20), 64.1 (d, C-16), 97.8 (d, C-3), 107.7 (d, C-1), 107.9 (s, C-5), 108.4 (d, C-8), 118.3 (t, C-18), 125.1 (d, C-6), 135.2 (d, C-17), 150.5 (s, C-2), 154.5 (s, C-4/C-19), 155.5 (s, C-4/C-19), 156.2 (s, C-9), 162.2 (s, C-7).

Elemental Analysis:

$C_{21}H_{27}NO_5$	Calculated	C 67.54	H 7.29	N 3.75
(373.45)	Found	C 68.39	H 6.84	N 3.43

HRMS (CI):	Calculated	Found
$C_{21}H_{27}NO_5$ $[M+5H]^+$:	378.2253	378.1353

Absorption max.: 371 nm

Exitation max.: 380 nm

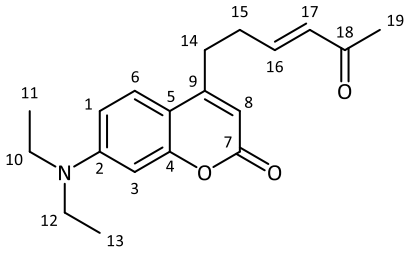
Emission max.: 420 nm

Lifetime: 3.06 ns

(E)-7-(Diethylamino)-4-(5-oxohex-3-en-1-yl)-2H-chromen-2-one (63)

To a suspension of 1.2 g (3.9 mmol, 1.03 eq.) 1-triphenylphosphoranylidene-2-propanone[162] in 21 ml THF 1 g (3.8 mmol, 1 eq.) fluorescenting aldehyde **60** was added. The reaction mixture was refluxed overnight. After solvent was removed in vacuo and column chromatography (silica gel, DCM:EA 95:5) 1.1 g (3.5 mmol, 92%) of **63** was isolated as a red solid compound.

R_f(63) = 0.18 (DCM:EA 9:1)



63

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 11-H, 13-H), 2.22 (s, 3 H, 19-H), 2.58 (td, $^3J_{15,14}$ = 14.3 Hz, $^3J_{15,16}$ = 6.8 Hz, 2 H, 15-H), 2.81 (m, 2 H, 14-H), 3.38 (t, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 10-H, 12-H), 5.89 (s, 1 H, 8-H), 6.11 (d, $^3J_{17,16}$ = 16.0 Hz, $^4J_{17,15}$ = 1.5 Hz, 1 H, 17-H), 6.47 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.56 (dd, $^4J_{1,3}$ = 2.6, $^3J_{1,6}$ = 9.0 Hz, 1 H, 1-H), 6.81 (dt, $^3J_{16,17}$ = 15.9 Hz, $^3J_{16,15}$ = 6.7 Hz, 1 H, 16-H), 7.34 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C-13), 27.1 (q, C-19), 30.0 (t, C-14), 30.9 (t, C-15), 44.7 (t, C-10/C-12), 97.9 (d, C-3), 107.6 (d, C-1), 107.8 (s, C-5), 108.6 (d, C-8), 125.0 (d, C-6), 132.1 (d, C-17), 145.3 (d, C-16), 150.6 (s, C-2), 154.8 (s, C-4), 156.3 (s, C-9), 162.1 (s, C-7), 198.2 (s, C-18).

Elemental Analysis:

$C_{19}H_{23}NO_3$	Calculated	C 72.82	H 7.40	N 4.47
(313.3970)	Found	C 72.95	H 7.42	N 4.42

HRMS (CI):	Calculated	Found
$C_{19}H_{24}NO_3$ $[M+H]^+$:	314.1751	314.1759

Absorption max.: 361 nm

Exitation max.: 369 nm

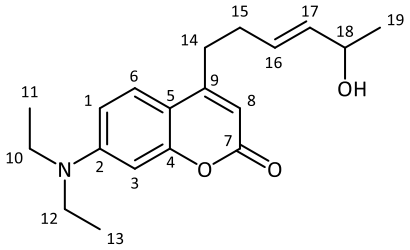
Emission max.: 422 nm

Melting point: 84-85 °C

(E)-7-(Diethylamino)-4-(5-hydroxyhex-3-en-1-yl)-2H-chromen-2-one (64)

To the solution of 627 mg (2 mmol, 1.0 eq.) fluorescenting ketone **63** and 768 mg (2.06 mmol, 1.03 eq.) $CeCl_3$*$7H_2O$ in 5 ml MeOH 83 mg (2.2 mmol, 1.1 eq.) sodium borohydride was added slowly. The reaction mixture was stirred for 5 minutes. Afterwards it was hydrolysed with 1M $KHSO_4$ at rt and extracted three times with DCM. The organic phase was washed with water and brine solution, and dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified on column (silica gel, DCM:MeOH 98:2) to furnish 555 mg (1.76 mmol, 88%) of **64** was isolated as a yellow oil.

R_f(64) = 0.17 (DCM:MeOH 95:5)



64

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.24 (d, $^3J_{19,18}$ = 6.4 Hz, 3 H, 19-H), 1.84 (bs, 1 H, OH), 2.37 (td, $^3J_{15,14}$ = 14.9 Hz, $^3J_{15,16}$ = 6.9 Hz, 2 H, 15-H), 2.73 (m, 2 H, 14-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 10-H, 12-H), 4.26 (qd, $^3J_{18,19}$ = $^3J_{18,17}$ = 6.2 Hz, 1 H, 18-H), 5.58 (dd, $^3J_{17,16}$ = 15.4 Hz, $^3J_{19,18}$ = 6.1 Hz, 1 H, 17-H), 5.68 (dt, $^3J_{16,17}$ = 15.3 Hz, 6.29, $^3J_{16,15}$ =

6.3 Hz, 1 H, 16-H), 5.9 (s, 1 H, 8-H), 6.48 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.56 (dd, $^4J_{1,3}$ = 2.6, $^3J_{1,6}$ = 9.0 Hz, 1 H, 1-), 7.37 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C-13), 23.5 (q, C-19), 30.9 (t, C-15), 31.3 (t, C-14), 44.7 (t, C-10/C-12), 68.6 (d, C-18), 97.9 (d, C-3), 107.8 (d, C-1), 108.2 (s, C-5), 108.1 (d, C-8), 125.2 (d, C-6), 132.5 (d, C-16), 135.8 (d, C-17), 150.5 (s, C-2), 155.8 (s, C-4), 156.3 (s, C-9), 162.3 (s, C-7).

Elemental Analysis:

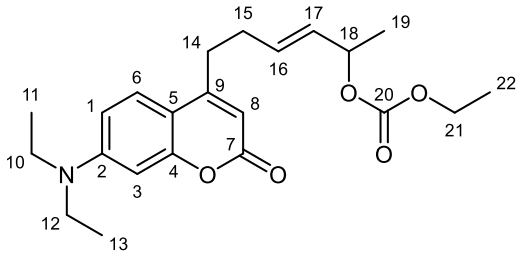
$C_{19}H_{25}NO_3$	Calculated	C 72.35	H 7.99	N 4.44
(315.4130)	Found	C 72.65	H 7.51	N 3.60

HRMS (CI):	Calculated	Found
$C_{19}H_{25}NO_3$ [M+]:	315.1834	315.1834
Absorption max.:	363 nm	
Exitation max.:	370 nm	
Emission max.:	420 nm	

(E)-6-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)hex-3-en-2-yl ethyl carbonate (65)

According to the **GP-8** 252 mg (0.8 mmol, 1 eq.) allylalcohol **64** was dissolved in 0.8 ml dry pyridine. 0.15 ml (174 mg, 1.6 mmol, 2 eq.) ethyl chloroformate was added at 0 °C. The reaction mixture was allowed to warm to rt overnight. After work-up and column chromatography (silica gel, PE:EA 7:3) 279 mg (0.72 mmol, 90%) of allylcarbonate **65** was isolated as a yellow oil.

R_f(65) = 0.52 (PE:EA 6:4)



65

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.32 (m, 6 H, 19-H, 22-H), 2.41 (dd, $^3J_{15,14}$ = 15.3 Hz, $^3J_{15,16}$ = 6.9 Hz, 2 H, 15-H), 2.75 (m, 2 H, 14-H), 3.41 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.18 (q, $^3J_{20,21}$ = 7.2 Hz, 2 H, 20-H), 5.16 (qd, $^3J_{18,19}$ = $^3J_{18,17}$ = 6.6 Hz, 1 H, 18-H), 5.56 (ddt, $^3J_{17,16}$ = 15.4 Hz, $^3J_{17,28}$ = 6.9 Hz, $^4J_{17,15}$ = 1.3 Hz, 1 H, 17-H), 5.81 (m, 1 H,

16-H), 5.91 (s, 1 H, 8-H), 6.50 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.57 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.37 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C-13), 14.3 (q, C-22) 20.4 (q, C-19), 30.9 (t, C-15/C-14), 31.0 (t, C-14/C-15), 44.7 (t, C-10/C-12), 63.8 (t, C-21), 74.7 (d, C-18), 97.9 (d, C-3), 107.8 (d, C-1), 108.1 (s, C-5), 108.4 (d, C-8), 125.2 (d, C-6), 130.6 (d, C-16/C-17), 131.6 (d, C-16/C-17), 150.5 (s, C-2), 154.5 (s, C-4/C-20), 155.6 (s, C-4/C-20), 156.3 (s, C-9), 162.2 (s, C-7).

Elemental Analysis:

$C_{22}H_{29}NO_5$	Calculated	C 68.20	H 7.54	N 3.61
(387.4760)	Found	C 67.95	H 7.61	N 3.37

HRMS (CI):	Calculated	Found
$C_{22}H_{29}NO_5$ $[M]^+$:	387.2046	387.2044

Absorption max.: 364 nm

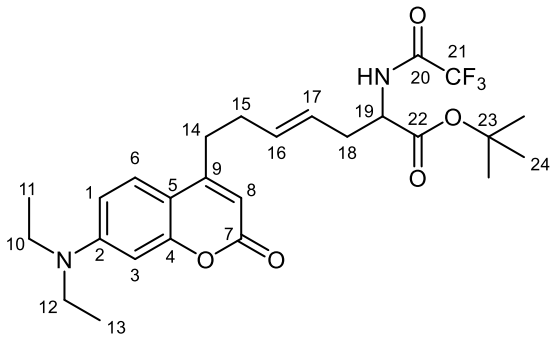
Exitation max.: 367 nm

Emission max.: 420 nm

tert-Butyl (E)-7-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-2-(2,2,2-trifluoro-acetamido)hept-4-enoate (66)

According to the **GP 9** 134 mg (0.59 mmol, 1 eq.) of TFA-Glycin-*tert*-butylester reacted with 154 mg (0.41 mmol, 0.7 eq.) 7-Aminocoumarin-carbonate **62**. After work-up and column chromatography (silica gel, PE:EA 7:3) 146.2 mg (0.28 mmol, 68%) of **66** was isolated as a yellow oil.

R_f(66) = 0.23 (PE:EA 7:3)



66

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.17 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.45 (s, 9 H, 24-H), 2.28-2.40 (m, 2 H, 15-H), 2.52 (td, $^3J_{18,17}$ = 14.9 Hz, $^3J_{18,19}$ = 6.0 Hz, 1 H, 18-H), 2.61-2.74 (sh, 3 H, 14 H, 18'-H), 3.38 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.50 (m, 1 H, 19-H), 5.34 (m, 2 H, 17-H), 5.61 (m, 2 H, 16-H),

5.87 (s, 1 H, 8-H), 6.46 (d, $^4J_{3,1}$ = 2.4 Hz, 1 H, 3-H), 6.52-6.58 (sh, 1 H, 1-H), 7.03 (d, $^3J_{NH,19}$ = 7.3 Hz, 1 H, NH), 7.33 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

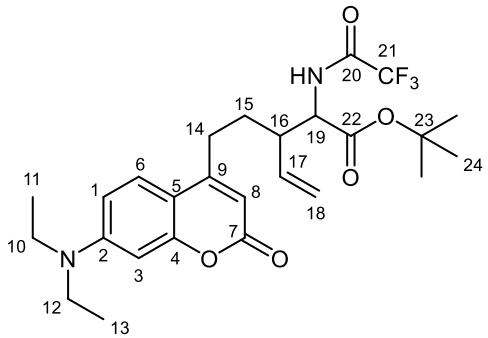
δ = 12.4 (q, C-11, C-13), 27.9 (q, C-24), 31.2 (2t, C-15/C-14), 34.9 (t, C-18), 44.7 (t, C-12, C-10), 52.7 (d, C-19), 83.4 (s, C-23), 97.8 (d, C-3), 107.5 (d, C-1/C-5), C-23 not seen, 108.5 (d, C-8), 125.1 (d, C-17/C-6), 132.5 (d, C-16), 155.7 (s, C-4/C-2), 156.3 (s, C-9/C-20), 162.3 (s, C-7), 169.3 (s, C-22).

HRMS (CI):	Calculated	Found
$C_{26}H_{33}N2O_5$ $[M]^+$:	510.2342	510.2336
Absorption max.:	364 nm	
Exitation max.:	367 nm	
Emission max.:	420 nm	
Lifetime:	3.06 ns	

tert-Butyl-3-(2-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)ethyl)-2-(2,2,2-trifluoroacetamido)pent-4-enoate (67)

According to the **GP 9** 134 mg (0.59 mmol, 1 eq.) of TFA-Glycin-*tert*-butylester reacted with 154 mg (0.41 mmol, 0.7 eq.) 7-Aminocoumarin-carbonate **62**. After work-up and column chromatography (silica gel, PE:EA 7:3) 23.8 mg (0.05 mmol, 12%) of **67** was isolated as a yellow oil.

R_f(67) = 0.23 (PE:EA 7:3)



67

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.17 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.45 (s, 9 H, 24-H), 2.28-2.40 (m, 2 H, 15-H), 2.61-2.74 (sh, 2 H, 14 H), 3.38 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.50 (m, 1 H, 19-H), 4.64 (m, 1 H, 16-H), 5.23 (d, $^3J_{18cis,17}$ = 17.1 Hz, 1 H, 18_{cis}-H), 5.30 (sh, 1H, 18_{trans}-H), 5.60 (sh, 1H, 17-H), 5.87 (s, 1 H, 8-H), 6.46 (d, $^4J_{3,1}$ = 2.4 Hz, 1 H, 3-H), 6.52-6.58 (sh, 1 H, 1-H), 7.03 (d, $^3J_{NH,19}$ = 7.3 Hz, 1 H, NH), 7.33 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

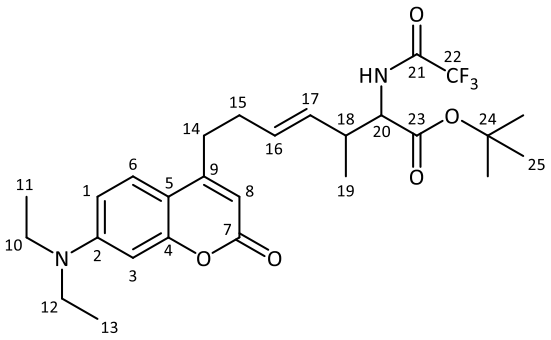
δ = 12.4 (q, C-11, C-13), 27.9 (q, C-24), 31.2 (2t, C-15/C-14), 44.7 (t, C-12, C-10), 52.7 (d, C-16), 60.4 (d, C-19), 83.4 (s, C-23), 97.8 (d, C-3), 107.5 (d, C-1/C-5), C-23 not seen, 108.5 (d, C-8), 114.3 (d, C-18), 125.1 (d, C-6), C-17 not seen, 155.7 (s, C-4/C-2), 156.3 (s, C-9/C-20), 162.3 (s, C-7), 169.3 (s, C-22).

