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Fotoaktivovatelné sloučeniny

Diplomová práce

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Vedoucí práce: prof. RNDr. Petr Klán, Ph.D.

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Abstrakt

Práce zahrnuje tři projekty zaměřené na syntézu fotoaktivovatelných molekul a studium jejich fotoreaktivity.

První projekt představuje nový koncept – fotoodstupitelnou chirální pomocnou látku. Benzoinová fotoodstupitelná chránicí skupina byla použita jako chirální pomocná látka indukující enantioselektivitu v Dielsově-Alderově reakci cyklopentadienu a chráněného akrylátu. Byly optimalizovány reakční podmínky a struktura chromoforu při dosažení až 96 % enantiomerního nadbytku vzniklého norbornenátu. Následné odstranění pomocné látky bylo provedeno pomocí ozáření UV světlem, bezestopým reagentem, ve vysokém chemickém výtěžku (>85 %).

Druhý projekt obsahuje pokusy o syntézu derivátu flavinu. Struktura fenantrolin-flavinu byla navržena tak, aby byla schopna fotokatalyzovat jak oxidace, tak redukce. Byly zkoumány tři různé vícekrokové reakční cesty vedoucí k jeho přípravě a bylo připraveno a plně charakterizováno několik nových uracilových a fenantrolinových derivátů. Poslední krok všech reakčních cest, kondenzace substituovaného uracilu s fenantrolinovým derivátem, byl neúspěšný.

V posledním projektu je prezentována syntéza komplexu (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl diethyl fosfátu s 2,3-dichlor-5,6-dikyano-1,4-benzochinonem a jeho fotoproduktu 6-hydroxy-3-oxo-3*H*-xanthen-9-karboxylové kyseliny. Komplex byl úspěšně připraven a použit jako fotoodstupitelná chránicí skupina absorbující nad 500 nm (první skupina s významnou absorpcí ve viditelné oblasti). 6-Hydroxy-3-oxo-3*H*-xanthen-9-karboxylová kyselina byla syntetizována fotochemicky.

Abstract

The work presents three projects focused on the syntheses of photoactivatable compounds and studying of their photochemical behaviour.

The first project introduces a novel concept – a photoremovable chiral auxiliary. The benzoin photoremovable protecting group has been used as a chiral auxiliary inducing enantioselectivity in the Diels-Alder reaction of cyclopentadiene and protected acrylate. The conditions and the structure of the chromophore have been optimized to display up to 96% enantiomeric excess of the created norbornenate. The subsequent removal of the auxiliary was accomplished by irradiation by UV light – as traceless reagent with a high chemical yield (>85%).

The second project summarizes attempts of the synthesis of flavin derivative. The structure of phenanthroline-flavin has been proposed to be able of catalyzing of both oxidative and reductive reactions. Three different multistep synthetic pathways have been investigated and several new uracil and phenanthroline derivatives have been synthetized and fully characterized. However, the last step in all pathways, the condensation of substituted uracil with phenanthroline derivative, was unsuccessful.

In the final project, the synthesis of (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl diethyl phosphate complex with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and its photoproduct 6-hydroxy-3-oxo-3*H*-xanthene-9-carboxylic acid is presented. The complex was successfully synthetized and used as a photoremovable protecting group absorbing over 500 nm (the first one with significant absorption in the visible region). 6-Hydroxy-3-oxo-3*H*-xanthene-9-carboxylic acid has been synthetized photochemically.

Masarykova univerzita v Brně

Přírodovědecká fakulta

ZADÁNÍ DIPLOMOVÉ PRÁCE

Student : **Tomáš Slanina**
Studijní program : **chemie**
Studijní obor : **organická chemie**

Ředitel Ústavu chemie PřF MU Vám ve smyslu Studijního a zkušebního řádu MU určuje diplomovou práci s tématem:

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Zásady pro vypracování	1. Literární rešerše: fotolabilní chránicí skupiny, benzoin a xanthenon jako chromofory. 2. Syntéza výchozích látek pro fotochemické reakce zájmu, studium mechanismů jejich fotochemických reakcí standardními fotochemickými metodikami, případně pomocí laserové záblesková spektroskopie. 3. Využití fototransformace vhodných derivátů k syntéze biologicky zajímavých sloučenin.
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pro věci pedagogické

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Podpis studenta

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Last but not least, I thank my family for demonstrating me the beauty of chemistry and for support during my studies.

"The only way of having a good idea is having a lot of ideas."

Amos B. Smith III

Organisches Kolloquium, Regensburg, November 2011

Declaration

Hereby I declare I wrote my diploma thesis on my own with the use of the quoted literature.

Brno, May 14, 2012 .....

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Theoretical part

Protecting Groups

The concept of protecting groups (PG) is one of the most important techniques in modern organic chemistry. Protecting group is a molecule that is attached to particular functional group of the polyfunctional substrate molecule preventing it from unwanted reaction. The concept is illustrated in Figure 1. A molecule with two functional groups A and B reacts with the reagent E to give a mixture of products. The target molecule would be formed in a very low yield assuming B is more reactive than A. By using the protecting group PG to block the reactivity of B, A can react with E to full conversion and with no byproduct. Mild deprotection of B (A-E bond must not be cleaved) gives in the target molecule.