HRMS (CI):	Calculated	Found
$C_{26}H_{33}F_3N_2O_5$ $[M]^+$:	510.2342	510.2338
Absorption max.:	364 nm	
Exitation max.:	367 nm	
Emission max.:	420 nm	
Lifetime:	3.06 ns	

tert-Butyl (E)-7-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-3-methyl-2-(2,2,2-trifluoroacetamido)hept-4-enoate (68)

According to the **GP 9** 81 mg (0.36 mmol, 1 eq.) of TFA-Glycin-*tert*-butylester reacted with 95 mg (0.25 mmol, 0.7 eq.) 7-Aminocoumarin-carbonate **65**. After work-up and column chromatography (silica gel, PE:EA 7:3) 81.8 mg (0.16 mmol, 63%, dr 91:9) of regioisomer **68** was isolated as a yellow oil.

R_f(68) = 0.24 (PE:EA 7:3)



68

Major diastereomer *anti* (72 %):

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.05 (d, $^3J_{19,18}$ = 6.9 Hz, 3-H, 19-H), 1,19 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.46 (s, 9 H, 25-H), 2.34-2.42 (sh, 2 H, 15-H), 2.68-2.75 (sh, 3 H, 14-H, 18-H), 3.39 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.43 (dd, $^3J_{20,NH}$ = 8.5 Hz, $^3J_{20,18}$ = 5.1 Hz, 1 H, 20-H), 5.37 (dt, $^3J_{17,16}$ = 15.4 Hz, $^3J_{17,18}$ = 7.8 Hz, 1 H, 17-H), 5.60 (m, 1 H, 16-H), 5.89 (s, 1 H, 8-H), 6.48 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55-6.58 (sh, 1 H, 1-H), 6.77 (d, $^3J_{NH,20}$ = 8.2 Hz, 1 H, NH), 7.35 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11, C-13), 16.6 (q, C-19), 28.0 (q, C-25), 31.3 (t, C-15), 39.9 (t, C-14), 44.7 (t, C-12, C-10), 63.8 (d, C-20), 83.4 (s, C-24), 97.8 (d, C-3), 107.6 (d, C-1/C-8/s, C-5), 108.5 (d, C-8/C-1/s, C-5), C-22 not seen, 125.1 (d, C-16/C-6), 131.6 (d, C-17), 150.5 (s, C-2), 155.6 (s, C-4), 156.3 (s, C-9/C-21), 162.3 (s, C-7), 169.0 (s, C-23).

Minor diastereomer *syn* (28 %):

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.05 (d, $^{3}J_{19,18}$ = 6.9 Hz, 3-H, 19-H), 1,19 (t, $^{3}J_{13,12}$ = $^{3}J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.46 (s, 9 H, 25-H), 2.34-2.42 (sh, 2 H, 15-H), 2.68-2.75 (sh, 3 H, 14-H, 18-H), 3.39 (q, $^{3}J_{12,13}$ = $^{3}J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.57 (dd, $^{3}J_{20,NH}$ = 8.7 Hz Hz, $^{3}J_{20,18}$ = 4.4 Hz, 1 H, 20-H), 5.37 (dt, $^{3}J_{17,16}$ = 15.4 Hz, $^{3}J_{17,18}$ = 7.8 Hz, 1 H, 17-H), 5.60 (m, 1 H, 16-H), 5.89 (s, 1 H, 8-H), 6.48 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55-6.58 (sh, 1 H, 1-H), 6.77 (d, $^{3}J_{NH,20}$ = 8.2 Hz, 1 H, NH), 7.35 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11, C-13), 15.5 (q, C-19), 28.0 (q, C-25), 30.9 (t, C-15), 39.6 (t, C-14), 44.7 (t, C-12, C-10), 63.8 (d, C-20), 83.3 (s, C-24), 97.8 (d, C-3), 107.6 (d, C-1/C-8/s, C-5), 108.5 (d, C-8/C-1/s, C-5), C-22 not seen, 125.1 (d, C-16/C-6), 131.6 (d, C-17), 150.5 (s, C-2), 155.6 (s, C-4), 156.3 (s, C-9/C-21), 162.3 (s, C-7), 168.8 (s, C-23).

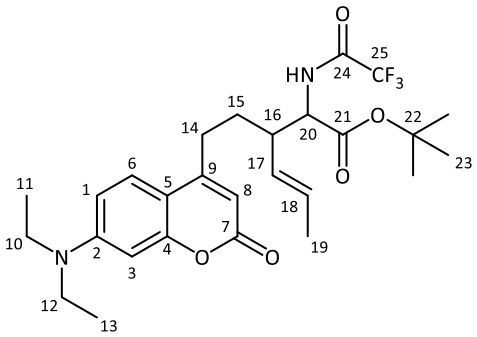
Elemental Analysis:

$C_{27}H_{35}F_3N_2O_5$	Calculated	C 61.82	H 6.73	N 5.34
(524.5812)	Found	C 62.93	H 7.06	N 4.97
HRMS (CI):	Calculated		Found	
$C_{27}H_{35}F_3N_2O_5$ $[M]^+$:	524.2498		524.2500	
Absorption max.:	364 nm			
Exitation max.:	373 nm			
Emission max.:	419 nm			
Lifetime:	3.29 ns			

tert-Butyl-(E)-3-(2-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)ethyl)-2-(2,2,2-trifluoroacetamido)hex-4-enoate (69)

According to the **GP 9** 81 mg (0.36 mmol, 1 eq.) TFA-Glycin-*tert*-butylester reacted with 95 mg (0.25 mmol, 0.7 eq.) 7-Aminocoumarin-carbonate **65**. After work-up and column chromatography (silica gel, PE:EA 7:3) 31.5 mg (0.06 mmol, 24%) of regioisomer **69** was isolated as a yellow oil.

R_f(69) = 0.23 (PE:EA 7:3)



69

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1,19 (t, $^{3}J_{13,12}$ = $^{3}J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.33 (d, $^{3}J_{19,18}$ = 6.5 Hz, 3 H, 19-H), 1.46 (s, 9 H, 23-H), 2.34-2.42 (sh, 2 H, 15-H), 2.68-2.75 (sh, 3 H, 14-H), 3.39 (q, $^{3}J_{12,13}$ = $^{3}J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.48 (dd, $^{3}J_{20,NH}$ = 8.4 Hz, $^{3}J_{20,16}$ = 4.5 Hz, 1 H, 20-H), 5.15 (m, 1 H, 16-H), 5.37 (m, 1 H, 18-H), 5.60 (m, 1 H, 17-H), 5.89 (s, 1 H, 8-H), 6.48 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55-6.58 (sh, 1 H, 1-H), 6.85 (d, $^{3}J_{NH,20}$ = 8.2 Hz, 1 H, NH), 7.35 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C-13), 16.6 (q, C-19), 28.0 (q, C-23), 31.3 (t, C-15), 39.9 (t, C-14), 44.7 (t, C-12, C-10), 57.1 (d, C-16), 63.8 (d, C-20), 83.4 (s, C-22), 97.8 (d, C-3), 107.6 (d, C-1/C-8/s, C-5), 108.5 (d, C-8/C-1/s, C-5), C-22 not seen, 125.1 (d, C-18/C-6), 131.6 (d, C-17), 150.5 (s, C-2), 155.6 (s, C-4), 156.3 (s, C-9/C-24), 162.3 (s, C-7), 169.0 (s, C-21).

Elemental Analysis:

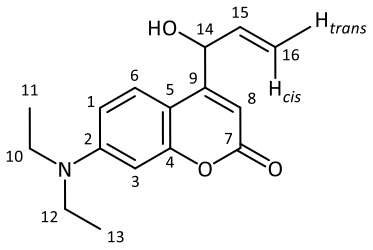
$C_{27}H_{35}F_3N_2O_5$	Calculated	C 61.82	H 6.73	N 5.34
(524.5812)	Found	C 62.93	H 7.06	N 4.97
HRMS (CI):	Calculated		Found	
$C_{27}H_{35}F_3N_2O_5$ $[M]^+$:	524.2498		524.2500	
Absorption max.:	364 nm			
Exitation max.:	373 nm			
Emission max.:	419 nm			
Lifetime:	3.29 ns			

7-(Diethylamino)-4-(1-hydroxyallyl)-2H-chromen-2-one (70)

According to the **GP-7** 1 g (4.3 mmol, 1 eq.) fluorescenting aldehyde **56** was dissolved in 45 ml abs. THF. 5.72 ml (5.72 mmol, 1.3 eq) 1M Vinylmagnesium-bromide-solution in THF was added slowly at –20 °C. The reaction mixture was

stirred for 3 h. After work-up solvent was removed in vacuo. The crude product was used raw without further purification.

R_f(70) = 0.16 (DCM:EA 9:1)



70

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{13,12}$ = $^3J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 3.40 (q, $^3J_{12,13}$ = $^3J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.32 (d, $^3J_{16trans,15}$ = 10.3 Hz, 1 H, 16_{trans}-H), 5.42 (m, 1 H, 14-H), 5.49 (d, $^3J_{16cis,15}$ = 17.2 Hz, 1 H, 16_{cis}-H), 6.06 (ddd, $^3J_{15,16cis}$ = 16.6 Hz, $^3J_{15,16trans}$ = 10.3 Hz, $^3J_{15,14}$ = 5.9 Hz, 1 H, 15-H), 6.26 (s, 1 H, 8-H), 6.50 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.48 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

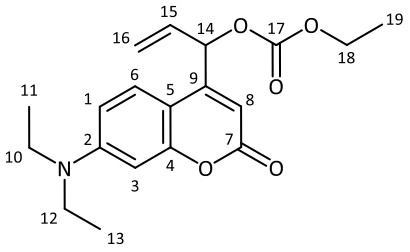
δ = 12.5 (q, C-11/C-13), 44.7 (t, C-10/C-12), 71.1 (d, C-14), 97.8 (d, C-3), 105.9 (d, C-1), 106.2 (s, C-5), 108.4 (d, C-8), 117.9 (t, C-16), 125.7 (d, C-6), 137.4 (d, C-15), 150.4 (s, C-2), 155.7 (s, C-4), 156.6 (s, C-9), 162.6 (s, C-7/C-20).

HRMS (CI):	Calculated	Found
$C_{16}H_{23}NO_3$ $[M]^+$:	273.1365	273.1399
Absorption max.:	366 nm	
Exitation max.:	369 nm	
Emission max.:	432 nm	
Lifetime:	3.46 ns	

1-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)allyl ethyl carbonate (71)

According to the **GP-8** allylalcohol **70** in 10 ml abs. DCM was dissolved in 5 ml dry pyridine. 1 ml (8.6 mmol, 2 eq.) ethyl chloroformate was added at 0 °C. The reaction mixture was allowed to warm to rt overnight. After work-up and column chromatography (silica gel, PE:EA 7:3) 848 mg (2.5 mmol, 57% over two steps) of allylcarbonate **71** was isolated as a yellow oil.

R_f(71) = 0.38 (PE:EA 7:3)



71

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.13 (t, $^{3}J_{13,12}$ = $^{3}J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.26 (t, $^{3}J_{19,18}$ = 7.1 Hz, 3 H, 19-H), 3.34 (q, $^{3}J_{12,13}$ = $^{3}J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.16 (q, $^{3}J_{18,19}$ = 7.1 Hz, 2 H, 18-H), 5.32 (d, $^{3}J_{16trans,15}$ = 10.4 Hz, 1 H, 16_{trans}-H), 5.42 (d, $^{3}J_{16cis,15}$ = 17.2 Hz, 1 H, 16_{cis}-H), 5.95 (ddd, $^{3}J_{15,16cis}$ = 16.7 Hz, $^{3}J_{15,16trans}$ = 10.4 Hz, $^{3}J_{15,14}$ = 6.1 Hz, 1 H, 15-H), 6.12 (s, 1 H, 8-H), 6.18 (d, $^{3}J_{14,15}$ = 6.1 Hz, 1 H), 6.44 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.50 (dd, $^{3}J_{1,6}$ = 9.1 Hz, $^{4}J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.36 (d, $^{3}J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

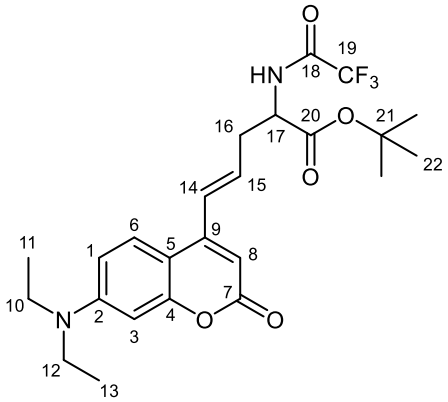
δ = 12.4 (q, C-11/C-13), 14.2 (q, C-19), 44.7 (t, C-10/C-12), 64.7 (t, C-18), 75.1 (d, C-14), 97.9 (d, C-3), 105.8 (d, C-1), 106.4 (s, C-5), 108.6 (d, C-8), 120.2 (t, C-16), 125.5 (d, C-6), 133.2 (d, C-15), 150.6 (s, C-2), 154.1 (s, C-4), 156.6 (s, C-17), 162.0 (s, C-7/C-9).

HRMS (CI):	Calculated	Found
$C_{19}H_{23}NO_5$ $[M]^+$:	345.1576	345.1575
Absorption max.:	377 nm	
Exitation max.:	372 nm	
Emission max.:	436 nm	
Lifetime:	4.62 ns	

tert-Butyl-(E)-5-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-2-(2,2,2-trifluoro-acetamido)pent-4-enoate (72)

According to the **GP 9** 81 mg (0.36 mmol, 1 eq.) TFA-Glycin-*tert*-butylester reacted with 95 mg (0.25 mmol, 0.7 eq.) 7-Aminocoumarin-carbonate **71**. After work-up and column chromatography (silica gel, PE:EA 7:3) 86.9 mg (0.18 mmol, 72%) of **72** was isolated as a yellow oil.

R_f(72) = 0.25 (PE:EA 7:3)



72

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^{3}J_{13,12}$ = $^{3}J_{11,10}$ = 7.1 Hz, 6 H, 11-H, 13-H), 1.49 (s, 9 H, 22-H), 2.82 (m, 1 H, 16-H), 2.95 (m, 1 H, 16'-H), 3.40 (q, $^{3}J_{12,13}$ = $^{3}J_{10,11}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.64 (dd, $^{3}J_{17,16}$ = 12.4 Hz, $^{3}J_{17,NH}$ = 5.7 Hz, 1 H, 17-H), 6.03 (s, 1 H, 8-H), 6.24 (dt, $^{3}J_{15,14}$ = 15.1 Hz, $^{3}J_{15,16}$ = 7.4 Hz, 1 H, 15-H), 6.49 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.68 (d, $^{3}J_{14,15}$ = 15.6 Hz, 1 H, 14-H), 7.14 (d, $^{3}J_{NH,17}$ = 6.7 Hz, 1 H, NH), 7.37 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C-13), 27.8 (q, C-22), 35.5 (t, C-16), 44.7 (t, C-12/C-10), 52.7 (d, C-17), 84.1 (s, C-21), 97.9 (d, C-3), 104.6 (d, C-8), 107.2 (d, C-1), 108.5 (s, C-5), 114.2 (s, C-19), 125.3 (d, C-6), 128.1 (d, C-14), 131.5 (d, C-15), 149.9 (s, C-9), 150.7 (s, C-4), 156.5 (s, C-18), 162.4 (s, C-7/C-9), 168.9 (s, C-20).

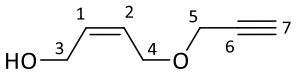
HRMS (CI):	Calculated	Found
$C_{24}H_{29}F_3N_2O_5$ $[M+H]^+$:	483.2101	483.2101
Absorption max.:	380 nm	
Exitation max.:	375 nm	
Emission max.:	443 nm	
Lifetime:	4.71 ns	

(Z)-4-(Prop-2-yn-1-yloxy)but-2-en-1-ol (73)

To solution of 2.67 ml (30 mmol, 3 eq.) cis-2-Butene-1,4-diol in 10 ml abs. THF 400 mg (10 mmol, 1 eq., 60% suspension in mineral oil) NaH suspension in 5 ml abs. THF was added at 0 °C. The reaction mixture was stirred at rt for 30 minutes and 1.11 ml (10 mmol, 1 eq., 80% w/v in toluene) propargylic bromide was added quickly. After refluxing overnight the solution was diluted with water and washed with diethylether. The organic layer was dried over Na_2SO_4. The solvent was removed in

vacuo and the crude product purified by column chromatography (silica gel, DCM:Et_2O 9:1) to furnish 980 mg (7.77 mmol, 78%) of **73** as a colourless liquid.

R_f(73) = 0.16 (DCM:Et_2O 9:1)



73

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ ppm 2.44 (t, $^4J_{7,5}$ = 2.4 Hz, 1 H, 7-H), 2.98 (bs, 1 H, OH), 4.07 (m, 4 H, 3-H, 4-H), 4.12 (m, 2 H, 5-H), 5.59 (m, 1 H, 2-H), 5.74 (m, 1 H, 1-H).

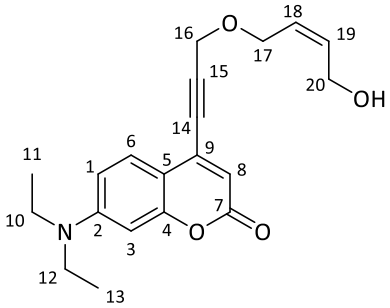
^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 57.2 (t, C-3), 58.2 (t, C-5), 64.9 (t, C-4), 74.9 (d, C-7), 79.4 (s, C-6), 126.9 (d, C-1), 133.2 (d, C-2).

(Z)-7-(Diethylamino)-4-(3-((4-hydroxybut-2-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (74)

According to the **GP 4** to the solution of 311 mg (248 µmol, 2 mol%) $Pd(PPh_3)_4$, 94.5 mg (496 µmol, 4 mol%) CuI and 4.5 g (12.4 mmol, 1eq.) enol triflate **12** in 62 ml abs. THF 1.7 ml (1.3 g, 12.4 mmol, 1 eq.) Et_3N and 3 g (24.8 mmol, 2 eq.) propargylic diol **73** were added at rt and the reaction mixture was stirred at rt for 2 h. Work-up and column chromatography (silicagel, DCM:MeOH 95:5) furnished 3.3 g (9.8 mmol, 79%) of the product **74** as a yellow oil.

R_f(74) = 0.23 (DCM:MeOH 95:5)



74

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.25 (t, $^3J_{17,18}$ = $^3J_{20,19}$ = 6.6 Hz, 4 H, 17-H, 20-H), 4.45 (s, 2 H, 16-H), 5.71 (m, 1 H, 18-H), 5.89 (m, 1 H, 19-H), 6.13 (s, 1 H, 8-H), 6.43 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.54 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

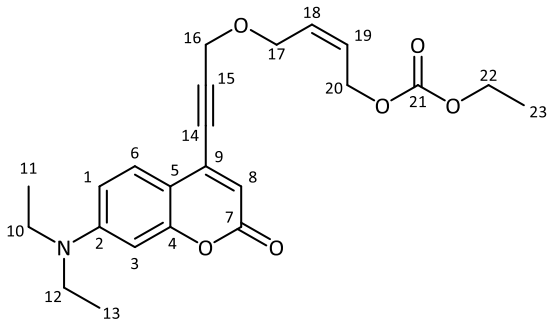
δ = 12.4 (q, C-11/C13), 44.9 (t, C-12/C-10), 57.2 (t, C-16/C-20), 58.7 (t, C-16/C-20), 64.9 (t, C-17), 80.8 (s, C-14), 96.2 (s, C-15), 97.4 (d, C-3), 107.7 (d, C-1), 108.8 (s, C-5), 111.8 (d, C-8) 127.2 (d, C-6/C-19), 127.5(d, C-6/C-19), 133.4 (d, C-18) 136.7 (s, C-9), 151.1 (s, C-2), 156.2 (s, C-4), 161.5 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{20}H_{23}NO_4$ $[M\text{-}OH]^+$:	326.1751	326.1770
Absorption max.:	393 nm	
Emission max.:	484 nm	
Lifetime:	5.53 ns	
Melting point:	68 °C	

(Z)-4-((3-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)oxy)but-2-en-1-yl ethyl carbonate (75)

According to the **GP 8** to 3.3 g (9.8 mmol, 1 eq.) fluorescenting allylalcohol **74** in 3.2 ml dry pyridine 4.5 ml (19.6 mmol, 2 eq.) ethyl chloroformate was added dropwise at 0 °C. After work-up and column chromatography (silicagel, DCM:EA 95:5) 4.4 g (10.7 mmol, 87%) of **75** was isolated as a yellow oil.

R_f(75) = 0.42 (DCM:EA 95:5)



75

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.30 (t, $^{3}J_{23,22}$ = 7.1 Hz, 3 H, 23-H), 3.41 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.19 (q, $^{3}J_{22,23}$ = 7.1 Hz, 2 H, 22-H), 4.28 (d, $^{3}J_{17,18}$ = 5.0 Hz, 2 H, 17-H), 4.47 (s, 2 H, 16-H), 4.75 (d, $^{3}J_{20,19}$ = 5.3 Hz, 2 H, 20-H), 5.86 (dtt, $^{3}J_{18,19}$ = $^{3}J_{19,18}$ = 11.3 Hz, $^{3}J_{18,17}$ = $^{3}J_{19,20}$ = 5.3 Hz, $^{4}J_{19,17}$ = $^{4}J_{18,20}$ = 2.5 Hz, 2 H, 18-H, 19-H), 6.17 (s, 1 H, 8-H), 6.46 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.60 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.57 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.3 (q, C-23), 44.8 (t, C-12/C-10), 58.1 (t, C-16), 63.2 (t, C-22/C-10/C-17), 64.2 (t, C-22/C-10/C-17), 65.6 (t, C-22/C-10/C-17), 80.8 (s, C-14), 96.1 (s, C-15), 97.4 (d, C-3), 107.8 (d, C-1), 108.8 (s, C-5), 111.9 (d, C-8) 127.3 (d, C-6/C-19), 127.5 (d, C-6/C-19), 130.1 (d, C-18) 136.6 (s, C-9), 151.0 (s, C-2), 155.0 (s, C-4), 156.2 (s, C-21), 161.4 (s, C-7).

Elemental Analysis:

$C_{23}H_{27}NO_6$	Calculated	C 66.81	H 6.58	N 3.39
(413.4636)	Found	C 67.11	H 6.61	N 3.13

HRMS (CI):	Calculated	Found
$C_{23}H_{27}NO_6$ $[M]^+$:	413.1824	413.1847

Absorption max.: 396 nm

Emission max.: 478 nm

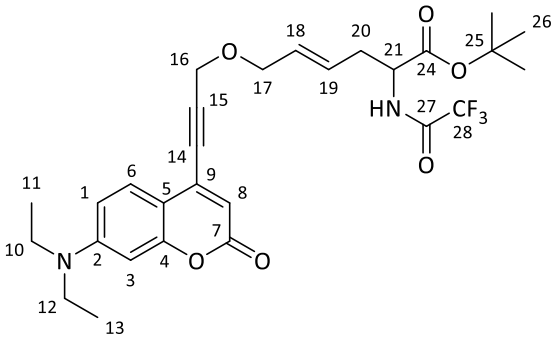
Lifetime: 5.57 ns

Melting point: 55-56 °C

tert-Butyl-(E)-6-((3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)oxy)-2-(2,2,2-trifluoroacetamido)hex-4-enoate (76)

According to the **GP 9** 227 mg (1 mmol, 1 eq.) TFA-glycin-*tert*-butylester reacted with 206 mg (0.5 mmol, 0.5 eq.) 7-Aminocoumarin-carbonate **75**. After work-up and column chromatography (silica gel, DCM:EA 95:5, 9:1) 189.5 mg (0.34 mmol, 69%) of **76** was isolated as a yellow oil.

R_f(76) = 0.41 (DCM:EA 95:5)



76

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.17 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.45 (s, 9 H, 26-H), 2.51 (m, 1 H, 20-H), 2.67 (m, 1 H, 20'-H), 3.37 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.07 (d, $^{3}J_{17,18}$ = 6.1 Hz, 2 H, 17-H), 4.52 (m, 1 H, 21-H), 5.26 (s, 2 H, 16-H), 5.59 (m, 2 H, 18-H, 19-H), 5.95 (s, 1 H, 8-H), 6.46 (d,

$^{4}J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.53 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.06 (d, $^{3}J_{NH,21}$ = 7.2 Hz, 1 H, NH), 7.25 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

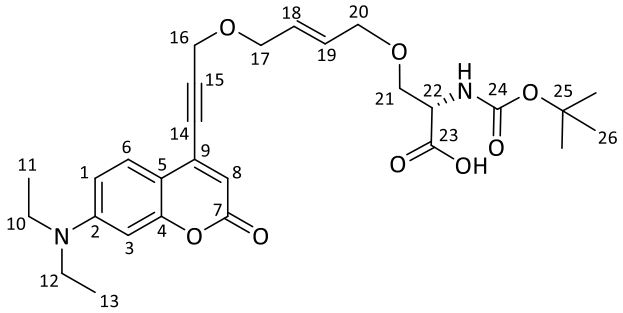
δ = 12.4 (q, C-11/C13), 27.9 (q, C-26), 34.7 (t, C-20), 44.8 (t, C-12/C-10), 52.3 (t, C-16), 53.4 (d, C-21), 71.4 (t, C-17), C-25 not seen, 83.7 (s, C-14), C-15 not seen, 97.5 (d, C-3), 108.0 (d, C-1), 108.7 (s, C-5), 108.8 (d, C-8), 125.7 (d, C-19), 129.2 (d, C-18), 148.3 (s, C-9), 148.2 (s, C-2), 150.8 (s, C-27/C-4), 161.8 (s, C-7), 169.1 (s, C-24).

HRMS (CI):	Calculated	Found
$C_{28}H_{36}F_3N_2O_6$ $[M+3H]^+$:	553.2509	553.1800
Absorption max.:	372 nm	
Exitation max.:	381 nm	
Emission max.:	446 nm	
Lifetime:	3.98 ns	

(E)-N-(tert-Butoxycarbonyl)-O-(4-((3-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl)oxy)but-2-en-1-yl)-L-serine (77)

To the solution of 1 mg (2.5 µmol, 1 mol%) $[AllylPdCl]_2$ and 3 mg (11.3 µmol, 4.5 mol%) PPh_3 in 0.5 ml abs. THF 103 mg (0.25 mmol, 0.5 eq.) fluorescenting carbonate **75** was added. The mixture was stirred for 15 minutes at rt and 110 mg (0.5 mmol, 1 eq.) Boc-serine was added. The reaction mixture was stirred overnight at rt. Afterwards the solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:MeOH 95:5) to furnish 90 mg (0.17 mmol, 68%) of **77** as a yellow oil.

R_f(77) = 0.27 (DCM:MeOH 95:5)



77

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.44 (s, 9 H, 26-H), 3.41 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.95 (m, 2 H, 21-H), 4.16-4.17 (sh, 2 H, 17-H), 4.39 (m, 1 H, 22-H), 4.46 (s, 2 H, 16-H), 4.70-4.71 (sh, 2 H, 20-H), 5.91 (m, 2 H, 18-H, 19-H), 6.16 (s, 1 H, 8-H), 6.46 (d,

$^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.60 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.56 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 8.01 (s, 1 H, OH).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

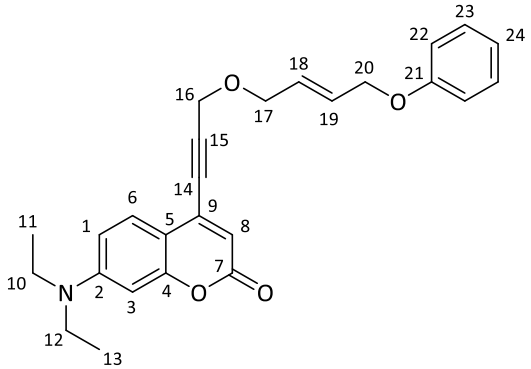
δ = 12.4 (q, C-11/C13), 28.3 (q, C-26), 44.9 (t, C-12/C-10), 58.0 (t, C-16/d, C-22), 69.7 (t, C-21/C-20), 77.2 (d, C-17), 80.8 (s, C-14/C-25), 96.1 (s, C-15), 97.4 (d, C-3), 107.7 (d, C-1), 108.8 (s, C-5), 111.8 (d, C-8) 127.5 (d, C-6), 130.3 (d, C-18), 132.1 (d, C-19), 136.7 (s, C-9), 151.0 (s, C-2/C-4), 156.2 (s, C-24), 161.6 (s, C-7), 170.5 (s, C-23).

HRMS (CI):	Calculated	Found
$C_{28}H_{37}N_2O_8$ $[M+H]^+$:	528.2472	528.2462
Absorption max.:	396 nm	
Exitation max.:	404 nm	
Emission max.:	484 nm	
Lifetime:	5.66 ns	

(E)-7-(Diethylamino)-4-(3-((4-phenoxybut-2-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (78)

To the solution of 6 mg (5 µmol, 5 mol%) $Pd(PPh_3)_4$ and 41 mg (0.1 mmol, 1 eq.) fluorescenting carbonate **75** in 0.6 ml THF 10.4 mg (0.11 mmol, 1.1 eq.) phenol was added. The reaction mixture was refluxed overnight. Afterwards the solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 24.2 mg (0.058 mmol, 58%) of **78** as a yellow oil.

R_f(78) = 0.3 (DCM:EA 95:5)



78

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.13 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.34 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.14 (d, $^{3}J_{17,18}$ = 4.8 Hz, 2 H, 17-H), 4.40 (s, 2 H, 16-H), 4.50 (d, $^{3}J_{20,19}$ = 4.2 Hz, 2 H, 20-H), 5.94 (m, 2 H, 18-H, 19-H), 6.10 (s, 1 H, 8-H), 6.39 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.51 (dd, $^{3}J_{1,6}$ = 9.0

Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.85 (m, 3 H, Phe), 7.19 (m, 2 H, Phe), 7.50 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

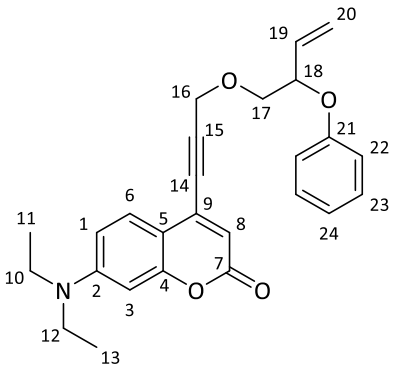
δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 57.9 (t, C-16), 67.6 (t, C-20), 70.0 (t, C-17), 80.8 (s, C-14), 96.4 (s, C-15), 97.5(d, C-3), 107.7 (d, C-1), 108.8 (d, C-22), 111.9 (d, C-8), 114.7 (s, C-5), 116,1 (d, C-24), 127.5 (d, C-6), 128.6 (d, C-18), 129.2 (d, C-23), 129.5 (d, C-19), 136.7 (s, C-9), 151.1 (s, C-2), 156.2 (s, C-4), 158.5 (s, C-21), 161.4 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{26}H_{27}NO_4$ $[M]^+$:	417.1940	417.1947
Absorption max.:	397 nm	
Exitation max.:	408 nm	
Emission max.:	482 nm	
Lifetime:	5.65 ns	

7-(Diethylamino)-4-(3-((2-phenoxybut-3-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (79)

To the solution of 6 mg (5 µmol, 5 mol%) $Pd(PPh_3)_4$ and 41 mg (0.1 mmol, 1 eq.) fluorescenting carbonate **75** in 0.6 ml THF 10.4 mg (0.11 mmol, 1.1 eq.) phenol was added. The reaction mixture was refluxed overnight. Afterwards the solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 5 mg (0.012 mmol, 12%) of **79** as a yellow oil.

R_f(79) = 0.3 (DCM:EA 95:5)



79

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.13 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.34 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.79 (dd, $^{3}J_{17,18}$ = 5.2 Hz, $^{2}J_{17,17'}$ = 4.0 Hz, 1 H, 17-H), 4.41 (s, 2 H, 16-H), 4.84 (m, 1 H, 18-H), 5.25 (dt, $^{3}J_{20trans,19}$ = 10.6 Hz, $^{2}J_{20trans,20cis}$ = 1.0 Hz, 1 H, 20_{trans}-H), 5.35 (dt, $^{3}J_{20cis,19}$ = 17.4 Hz, $^{2}J_{20cis,20trans}$ = 1.2 Hz, 1 H, 20_{cis}-H), 5.83 (m, 1 H, 19-H), 6.10 (s, 1 H, 8-H),

6.39 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.51 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.85 (m, 3 H, Phe), 7.19 (m, 2 H, Phe), 7.50 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

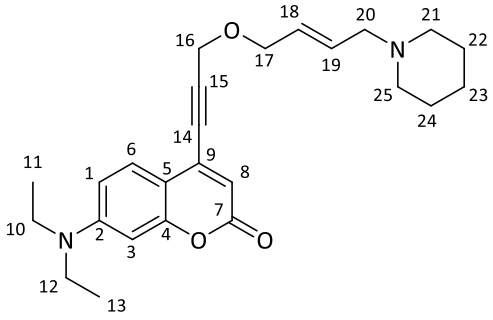
δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 59.4 (t, C-16), 72.6 (t, C-17), 78.1 (d, C-18), 80.8 (s, C-14), 96.4 (s, C-15), 97.5 (d, C-3), 107.7 (d, C-1), 108.8 (d, C-22), 111.9 (d, C-8), 114.7 (s, C-5), 116,1 (d, C-24), 127.5 (d, C-6), 118.4 (t, C-20), 129.2 (d, C-23), 134.3 (d, C-19), 136.7 (s, C-9), 151.1 (s, C-2), 156.2 (s, C-4), 158.5 (s, C-21), 161.4 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{26}H_{27}NO_4$ $[M]^+$:	417.1940	417.1947
Absorption max.:	397 nm	
Exitation max.:	408 nm	
Emission max.:	482 nm	
Lifetime:	5.65 ns	

(E)-7-(diethylamino)-4-(3-((4-(piperidin-1-yl)but-2-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (80)

To the solution of 4.8 mg (5 µmol, 5 mol%) $[AllylPdCl]_2$ and 5.2 mg (20 µmol, 20 mol%) PPh_3 in 2 ml DMF 41 mg (0.1 mmol, 1 eq.) fluorescenting carbonate **75** was added and the mixture was stirred at rt for 15 minutes before it was cooled down to –20 °C and 15 µl (0.15 mmol, 1.5 eq.) piperidine was added dropwise. The reaction mixture was allowed to warm up to rt overnight. Afterwards it was dissolved with EA and washed with water. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:MeOH, 95:5) to furnish 35.9 mg (0.088 mmol, 88%) of **80** as a brown oil.

R_f(80) = 0.19 (DCM:MeOH 9:1)



80

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.49 (sh, 2 H, 23-H), 1.71 (m, 4 H, 22-H, 24-H), 2.63 (sh, 4 H, 21-H, 25-H), 3.21 (d, $^3J_{20,19}$ = 6.6 Hz, 2 H, 20-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.15 (d, $^3J_{17,18}$ = 5.2 Hz, 2 H, 17-H), 4.45 (s, 2 H, 16-H), 5.92 (dtt, $^3J_{18,19}$ = $^3J_{19,18}$ = 16.8 Hz, $^3J_{19,20}$ = 6.6 Hz, $^3J_{18,17}$ = 5.6 Hz, 2 H, 18-H, 19-H), 6.14 (s, 1 H, 8-H), 6.44 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.60 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.55 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

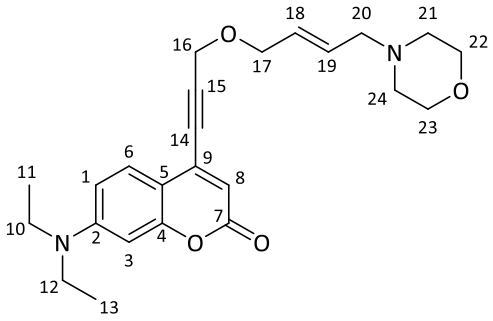
δ = 12.4 (q, C-11/C13), 23.4 (t, C-23), 24.5 (t, C-22/C-24), 44.8 (t, C-12/C-10), 53.7 (t, C-21/C-25), 58.0 (t, C-16), 60.0 (t, C-20), 70.0 (t, C-17), 80.8 (s, C-14), 96.3 (s, C-15), 97.4 (d, C-3), 107.7 (d, C-1), 108.8 (s, C-5), 111.8(d, C-8) 127.4 (d, C-6), 131.91 (d, C-18) 136.7 (d, C-19), C-9 not seen, 151.1 (s, C-2), 156.2 (s, C-4), 161.5 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{25}H_{33}N_2O_3$ $[M+H]^+$:	409.2486	409.2497
Absorption max.:	374 nm	
Exitation max.:	380 nm	
Emission max.:	435 nm	
Lifetime:	4.50/1.37 ns	

(E)-7-(diethylamino)-4-(3-((4-morpholinobut-2-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (81)

To the solution of 3.7 mg (10 µmol, 5 mol%) $[AllylPdCl]_2$ and 10.5 mg (40 µmol, 20 mol%) PPh_3 in 2 ml DMF 83 mg (0.2 mmol, 1 eq.) fluorescenting carbonate **75** was added and the mixture was stirred at rt for 15 minutes before it was cooled down to –20 °C and 22.4 µl (22.6 mg, 0.26 mmol, 1.3 eq.) morpholine was added dropwise. The reaction mixture was allowed to warm up to rt overnight. Afterwards it was dissolved with EA and washed with water. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:MeOH 95:5) to furnish 68.7 mg (0.168 mmol, 84%) of **81** as a brown solid compound.

R_f(81) = 0.28 (DCM:MeOH 9:1)



81

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.89 (sh, 4 H, 21-H,24-H), 2.76 (sh, 4 H, 22-H, 23-H), 3.32 (d, $^{3}J_{20,19}$ = 6.4 Hz, 2 H, 20-H), 3.39 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.14 (dd, $^{3}J_{17,18}$ = 5.6 Hz, $^{4}J_{17,16}$ = 0.7 Hz, 2 H, 17-H), 4.44 (s, 2 H, 16-H), 5.94 (dtt, $^{3}J_{18,19}$ = $^{3}J_{19,18}$ = 15.6 Hz, $^{3}J_{19,20}$ = 6.5 Hz, $^{3}J_{18,17}$ = 5.6 Hz, 2 H, 18-H, 19-H), 6.13 (s, 1 H, 8-H), 6.43 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.59 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.54 (d, $^{3}J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

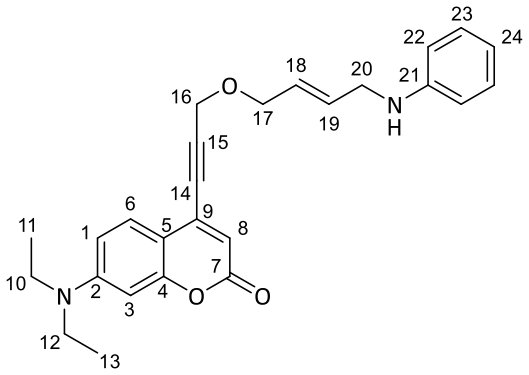
δ = 12.4 (q, C-11/C13), 23.4 (t, C-21/C24), 44.8 (t, C-12/C-10), 53.5 (t, C-22/C-23), 57.0 (t, C-16/C-20), 57.9 (t, C-16/C-20), 70.0 (t, C-17), 80.7 (s, C-14), 96.4 (s, C-15), 97.4 (d, C-3), 107.7 (d, C-1), 108.9 (s, C-5), 111.7 (d, C-8) 127.5 (d, C-6), 128.6 (d, C-18) 130.9 (d, C-19), 136.7 (s, C-9), 151.1 (s, C-2), 156.2 (s, C-4), 161.4 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{24}H_{30}N_2O_4$ $[M+3H]^+$:	413.2435	413.2446
Absorption max.:	370 nm	
Exitation max.:	373 nm	
Emission max.:	432 nm	
Lifetime:	3.68/1.25 ns	
Melting point:	73-75 °C	

(E)-7-(diethylamino)-4-(3-((4-(phenylamino)but-2-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (82)

To the solution of 5.8 mg (5 µmol, 5 mol%) $Pd(PPh_3)_4$ and 41 mg (0.1 mmol, 1 eq.) fluorescenting carbonate **75** in 0.6 ml THF 10 µl (10.2 mg, 0.11 mmol, 1.1 eq.) aniline was added. The reaction mixture was stirred at rt overnight. Afterwards the solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 25.9 mg (0.062 mmol, 62%) of **82** as a yellow oil.

R_f(82) = 0.28 (DCM:EA 9:1)



82

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.82 (d, $^{3}J_{17,18}$ = 4.8 Hz, 2 H, 17-H), 4.16 (d, $^{3}J_{20,19}$ = 5.7 Hz, 2 H, 20-H), 4.45 (s, 2 H, 16-H), 5.91 (m, 2 H, 18-H, 19-H), 6.16 (s, 1 H, 8-H), 6.46 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.58 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H),), 6.85 (m, 3 H, Phe), 7.19 (m, 2 H, Phe), 7.41 (d, $^{3}J_{NH,20}$ = 7.9 Hz, 1 H, NH7.56 (d, $^{3}J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

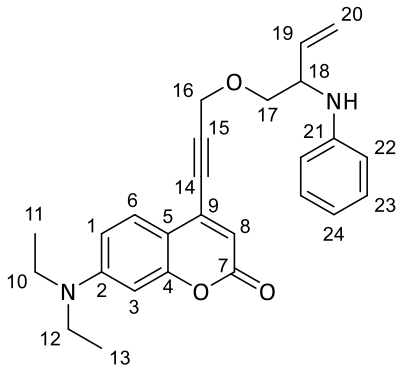
δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 45.4 (t, C-20), 57.7 (t, C-16), 70.0 (t, C-17), 80.7(s, C-14), 96.5 (s, C-15), 97.5(d, C-3), 107.8 (d, C-1), 108.8 (d, C-8, s, C-5), 111.8 (d, C-22), 117.7 (d, C-24), 127.5 (d, C-6), 129.3 (d, C-23/C-18), 131.9 (d, C-19), 136.7 (s, C-9), 147.9 (s, C-21), 151.1 (s, C-2), 156.2 (s, C-4), 161.4 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{26}H_{28}N_2O_3$ $[M]^+$:	416.2100	416.2095
Absorption max.:	377 nm	
Exitation max.:	373 nm	
Emission max.:	436 nm	
Lifetime:	4.60/1.82 ns	

7-(Diethylamino)-4-(3-((2-(phenylamino)but-3-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (83)

To the solution of 5.8 mg (5 µmol, 5 mol%) $Pd(PPh_3)_4$ and 41 mg (0.1 mmol, 1 eq.) fluorescenting carbonate **75** in 0.6 ml THF 10 µl (10.2 mg, 0.11 mmol, 1.1 eq.) aniline was added. The reaction mixture was stirred at rt overnight. Afterwards the solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 3.7 mg (0.009 mmol, 9%) of **83** as a yellow oil.

R_f(83) = 0.33 (DCM:EA 9:1)



83

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^{3}J_{11,10}$ = $^{3}J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.41 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.77 (m, 2 H, 17-H), 4.14 (dd, $^{3}J_{18,17}$ = 10.2 Hz, $^{3}J_{18,19}$ = 5.7 Hz, 1 H, 18-H), 4.52 (s, 2 H, 16-H), 5.25 (dt, $^{3}J_{20trans,19}$ = 10.5 Hz, $^{2}J_{20trans,20cis}$ = 1.2 Hz, 1 H, 20_{trans}-H), 5.38 (dt, $^{3}J_{20cis,19}$ = 17.3 Hz, $^{2}J_{20cis,20trans}$ = 1.4 Hz, 1 H, 20_{cis}-H), 5.89 (ddd, $^{3}J_{19,20cis}$ = 17.2 Hz, $^{3}J_{19,20trans}$ = 10.4 Hz, $^{3}J_{19,18}$ = 5.7 Hz, 1 H, 19-H), 6.14 (s, 1 H, 8-H), 6.46 (d, $^{4}J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.53 (dd, $^{3}J_{1,6}$ = 9.0 Hz, $^{4}J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 6.69 (m, 3 H, Phe), 7.16 (m, 2 H, Phe), 7.55 (d, $^{3}J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 44.8 (t, C-12/C-10), 59.4 (t, C-16), 64.4 (d, C-18), 70.2 (t, C-17), 80.9 (s, C-14), 96.5 (s, C-15), 97.5 (d, C-3), 107.7 (d, C-1), 108.0 (d, C-22), 111.9 (d, C-8), 114.7 (s, C-5), 116,1 (d, C-24), 127.5 (d, C-6), 118.4 (t, C-20), 129.2 (d, C-23), 131.9 (d, C-19), 136.7 (s, C-9), 151.1 (s, C-2), 156.2 (s, C-4), 158.5 (s, C-21), 161.4 (s, C-7).

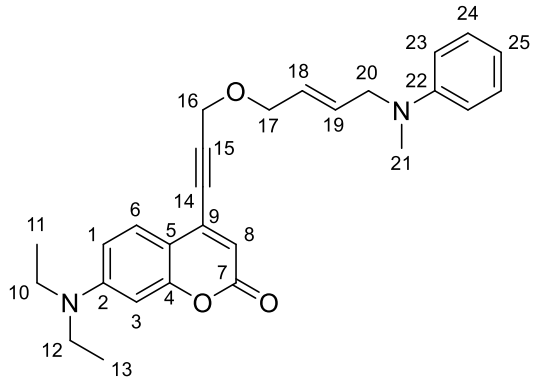
HRMS (CI):	Calculated	Found
$C_{26}H_{28}N_2O_3$ $[M]^+$:	416.2100	416.2099
Absorption max.:	387 nm	
Exitation max.:	382 nm	
Emission max.:	449 nm	
Lifetime:	5.72/2.66 ns	

(E)-7-(Diethylamino)-4-(3-((4-(methyl(phenyl)amino)but-2-en-1-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (84)

To the solution of 5.8 mg (5 µmol, 5 mol%) $Pd(PPh_3)_4$ and 41 mg (0.1 mmol, 1 eq.) fluorescenting carbonate **75** in 0.6 ml THF 11 µl (10.2 mg, 0.11 mmol, 1.1 eq.) N-methylanilin was added. The reaction mixture was stirred at rt overnight. Afterwards the solvent was removed in vacuo and the crude product purified by column

chromatography (silica gel, DCM:EA 95:5) to furnish 32.4 mg (0.075 mmol, 75%) of **84** as a brown oil.

R_f(84) = 0.38 (DCM:EA 9:1)



84

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.89 (sh, 4 H, 21-H,24-H), 2.76 (sh, 4 H, 22-H, 23-H), 3.32 (d, $^3J_{20,19}$ = 6.4 Hz, 2 H, 20-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.14 (dd, $^3J_{17,18}$ = 5.6 Hz, $^4J_{17,16}$ = 0.7 Hz, 2 H, 17-H), 4.44 (s, 2 H, 16-H), 5.94 (dtt, $^3J_{18,19}$ = $^3J_{19,18}$ = 15.6 Hz, $^3J_{19,20}$ = 6.5 Hz, $^3J_{18,17}$ = 5.6 Hz, 2 H, 18-H, 19-H), 6.13 (s, 1 H, 8-H), 6.43 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.59 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.54 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 23.4 (t, C-21/C24), 44.8 (t, C-12/C-10), 53.5 (t, C-22/C-23), 57.0 (t, C-16/C-20), 57.9 (t, C-16/C-20), 70.0 (t, C-17), 80.7 (s, C-14), 96.4 (s, C-15), 97.4 (d, C-3), 107.7 (d, C-1), 108.9 (s, C-5), 111.7 (d, C-8) 127.5 (d, C-6), 128.6 (d, C-18) 130.9 (d, C-19), 136.7 (s, C-9), 151.1 (s, C-2), 156.2 (s, C-4), 161.4 (s, C-7).

Elemental Analysis:

$C_{27}H_{30}N_2O_3$	Calculated	C 75.32	H 7.02	N 6.51
(430.5480)	Found	C 75.02	H 7.20	N 5.90

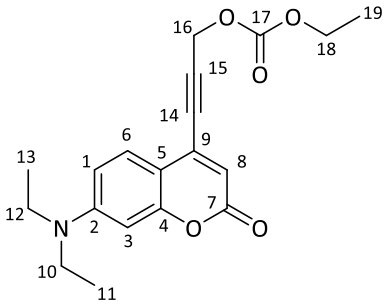
HRMS (CI):	Calculated	Found
$C_{27}H_{31}N_2O_3$ $[M+H]^+$:	431.2329	431.2343

3-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)prop-2-yn-1-yl ethyl carbonate (85).

According to the **GP-4** to the solution of 2.8 mg (240 µmol, 2 mol%) $Pd(PPh_3)_4$, 90 mg (480 µmol, 4 mol%) CuI and 4.4 g (12 mmol, 1eq.) enol triflate **12** in 60 ml abs. THF under nitrogen atmosphere at rt 2 ml (12 mmol, 1 eq) Et_3N and 3.1 g (24 mmol, 2 eq.) ethyl propargyl carbonate were added. The reaction mixture was stirred for 2

h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 4.049 g (11.8 mmol, 98%) of **85** was isolated as a yellow solid compound.

R_f(85) = 0.13 (DCM:EA 95:5)



85

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.35 (t, $^3J_{19.18}$ = 7.1 Hz, 3 H, 19-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.28 (q, $^3J_{18.19}$ = 7.1 Hz, 2 H, 18-H), 5.03 (s, 2 H, 16-H), 6.17 (s, 1 H, 8-H), 6.46 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.59 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.55 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.3 (q, C-19), 44.9 (t, C-12/C-10), 55.4 (t, C-16), 64.9 (t, C-18), 81.3 (s, C-14), 93.2 (s, C-15), 97.4 (d, C-3), 107.6 (d, C-1), 108.8 (s, C-5), 112.1 (d, C-8) 127.5 (d, C-6), 136.1 (s, C-9), 151.1 (s, C-2), 154.5 (s, C-4), 156.2 (s, C-17), 161.3 (s, C-7).

Elemental Analysis:

$C_{19}H_{21}NO_5$	Calculated	C 66.46	H 6.16	N 4.08
(343.3790)	Found	C 66.91	H 5.48	N 4.21

HRMS (CI):	Calculated	Found
$C_{19}H_{22}NO_5$ $[M]^+$:	343.1414	343.1422

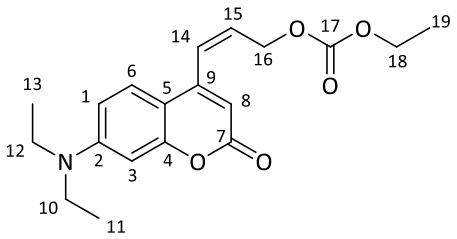
Absorption max.:	405 nm
Exitation max.:	410 nm
Emission max.:	502 nm
Lifetime:	6.05 ns
Melting point:	135-138 °C

(Z)-3-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)allyl ethyl carbonate (86) [163]

162 mg (0.49 mmol, 1 eq.) fluorescenting ester **85** was dissolved in EA (4 ml), and 25 mg (40 wt%) Lindlar catalyst was added. The mixture was stirred under H_2

atmosphere for 2 h until the complete conversion of the starting material, filtered through celite. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 140 mg (0.4 mmol, 83%) of **86** as a yellow oil.

R_f(86) = 0.39 (DCM:EA 9:1)



86

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.29 (t, $^3J_{19.18}$ = 7.1 Hz, 3 H, 19-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.19 (q, $^3J_{18.19}$ = 7.2 Hz, 2 H, 18-H), 4.76 (dd, $^3J_{16,15}$ = 6.6 Hz, $^4J_{16.14}$ = 1.2 Hz, 2 H, 16-H), 5.9 (sh, 1 H, 8-H), 6.15 (dt, $^3J_{15.14}$ = 11.9 Hz, $^3J_{15,16}$ = 6.6 Hz, 1 H, 15-H), 6.50 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.55 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.65 (ddd, $^3J_{14.15}$ = 11.8 Hz, $^4J_{14.16}$ = 1.5 Hz, 1 H, 14-H), 7.29 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H)

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.2 (q, C-19), 44.8 (t, C-12/C-10), 64.0 (t, C-18/C-16), 64.2 (t, C-18/C-16), 97.8 (d, C-3), 107.4 (d, C-1/C-8), 108.2 (d, C-1/C-8), 108.4 (s, C-5), 126.3 (d, C-15/C14), 127.3 (d, C-15/C14), 131.0 (d, C-6), 149.4 (s, C-2), 150.8 (s, C-4), 154.8 (s, C-17), 156.3 (s, C-7), 161.7 (s, C-9).

Elemental Analysis:

$C_{19}H_{23}NO_5$	Calculated	C 66.07	H 6.71	N 4.06
(345.3896)	Found	C 66.72	H 6.94	N 4.57

HRMS (CI):	Calculated	Found
$C_{19}H_{24}NO_5$ $[M+H]^+$:	346.1649	346.1659
Absorption max.:	379 nm	
Exitation max.:	378 nm	
Emission max.:	516 nm	
Lifetime:	4.83/3.2 ns	

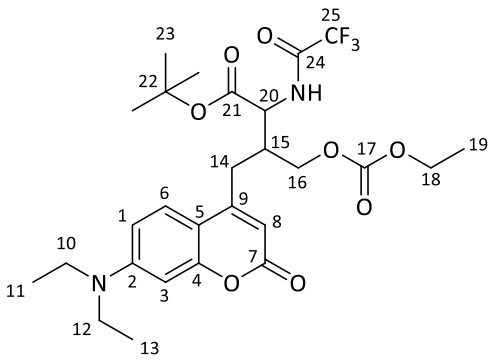
tert-Butyl-4-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-3-[ethoxycarbonyl)-oxy)methyl)-2-(2,2,2-trifluoroacetamido]butanoate (88) [99]

In a Schlenk flask 228 mg (1 mmol, 1 eq.) TFA-glycine-*tert*-butylester was dissolved in abs. THF (5 ml), 2.5 ml (1M solution in THF, 2.5 mmol, 2.5 eq) LHMDS was added dropwise at –78 °C. The mixture was stirred for another 15 minutes. In the second Schlenk flask 158 mg (1.1 mmol, 1.1 eq) $ZnCl_2$ was heated carefully in vacuo, and after cooling down to room temperature dissolved in 2 ml abs. THF. The $ZnCl_2$-solution was added dropwise at –78 °C to Li-enolate solution and the resulting mixture was stirred for additional 45 minutes for the complete transmetallation.

Meanwhile 4 mg (10 µmol, 1 mol%) allylpalldium(II) chloride dimer and 15 mg (46 µmol, 4.5 mol%) triphenylphosphine were dissolved in THF (2 ml) stirred for 10 minutes at rt before 242 mg (0.7 mmol, 0.7 eq.) fluorescenting allylcarbonate **86** was added.

The catalyst-substrate solution was added dropwise to Zink-enolat solution at –78 °C. The reaction mixture was warmed up to –50 °C over 4 h, after monitoring by TLC, the reaction mixture was diluted with EA and quenched with 1N $KHSO_4$-solution. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 237 mg (0.41 mmol, 59%) of **88** as a yellow solid compound.

R_f(88) = 0.41 (DCM:EA 9:1)



88

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.28 (t, $^3J_{19,18}$ = 7.1 Hz, 3 H, 19-H), 1.54 (s, 9 H, 23-H), 2.71-2.79 (sh, 1 H, 15-H), 2.84 (dd, $^2J_{14,14'}$ = 13.7 Hz, $^3J_{14,15}$ = 9.6 Hz, 1 H, 14-H), 2.94 (dd, $^2J_{14',14}$ = 13.7 Hz, $^3J_{14',15}$ = 5.4 Hz, 1 H, 14'-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.02 (dd, $^2J_{16,16'}$ = 11.5 Hz, $^3J_{16,15}$ = 6.4 Hz, 1 H, 16-H), 4.17 (m, 3 H, 16'-H, 18-H), 4.71 (dd, $^3J_{20,NH}$ = 7.5 Hz, $^3J_{20,15}$ = 3.4 Hz, 1 H, 20-H), 5.97 (s, 1 H, 8-H), 6.49 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.59 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ =

2.6 Hz, 1 H, 1-H), 7.30 (d, $^3J_{NH,20}$ = 6.9 Hz, 1 H, NH), 7.40 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 14.1 (q, C-19), 28.0 (q, C-23), 30.4 (d, C-15), 39.9 (t, C-14), 44.7 (t, C-12/C-10), 54.4 (d, C-20), 64.4 (t, C-18/C-16), 65.5 (t, C-18/C-16), 84.8 (s, C-22), 98.0 (d, C-3), 107.5 (d, C-1), 108.8 (s, C-5), 109.3 (d, C-8), 114.2 (s, C-25), 125 (d, C-6), 150.8 (s, C-2), 152.4 (s, C-4), 154.5 (s, C-17/C-24), 156.6 (s, C-9), 161.7 (s, C-7), 168.1 (s, C-21).

Elemental Analysis:

$C_{27}H_{35}F_3N_2O_8$	Calculated	C 56.64	H 6.16	N 4.89
(572.5782)	Found	C 55.67	H 5.99	N 4.69

HRMS (CI):	Calculated	Found
$C_{27}H_{34}F_3N_2O_8$ $[M-H]^+$:	571.2340	571.2258
Absorption max.:	363 nm	
Exitation max.:	370 nm	
Emission max.:	442 nm	
Lifetime:	3.24 ns	
Melting poing:	67-69 °C	

tert-Butyl-2-(4-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-5-oxotetrahydro-furan-3-yl)-2-(2,2,2-trifluoroacetamido)acetate (89) [99]

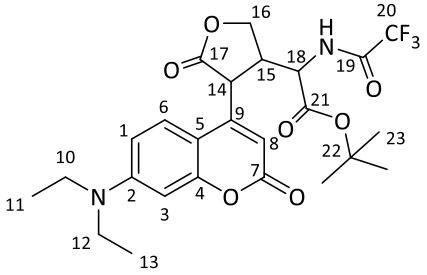
In a Schlenk flask 228 mg (1 mmol, 1 eq.) TFA-glycine-*tert*-butylester was dissolved in abs. THF (5 ml), 2.5 ml (1M solution in THF, 2.5 mmol, 2.5 eq) LHMDS was added dropwise at –78 °C. The mixture was stirred for another 15 minutes. In the second Schlenk flask 158 mg (1.1 mmol, 1.1 eq) $ZnCl_2$ was heated carefully in vacuo, and after cooling down to room temperature dissolved in 2 ml abs. THF. The $ZnCl_2$ solution was added dropwise at –78 °C to Li-enolate solution and the resulting mixture was stirred for additional 45 minutes for the complete transmetallation.

Meanwhile 4 mg (10 µmol, 1 mol%) allylpalldium(II) chloride dimer and 15 mg (46 µmol, 4.5 mol%) triphenylphosphine were dissolved in THF (2 ml) stirred for 10 minutes at rt before 242 mg (0.7 mmol, 0.7 eq.) fluorescenting allylcarbonate **86** was added.

The catalyst-substrate solution was added dropwise to Zink-enolate solution at –78 °C. The reaction mixture was warmed up overnight to rt, after monitoring by TLC, the reaction mixture was diluted with EA and quenched with 1N $KHSO_4$-solution. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified

by column chromatography (silica gel, DCM:EA 95:5) to furnish 226 mg (0.43 mmol, 60 %) of **89** as a yellow solid.

R_f(89) = 0.16 (DCM:EA 9:1)



89

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.18 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.43 (s, 9 H, 23-H), 3.39 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.55 (m, 1 H, 15-H), 4.16 (d, $^3J_{14,15}$ = 9.4 Hz, 1 H, 14-H), 4.35 (dd, $^3J_{16,16'}$ = 9.8 Hz, $^3J_{16,15}$ = 8.4 Hz, 1 H, 16-H), 4.75 (m, 2 H, 18-H, 16'-H), 5.93 (s, 1 H, 8-H), 6.45 (d, , $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.61 (dd, $^3J_{1,6}$ = 9.1 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.30 (d, $^3J_{6,1}$ = 9.2 Hz, 1 H, 6-H), 8.11 (d, $^3J_{NH,18}$ = 8.2 Hz, 1 H, NH)

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

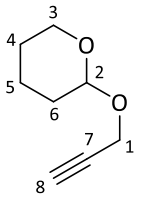
δ = 12.4 (q, C-11/C13), 27.7 (q, C-23), 43.9 (d, C-15), 44.7 (t, C-12/C-10), 53.4 (d, C-14), 54.6 (d, C-18), 69.1 (t, C-16), 85.0 (s, C-22), 97.7 (d, C-3), 106.9 (d, C-1), 107.7 (s, C-5), 109.4 (d, C-8), 114.0 (s, C-20) 125.3 (d, C-6), 150.5 (s, C-2), 156.5 (s, C-4), 157.4 (s, C-19/C-9), 157.8 (s, C-19/C-9), 162.0 (s, C-7), 167.1 (s, C-17), 173.5 (s, C-21).

HRMS (CI):	Calculated	Found
$C_{25}H_{29}F_3N_2O_7$ $[M]^+$:	526.1927	526.1951
Absorption max.:	375 nm	
Exitation max.:	386 nm	
Emission max.:	213 nm	
Lifetime:	4.78/2.27 ns	

2-(Prop-2-yn-1-yloxy)tetrahydro-2H-pyran (91) [164]

0.8 ml (13.9 mmol, 1 eq.) propargylic alcohol and 352 mg (1.4 mmol, 0.1 eq.) PPTS were suspended in DCM (55 ml). 1.4 ml (15.3 mmol, 1.1 eq.) 3,4-dihydro-*2H*-pyran was added dropwise. The reaction was allowed to stir over night at rt and afterwards it was washed with sat. NaCl-solution. The organic layer was dried over Na_2SO_4 and the solvent was removed in vacuo. The resulted white liquid product **91** 1.9 g (13.6 mmol, 98%) was used raw without further purification.

R_f(91) = 0.63 (PE:EA 8:2)



91

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.44-1.81 (sh, 6 H, 4-H, 5-H, 6-H), 2.38 (t, $^2J_{8.1}$ = 2.4 Hz, 1 H, 8-H), 4.20 (m, 2 H, 3-H), 4.76 (m, 1 H, 2-H), 5.26 (s, 2 H, 1-H).

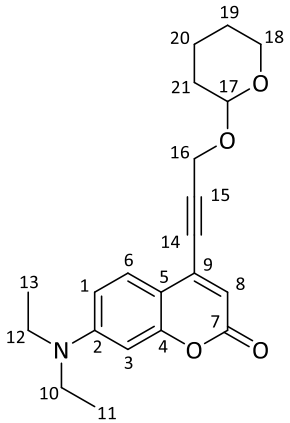
^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 20.4 (t, C-5), 25.4 (t, C-4), 30.2 (t, C-6), 54.6 (t, C-1), 62.1 (t, C-3), 80.1 (s, C-14), 96.7 (s, C-15), 104.2 (d, C-2).

7-(Diethylamino)-4-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-yn-1-yl)-2H-chromen-2-one (92)

According to the **GP-4** to the solution of 17 mg (15 µmol, 2 mol%) $Pd(PPh_3)_4$, 5.7 mg (30 µmol, 4 mol%) CuI and 271 mg (0.74 mmol, 1eq.) enol triflate **12** in 3.7 ml abs. THF under nitrogen atmosphere 0.1 ml (0.74 mmol, 1 eq) Et_3N and 207 mg (1.48 mmol, 2 eq.) propargyl ester **91** were added at rt. The reaction mixture was stirred for 2 h. After work-up and column chromatography (silica gel, DCM:EA 95:5) 278 mg (0.72 mmol, 97 %) of **92** was isolated as a yellow solid.

R_f(92) = 0.16 (DCM:EA 95:5)



92

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.21 (t, $^3J_{11.10}$ = $^3J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.54-1.8 (sh, 6 H, 19-H, 20-H, 21-H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.56-3.61 (m, 1 H, 18'-H), 3.87-3.92 (m, 1 H, 18-H), 4.58 (d, $^4J_{16.17}$ = 2.0 Hz, 2 H, 16-H), 4.90 (t, $^3J_{17.21}$ = 3.4 Hz, 17-H), 6.17 (s, 1 H, 8-H), 6.46 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.59 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.58 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 19.0 (t, C-20), 25.3 (t, C-19), 30.2 (t, C-21), 44.8 (t, C-12/C-10), 54.6 (t, C-16), 62.1 (t, C-18), 80.1 (s, C-14), 96.7 (s, C-15), 97.3 (d, C-3/C-17), 97.4 (d, C-3/C-17), 107.9 (d, C-1), 108.8 (s, C-5), 111.9 (d, C-8) 127.6 (d, C-6), 136.9 (s, C-9), 151.0 (s, C-2), 156.2 (s, C-4), 161.5 (s, C-7).

Elemental Analysis:

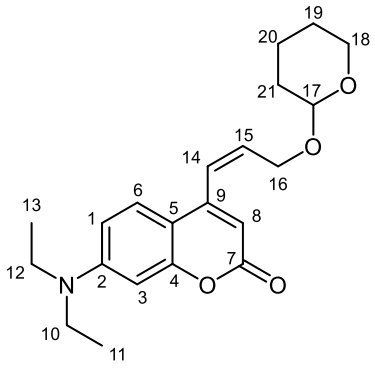
$C_{21}H_{25}NO_4$	Calculated	C 70.96	H 7.09	N 3.94
(355.4275)	Found	C 70.66	H 7.19	N 4.05

HRMS (CI):	Calculated	Found
$C_{21}H_{26}NO_4$ $[M+H]^+$:	356.1856	356.1872
Absorption max.:	397 nm	
Exitation max.:	404 nm	
Emission max.:	481 nm	
Lifetime:	5.523 ns	

(Z)-7-(Diethylamino)-4-(3-((tetrahydro-2H-pyran-2-yl)oxy)prop-1-en-1-yl)-2H-chromen-2-one (93) [163]

109.6 mg (0.3 mmol, 1 eq.) fluorescenting ester **92** was dissolved in EA (1 ml), and 50 mg (45 wt%) Lindlar catalyst was added. The mixture was stirred under H_2 atmosphere for 2 h (until the complete conversion) and filtered through celite. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 102.6 mg (0.29 mmol, 97%) of **93** as a yellow oil.

R_f(93) = 0.16 (DCM:EA 95:5)



93

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.19 (t, $^{3}J_{11.10}$ = $^{3}J_{13.12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.47-1.84 (sh, 6 H, 19-H, 20-H, 21-H), 3.39 (q, $^{3}J_{10,11}$ = $^{3}J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.44-3.48 (m, 1 H, 18'-H), 3.77-3.83 (m, 1 H, 18-H), 4.38 (ddd, $^{3}J_{16,15}$ = 5.9 Hz, $^{4}J_{16.14}$ = 1.8 Hz, 2 H, 16-H), 4.56-4.58 (sh, 1 H, 17-H), 5.84 (s, 1 H, 8-H), 6.20 (m, 1 H, 15-H), 6.48 (d, $^{4}J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.54 (dd, $^{3}J_{1,6}$ = 8.9 Hz, $^{4}J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 6.58 (m, 1 H, 14-H), 7.33 (d, $^{3}J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 19.0 (t, C-20), 25.3 (t, C-19), 30.2 (t, C-21), 44.8 (t, C-12/C-10), 64.0 (t, C-16), 62.1 (t, C-18), 97.3 (d, C-3/C-17), 97.4 (d, C-3/C-17), 107.9 (d, C-1), 108.8 (s, C-5), 111.9 (d, C-8), 126.3 (d, C-15/C14), 127.3 (d, C-15/C14), 127.6 (d, C-6), 136.9 (s, C-9), 151.0 (s, C-2), 156.2 (s, C-4), 161.5 (s, C-7).

HRMS (CI):	Calculated	Found
$C_{17}H_{21}NO_2$ $[M\text{-}OTHP]^+$:	271.1572	271.1490
Absorption max.:	397 nm	
Exitation max.:	402 nm	
Emission max.:	479 nm	
Lifetime:	5.41 ns	

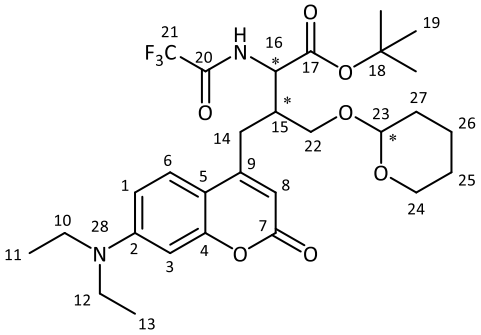
tert-Butyl-4-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-3-[tetrahydro-2H-pyran-2-yl)oxy)methyl)-2-(2,2,2-trifluoroacetamido]butanoate (94) [99]

To the solution of 0.2 ml (132 mg, 0.82 mmol, 2.75 eq.) HMDS in 1 ml abs. THF was added dropwise 1.6M solution of 0.46 ml (0.74 mmol, 2.5 eq.) *n*-BuLi at –78 °C. The cooling bath was removed and the mixture was stirred for another 15 minutes at rt. In the second Schlenk flask 45 mg (0.33 mmol, 1.1 eq) $ZnCl_2$ and 67 mg (0.297 mmol, 1.0 eq.) TFA-glycine-*tert*-butylester were dissolved in 1 ml abs. THF. The freshly prepared LHMDS-solution was added dropwise to glycineester-metall salt-

solution at –78 °C and the reaction mixture was stirred at this temperature for 30 minutes for the complete transmetallation.

97 mg (0.27 mmol, 0.9 eq.) fluorescing coumarin **93** was dissolved in 0.5 ml abs. THF and added dropwise to Zn-enolate solution at –78 °C. The reaction mixture was allowed to warm overnight to rt, diluted with EA and quenched with 1N $KHSO_4$-solution. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 83.1 mg (0.14 mmol, 53 %) of **94** as a yellow oil.

R_f(94) = 0.1 (DCM:MeOH 95:5)



94

Isolated as a mixture of diastereomers.

Selected signals:

^{1}H-NMR (400 MHz, *$CDCl_3$*)**:**

δ = 1.19 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.57 (m, 1 H, 16-H), 5.84 (s, 1 H, 8-H), 6.49 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.54 (dd, $^3J_{1,6}$ = 8.9 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.33 (d, $^3J_{6,1}$ = 8.9 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, *$CDCl_3$*)**:**

δ = 12.4 (q, C-11/C13), 27.3 (q, C-19), 31.1 (t, C-14), 44.7 (t, C-12/C-10), 53.0 (d, C-16), 84.2 (s, C-18), 97.9 (d, C-3), 107.6 (d, C-1/C-8), 107.7 (d, C-1/C-8), 108.73 (s, C-5), 114.2 (s, C-21), 124.8 (d, C-6), 150.7 (s, C-2), 154.6 (s, C-4), 156.4 (s, C-20), 162.0 (s, C-7), 169.4 (s, C-17).

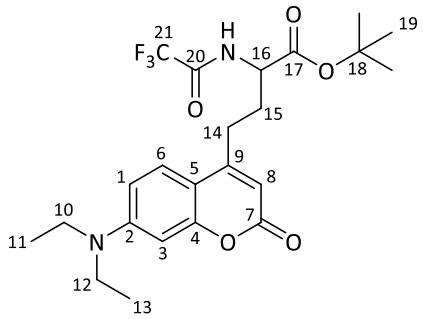
tert-Butyl-4-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-2-(2,2,2-trifluoroacetamido)butanoate (95) [99]

To the solution of 0.15 ml (113 mg, 0.7 mmol, 2.31 eq.) HMDS in 1 ml abs. THF 0.4 ml (0.63 mmol, 2.1 eq.) 1.6M solution of *n*-BuLi was added dropwise at –78 °C. The cooling bath was removed and the mixture was stirred for another 15 minutes at rt. In the second Schlenk flask 45 mg (0.33 mmol, 1.1 eq) $ZnCl_2$ and 75 mg (0.33 mmol,

1.1 eq.) TFA-glycine-*tert*-butylester were dissolved in 1 ml abs. THF. The freshly prepared LHMDS-solution was added dropwise to glycineester-metallsalt-solution at –78 °C and the reaction mixture was stirred at this temperature for 30 minutes for the complete transmetallation.

68 mg (0.3 mmol, 1 eq.) vinyl coumarin **58** was dissolved in 0.5 ml abs. THF and added dropwise to Zn-enolate solution at –78 °C. The reaction mixture was warmed up overnight to rt, after monitoring by TLC, the reaction mixture was diluted with EA and quenched with 1N $KHSO_4$-solution. The aqueous layer was extracted three times with EA and combined organic layers were dried over Na_2SO_4. The solvent was removed in vacuo and the crude product purified by column chromatography (silica gel, DCM:EA 95:5) to furnish 60 mg (0.13 mmol, 60 %) of **95** as a yellow solid compound.

R_f(95) = 0.18 (DCM:EA 95:5)



95

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.53 (s, 9 H, 19-H), 2.09 (s, 1 H), 2.06-2.14 (sh, 1 H), 2.29-2.38 (sh, 1 H), 2.59-2.67 (sh, 1 H), 2.74-2.82 (sh, 1 H), 3.41 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 4.60 (dd, $^3J_{16,15}$ = 12.3 Hz, $^3J_{16,NH}$ = 6.5 Hz, 1 H, 16-H), 5.91 (s, 1 H, 8-H), 6.50 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.58 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.11 (d, $^3J_{NH,16}$ = 6.90 Hz, 1 H, NH), 7.30 (d, $^3J_{6,1}$ = 9.1 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 27.3 (q, C-19), 31.1 (t, C-14), 44.7 (t, C-12/C-10), 53.0 (d, C-16), 84.2 (s, C-18), 97.9 (d, C-3), 107.6 (d, C-1/C-8), 107.7 (d, C-1/C-8), 108.73 (s, C-5), 114.2 (s, C-21), 124.8 (d, C-6), 150.7 (s, C-2), 154.6 (s, C-4), 156.4 (s, C-20), 162.0 (s, C-7), 169.4 (s, C-17).

Elemental Analysis:

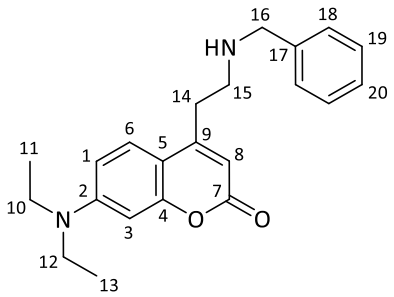
$C_{23}H_{29}F_3N_2O_5$	Calculated	C 58.72	H 6.21	N 5.95
(471.4967)	Found	C 59.58	H 6.43	N 5.77
HRMS (CI):	Calculated		Found	

$C_{23}H_{30}F_3N_2O_5$ $[M+H]^+$:	471.2101	471.2076
Absorption max.:	371 nm	
Exitation max.:	367 nm	
Emission max.:	421 nm	
Lifetime:	3.11 ns	
Melting poing:	135-138 °C	

4-(2-(Benzylamino)ethyl)-7-(diethylamino)-2H-chromen-2-one (96)

According to the **GP 10** to 27 µl (26.8 mg, 0.25 mmol, 2 eq) benzylamine in 0.3 ml 1.4-dioxane 38 µl (0.25 mmol, 2 eq.) Et_3N, 5.3 mg (0.125 mmol, 1 eq.) LiCl and 30 mg (0.125 mmol, 1 eq.) vinyl coumarin **58** in 0.3 ml 1.4-dioxane were added. The resulting mixture was refluxed overnight. After work-up and column chromatography (silicagel, DCM:EA 95:5, 9:1) 36.5 mg (0.1 mmol, 83%) of **96** was isolated as a yellow oil.

R_f(96) = 0.07 (DCM:EA 9:1)



96

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.15 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.64 (bs, 1 H, NH), 2.83 (t, $^3J_{14,15}$ = 7.0 Hz, 2 H, 14-H), 2.93 (t, $^3J_{15,14}$ = 6.7 Hz, 2 H, 15-H), 3.36 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.78 (s, 2 H, 16-H), 5.90 (s, 1 H, 8-H), 6.44 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.51 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.19-7.27 (m, 5 H, Phe), 7.35 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 32.0 (t, C-14), 44.7 (t, C-12/C-10), 47.8 (t, C-15), 53.7 (t, C-16), 97.8 (d, C-3), 108.1 (d, C-1), 108.2 (s, C-5), 108.5 (d, C-8), 125.4 (d, C-6), 127.3-128.8 (d, C-18/C-19/C-20), 139.2 (s, C-17), 150.5 (s, C-2), 154.4 (s, C-4), 156.3 (s, C-9), 162.2 (s, C-7).

Elemental Analysis:

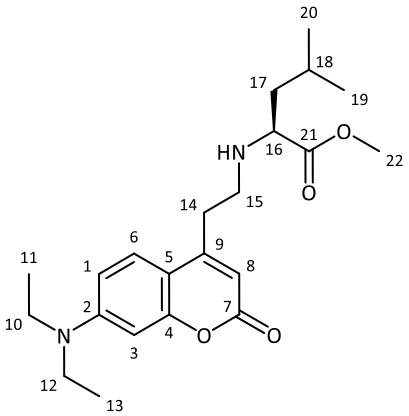
$C_{22}H_{26}N_2O_2$	Calculated	C 75.40	H 7.48	N 7.99

(350.4620)	Found	C 76.56	H 7.93	N 7.70
HRMS (CI):	Calculated		Found	
$C_{22}H_{27}N_2O_2$ $[M+H]^+$:	351.2067		351.2074	
Absorption max.:	364 nm			
Exitation max.:	370 nm			
Emission max.:	420 nm			

2-(7-(Diethylamino)-2-oxo-2H-chromen-4-yl)ethyl)-L-leucine methyl ester (97)

According to the **GP 10** to 54.5 mg (0.3 mmol, 1.2 eq) leucine methyl ester hydrochloride in 0.6 ml 1.4-dioxane 70 µl (0.5 mmol, 2 eq.) Et_3N, 10.6 mg (0.25 mmol, 1 eq.) LiCl and 60.8 mg (0.25 mmol, 1 eq.) vinyl coumarin **58** in 0.6 ml 1.4-dioxane were added. The resulting mixture was refluxed overnight. After work-up and column chromatography (silicagel, DCM:MeOH 98:2) 58.2 mg (0.15 mmol, 60%) of **97** was isolated as a yellow solid compound.

R_f(97) = 0.21 (DCM:MeOH 95:5)



97

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.92 (2d, $^3J_{20,18}$ = 6.6 Hz, 6 H, 20-H, 19-H), 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.46 (m, 2 H, 17-H, 18-H), 1.72 (m, 2 H, 17'-H, NH), 2.86 (m, 4 H, 15-H, 14-H), 3.31 (t, $^3J_{16,17}$ = $^3J_{16,NH7}$ = 7.3 Hz, 1 H, 16-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.69 (s, 3 H, 22-H), 5.96 (s, 1 H, 8-H), 6.49 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.57 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.40 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 22.4 (q, C-20/C-19), 22.7 (q, C-20/C-19), 24.9 (d, C-18), 32.5 (t, C-14), 42.7 (t, C-17), 44.7 (t, C-12/C-10), 46.8 (t, C-15), 51.7 (q, C-22), 60.1 (d, C-16), 97.9 (d, C-3), 108.1 (d, C-1), 108.2 (s, C-5),

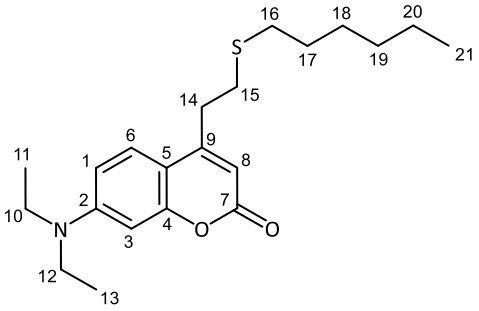
108.5 (d, C-8), 125.3 (d, C-6), 150.5 (s, C-2), 154.3 (s, C-4), 156.3 (s, C-9), 162.2 (s, C-7), 171.5 (s, C-21).

HRMS (CI):	Calculated	Found
$C_{22}H_{32}N_2O_4$ $[M]^+$:	388.2362	388.2369
Absorption max.:	363 nm	
Exitation max.:	368 nm	
Emission max.:	421 nm	
Lifetime:	3.13 ns	

7-(Diethylamino)-4-(2-(hexylthio)ethyl)-2H-chromen-2-one (98)

According to the **GP 10** to 71 µl (59 mg, 0.5 mmol, 2 eq) 1-hexanthiol in 0.6 ml 1.4-dioxane 70 µl (0.5 mmol, 2 eq.) Et_3N and 60.8 mg (0.25 mmol, 1 eq.) vinyl coumarin **58** in 0.6 ml 1.4-Dioxane were added. The resulting mixture was stirred overnight at rt. After work-up and column chromatography (silicagel, PE:EA 7:3) 79.3 mg (0.22 mmol, 88%) of **98** was isolated as a yellow solid compound.

R_f(98) = 0.41 (DCM:EA 95:5)



98

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 0.88 (t, $^3J_{21,20}$ = 6.9 Hz, 3 H, 21-H), 1.20 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.24-1.31 (sh, 4 H, 19-H, 20-H), 1.33-1.41 (sh, 2 H, 18-H), 1.59 (m, 2 H, 17-H), 2.56 (m, 2 H, 16-H), 2.78-2.82 (sh, 2 H, 14-H), 2.94 (t, $^3J_{15,14}$ = 7.3 Hz, 2 H, 15-H), 3.40 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 5.95 (s, 1 H, 8-H), 6.49 (d, $^4J_{3,1}$ = 2.6 Hz, 1 H, 3-H), 6.57 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.38 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 14.0 (q, C-21), 22.5 (t, C-20), 28.6-32.5 (5t, C-14/ C-15/C-16/C-17/C-18/C-19), 44.7 (t, C-12/C-10), 97.9 (d, C-3), 107.9 (d, C-1), 108.0 (s, C-5), 108.5 (d, C-8), 125.1 (d, C-6), 150.6 (s, C-2), 154.6 (s, C-4), 156.4(s, C-9), 162.1 (s, C-7).

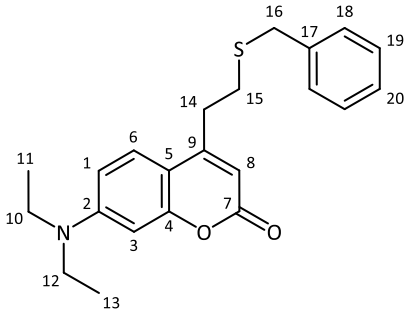
Elemental Analysis:

$C_{21}H_{31}NO_2S$	Calculated	C 69.76	H 8.64	N 3.87
(361.5440)	Found	C 69.64	H 8.63	N 3.77
HRMS (CI):	Calculated		Found	
$C_{21}H_{31}NO_2S$ $[M]^+$:	361.2075		361.2078	
Absorption max.:	370 nm			
Exitation max.:	369 nm			
Emission max.:	423 nm			
Melting point:	62-63 °C			

4-(2-(Benzylthio)ethyl)-7-(diethylamino)-2H-chromen-2-one (99)

According to the **GP 10** to 67 µl (70.8 mg, 0.5 mmol, 2 eq) benzyl mercaptan in 0.6 ml 1.4-dioxane 70 µl (0.5 mmol, 2 eq.) Et_3N and 60.8 mg (0.25 mmol, 1 eq.) vinyl coumarin **58** in 0.6 ml 1.4-dioxane were added. The resulting mixture was stirred at rt overnight. After work-up and column chromatography (silicagel, PE:EA 7:3) 78 mg (0.21 mmol, 85%) of **99** was isolated as a yellow solid compound.

R_f(99) = 0.24 (PE:EA 7:3)



99

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.11 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 2.60 (m, 2 H, 14-H), 2.75 (m, 2 H, 15-H), 3.30 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.68 (s, 2 H, 16-H), 5.77 (s, 1 H, 8-H), 6.38 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.43 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.6 Hz, 1 H, 1-H), 7.13 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H), 7.17 (m, 1 H, 20-H), 7.23 (sh, 4 H, 18-H, 19-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.5 (q, C-11/C13), 30.0 (t, C-15), 32.0 (t, C-16), 36.6 (t, C-14), 44.7 (t, C-12/C-10), 97.8 (d, C-3), 107.8 (d, C-1), 108.0 (s, C-5), 108.5 (d, C-8), 125.1 (d, C-6), 127.2 (d, C-20), 128.6 (d, C-18/C-19), 128.9 (d, C-18/C-19), 138.0 (s, C-17), 150.5 (s, C-2), 154.5 (s, C-4), 156.3 (s, C-9), 162.1 (s, C-7).

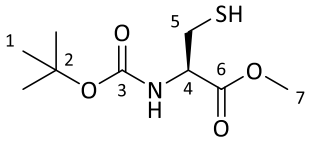
Elemental Analysis:

$C_{22}H_{25}NO_2S$	Calculated	C 71.90	H 6.86	N 3.81
(367.5070)	Found	C 71.80	H 6.96	N 3.68
HRMS (CI):	Calculated		Found	
$C_{21}H_{31}NO_2S$ $[M]^+$:	367.1606		367.1614	
Absorption max.:	368 nm			
Exitation max.:	370 nm			
Emission max.:	424 nm			
Melting point:	65-67 °C			

N-tert-Butoxycarbonyl-L-cystein methyl ester (100) [165]

1.49 g (3.18 mmol, 1 eq.) Boc-cystin was suspended in 12 ml MeOH, 5 ml H_2O and 0.1 ml glacial acetic acid. After 875 mg (3.34 mmol, 1.05 eq.) PPh_3 and 98.5 mg (1.2 mmol, 0.34 eq.) sodium acetate were added, the reaction mixture was refluxed for 20 h. Subsequently it was diluted with DCM, the organic layer was separated and the aqueous phase was extracted 3x with DCM. The combined organic layers were washed with sat. NaCl-solution and dried over Na_2SO_4. The solvent was removed in vacuo. The crude product was purified by column chromatography (silica gel, PE:EA 8:2) to furnish 1.2 mg (5.13 mmol, 81%) of **100** as a colourless oil.

R_f(100) = 0.19 (PE:EA 8:2)



100

^{1}H-NMR (400 MHz, *$CDCl_3$*)**:**

δ = 1.38 (t, $^3J_{SH,5}$ = 8.9 Hz, 1 H, SH), 1.44 (s, 9 H, 1-H), 2.96 (m, 2 H, 5-H), 3.77 (s, 3 H, 7-H), 4.59 (sh, 1 H, 4-H), 5.41 (bs, 1H, NH).

^{13}C-NMR (100 MHz, *$CDCl_3$*)**:**

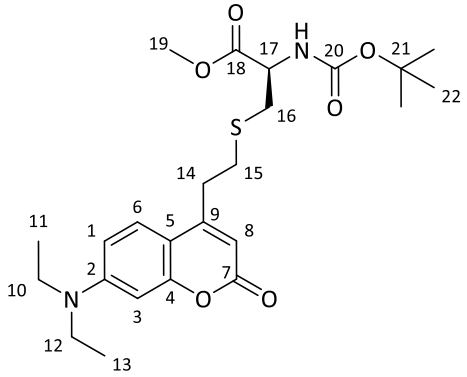
δ = 27.3 (t, 5-C), 28.3 (q, C-1), 52.7 (q, C-7), 54.8 (d, C-4), 80.3 (s, C-2), 155.1 (s, C-3), 170.8 (s, C-6).

N-(tert-Butoxycarbonyl)-S-(2-(7-(diethylamino)-2-oxo-2H-chromen-4-yl)-ethyl)-L-cysteine methyl ester (101).

According to the **GP 10** to 70.8 mg (0.3 mmol, 1.2 eq) methyl (*tert*-butoxycarbonyl)-L-cysteinester **100** in 0.6 ml 1.4-dioxane 35 µl (25 mg, 0.25 mmol, 1 eq.) Et_3N and 60.8 mg (0.25 mmol, 1 eq.) vinyl coumarin **58** solution in 0.6 ml 1.4-

dioxane were added at rt. The resulting solution was stirred for 3 h at rt. After work-up and column chromatography (silicagel, PE:EA 7:3, 6:4) 107.9 mg (0.23 mmol, 92%) of **101** was isolated as a yellow oil.

R_f(101) = 0.28 (PE:EA 6:4)



101

^{1}H-NMR (400 MHz, $CDCl_3$)**:**

δ = 1.17 (t, $^3J_{11,10}$ = $^3J_{13,12}$ = 7.1 Hz, 6 H, 13-H, 11-H), 1.40 (s, 9 H, 22-H), 2.78-2.83 (m, 2 H, 15-H), 2.88-2.92 (m, 2 H, 14-H), 2.98-3.02 (m, 2 H, 16-H), 3.37 (q, $^3J_{10,11}$ = $^3J_{12,13}$ = 7.1 Hz, 4 H, 12-H, 10-H), 3.73 (s, 3 H, 19-H), 4.53 (m, 1 H, 17-H), 5.37 (d, $^3J_{NH,17}$ = 7.3 Hz, 1 H, NH), 5.90 (s, 1 H, 8-H), 6.46 (d, $^4J_{3,1}$ = 2.5 Hz, 1 H, 3-H), 6.56 (dd, $^3J_{1,6}$ = 9.0 Hz, $^4J_{1,3}$ = 2.5 Hz, 1 H, 1-H), 7.35 (d, $^3J_{6,1}$ = 9.0 Hz, 1 H, 6-H).

^{13}C-NMR (100 MHz, $CDCl_3$)**:**

δ = 12.4 (q, C-11/C13), 28.3 (q, C-22), 28.3 (t, C-15), 29.7 (t, C-16), 34.9 (t, C-14), 44.8 (t, C-12/C-10), 53.4 (q, C-19), 60.4 (s, C-17), 80.3 (s, C-21), 97.9 (d, C-3), 107.6 (d, C-1), 108.0 (d, C-8), 108.6 (s, C-5), 125.0 (d, C-6), 150.6 (s, C-2), 154.1 (s, C-4), 162.0 (s, C-7/C-20), 171.4 (s, C-18).

Elemental Analysis:

$C_{24}H_{34}N_2O_6S$	Calculated	C 60.23	H 7.16	N 5.85
(478.6040)	Found	C 59.70	H 7.13	N 5.90

HRMS (CI):	Calculated	Found
$C_{24}H_{34}N_2O_6S$ $[M]^+$:	478.2132	478.2134
Absorption max.:	368 nm	
Exitation max.:	370 nm	
Emission max.:	523 nm	
Lifetime:	3.192 ns	

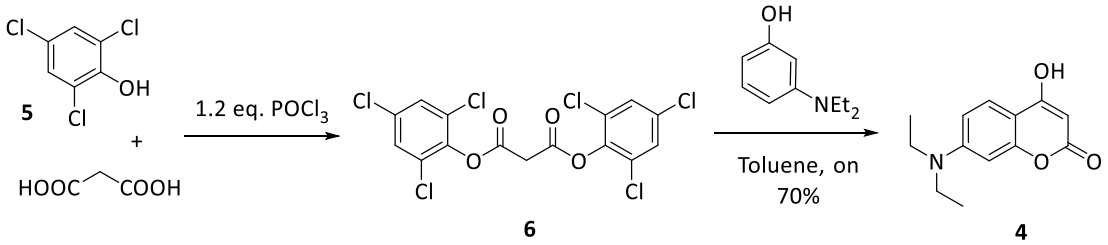
5

Summary

The aim of this thesis was the development of new fluorescent labels based on 7-diethylamino-coumarin moiety and to stuty their application in biorthogonal labellining of amino acids and peptides.

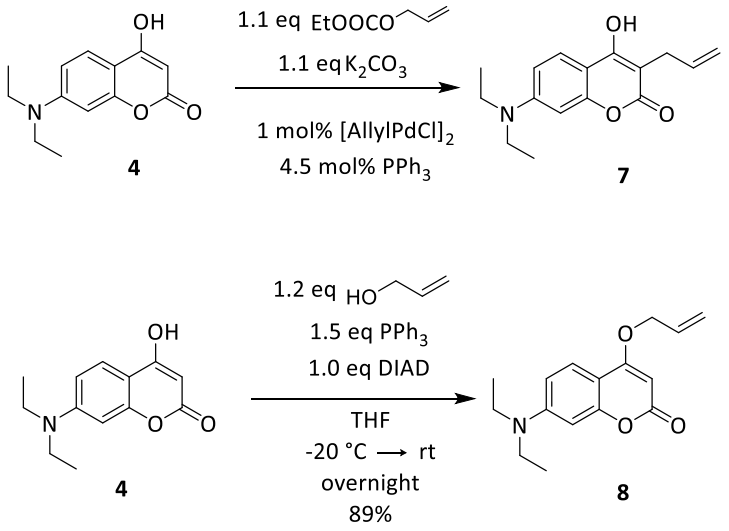
5.1 Synthesis of 7-diethylaminocoumarin

Unfortunately the previously developed methodology[19] using Ti-catalyzed Pechmann condensation did not afford the target aminocoumarin in preparative ammount for further modifications because of the side reaction – transesterification of the keto-ester. Though 4-hydroxycoumarin could be isolated in 70% yield according to the reported procedure. Malonic acid was converted to the activated bisphenylester **6**, which was subsequently condensed with commercially available 3-diethylaminophenol to afford the desired compound **4** (Scheme 5.1).



Scheme 5.1 Synthesis of 4-hydroxycoumarin **4.**

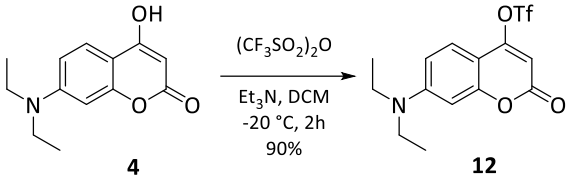
Further studies towards the selective *O*- and *C*-allylation gave satisfying results as well. It was possible to isolate *C*-allylated coumarin **7** in 87% yield under Pd-catalyzed allylic alkylation conditions; Mitsunobu conditions afforded the selective *O*-allylation of coumarin **4** in 89% yield (Scheme 5.2).



Scheme 5.2: Selective *C*- and *O*-allylation of hydroxycoumarin **4**.

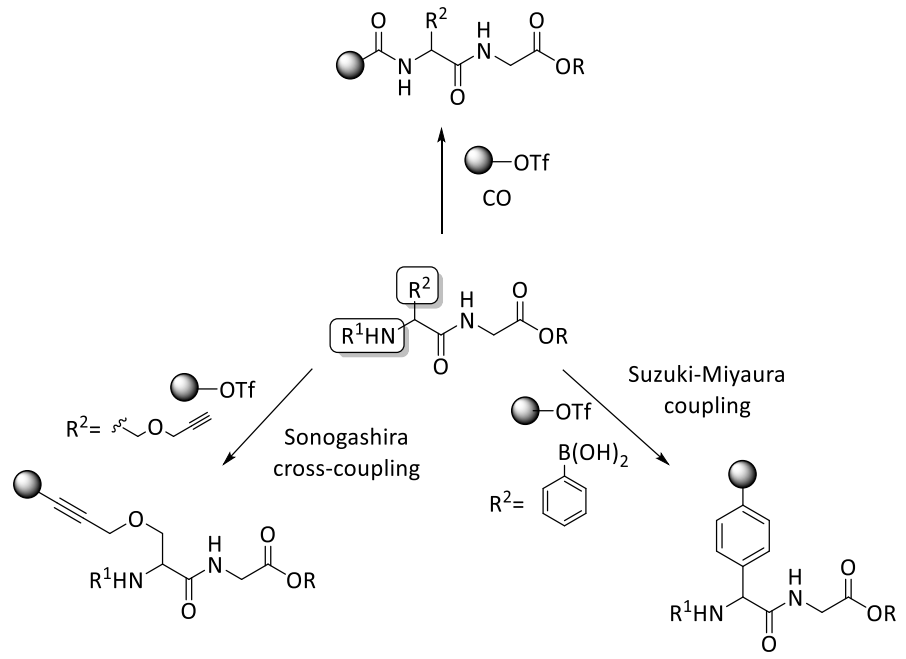
5.2 Labelling through cross-coupling reactions

Functionalization of 4-hydroxycoumarin **4** gave desired vinyl triflate **12** in high yield. This compound was found to be an excellend candidate for various transition metal-catalyzed cross-coupling reactions, such as Sonogashira-, Suzuki- and Buchwald-Hartwig coupling (Scheme 5.3).



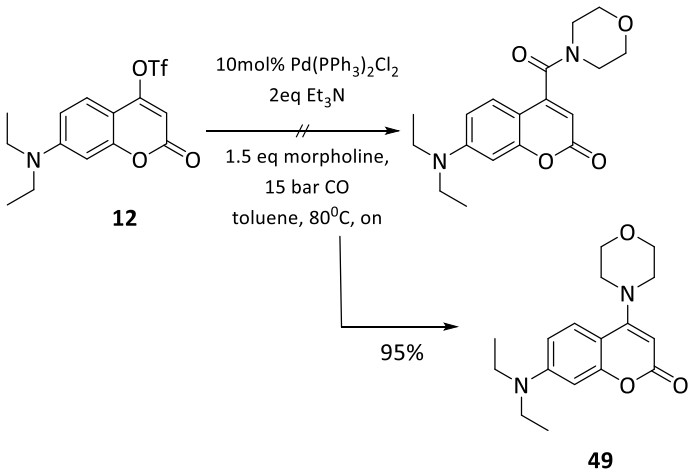
Scheme 5.3: Synthesis of 7-diethylamino-4-trifluoromethylsulfonyloxy-coumarin **12**.

It was possible to get access to molecules with coumarin fluorescent tag in good to excellent yields under Sonogashira and Suzuki-Miyaura, as well as Buchwald-Hartwig coupling conditions (Scheme 5.4). Several amino acids, as well as peptides, were screened in transition metal-catalyzed couplings to afford labelled molecules in high yields.



Scheme 5.4: Transition metal-catalyzed cross-coupling reactions with coumarin **12.**

More nucleophilic amines (e.g. morpholine) provided enamines of type **49** without CO-insertion under Buchwald-Hartwig coupling conditions (Scheme 5.5). The formation of **49** was also achieved under transition metal-free conditions. Therefore, in this case conjugate substitution (Michael addition and elimination of TfOH) leading to **49** must be significantly faster then cross-coupling reaction.



Scheme 5.5: Buchwald-Hartwig amination.

Various secondary amines were screened under substitution conditions affording the substitution products in good to excellent yield.

5.3 Labelling through Pd-catalyzed allylic alkylation

In the scope of this thesis several allylic fluorescent dyes (Figure 5.1) were developed and applied in Palladium-catalyzed allylic alkylation as one of the possible labelling method of the backbone of a peptide.

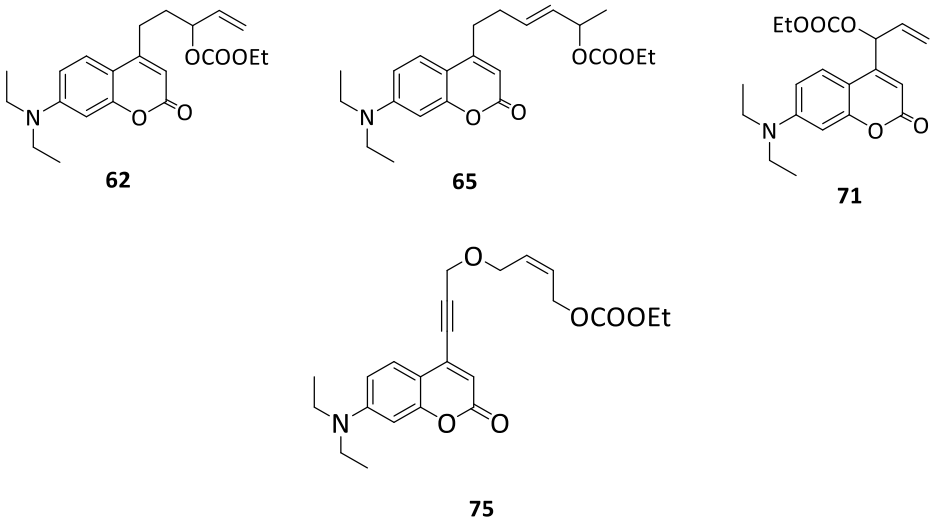
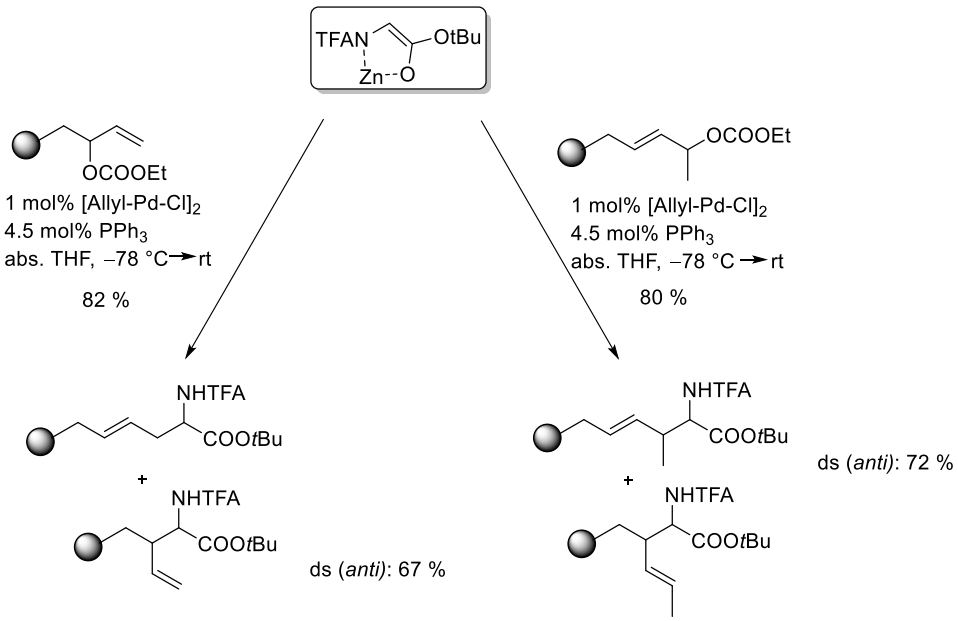


Figure 5.1: Fluorescent dyes with allylic residues.

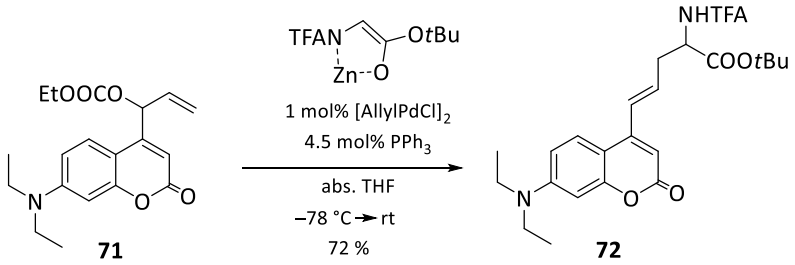
The synthesized allylcarbonate-labels **62** and **65** were subjected to palladium-catalyzed allylic alkylation of TFA-glycine-*tert*-butyl ester as a nucleophile. Here, the allylic substitution reaction again proved to be an excellent method for fluorescent labeling of amino acid esters. Both allylic alkylations provided the fluorescence labeled amino acid esters **66, 67, 68** and **69** in good yields (around 80%). In the reaction with the carbonate **62** and **65** the formation of the both - linear and branched products was observed (Scheme 5.6).

The fluorescent dye **75** with an expanded π-system was screened in allylic alkylation reactions with various nucleophiles (*C*-, *N*- and *O*-nucleophiles), what provided the target compounds (*Z*- and *E*-regioisomers) with aminocoumarin fluorescent tags in good yields.



Scheme 5.6: Pd-catalyzed allylic alkylaitons.

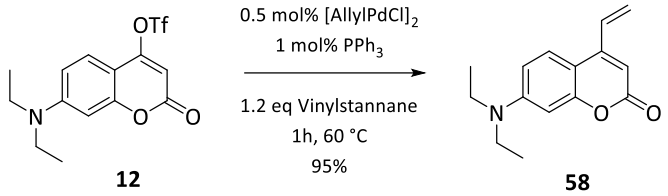
Fluorescent dye **71** with a shorter side chain gave rise to selective allylation furnishing only one regioisomer **72** (Scheme 5.7). In this case the branched position of monosubstituted π-allyl complex is prevented by the coumarin ring for an attack of the nucleophile, which resulted in regioselective allylation of the aminoacid.



Scheme 5.7: Pd-catalyzed allylic alkylation with fluorescent dye **71**.

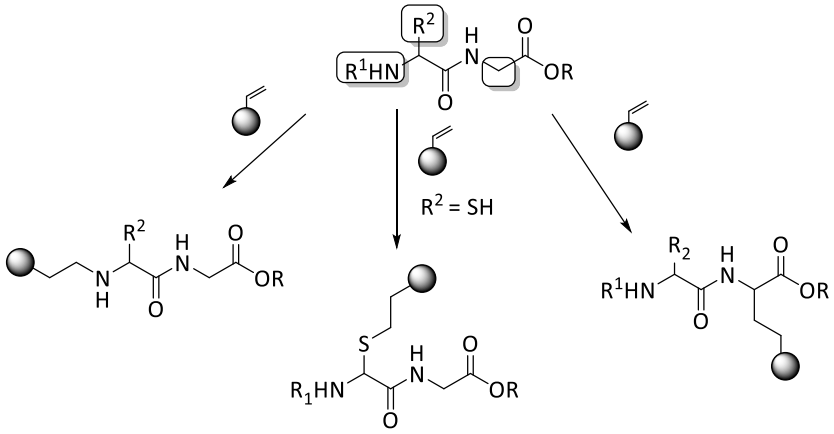
5.4 Labelling through Michael addition reaction

Dyes based on 7-aminocoumarin moiety turned out to be good Michael acceptors and were applied subsequently for various addition reactions (Scheme 5.8). Several dyes have been developed as possible Michael acceptors in fluorescent labelling. Amines and thiols were chosen as possible nucleophiles, as well as TFA-glycine-*tert*-butyl ester. The Michael acceptor was synthesized according to the scheme 5.9.



Scheme 5.9: Synthesis of Michael acceptor **58**.

The obtained vinyl-coumarin **58** was succefully applied as a Michael acceptor in various labelling reaction of amines and thiols. The molecules with fluorescent tag were isolated in good yields.



Scheme 5.8: Fluorescent labelling of peptide through Michael addition**.**

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