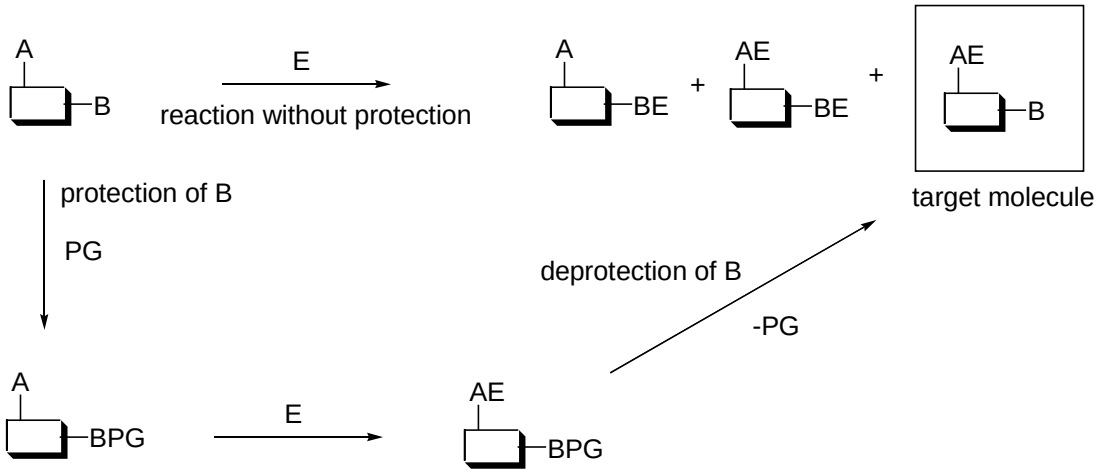


Figure 1: The use of protecting groups on bifunctional molecule

The more complex the target molecule, the better chemo-, regio-, stereo-, and enantioselectivity is usually needed. Therefore, many protecting groups, which are described and summarized in a lot of textbooks[1,2] and compendia have been developed. The synthesis of natural products[3] is a field of organic chemistry which often introduces the new synthetic techniques which are afterwards used in general. A precise selectivity in protecting substrates with subtle differences in reactivity of their functionalities has been demonstrated. By a proper choice of the protecting groups we can selectively protect, for example, allylic, phenolic, primary, secondary or tertiary alcohols in the presence of the other functionalities[4], or we can distinguish between *cis* and *trans* vicinal diols on cyclic systems.[5] The ideal synthesis would not need any protecting groups as all synthetical transformations would be highly selective.[6,7] The models of such a synthesis are enzymatic processes with their high substrate specificity and high chemo-, regio- and

stereoselectivity. Having such selective reagents would not be much practical because it requires enormous amounts of reagents needed, and reaction conditions would have to be tuned very carefully. Every protecting group should be attached easily and selectively to the substrate without enabling reactions with other functionalities. It should be cheap, easily available, and stable under reaction conditions of the subsequent reaction steps. Moreover, it should be selectively cleaved under mild conditions, ideally by workup of the last reaction step of the synthesis. The molecule being protected should also be simply isolated from the reaction mixture after (de)protection. The use of PG in the synthetic pathway should be carefully planned because every use of a protecting group prolongs the pathway by two additional steps (protection and deprotection). Protecting groups can be used also to increase (usually by electronic effects) or decrease (both electronic and steric effects) reactivities of functionalities in contact (conjugation, steric hindrance) with the protected functional group. Many protecting groups for basic functionalities are orthogonal, that means they can be removed selectively with the others being attached[8]. This removal is usually accomplished by chemical methods. The most common is solvolysis catalyzed by an acid or base, redox reactions (hydrogenation, oxidative cleavage) or specific reactions (for example, deprotection of silyl groups by fluoride ion).

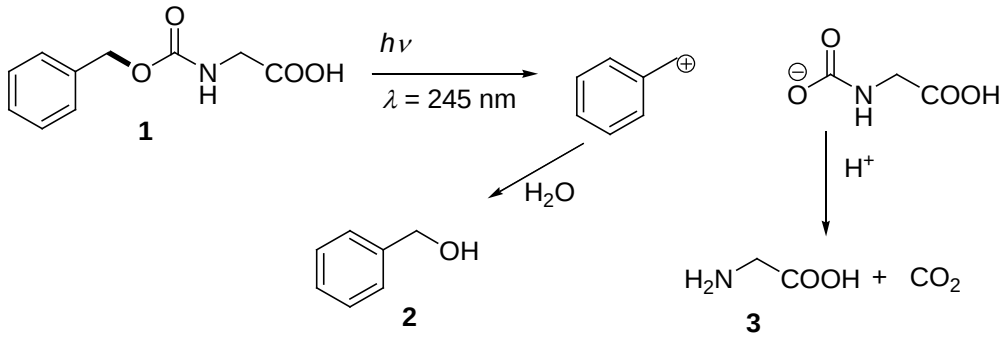
Photoremovable Protecting Groups

Theoretical Concepts

Introduction

Photoremovable protecting groups (PPGs) are protecting groups which are cleaved upon absorption of light – a traceless reagent.[9]

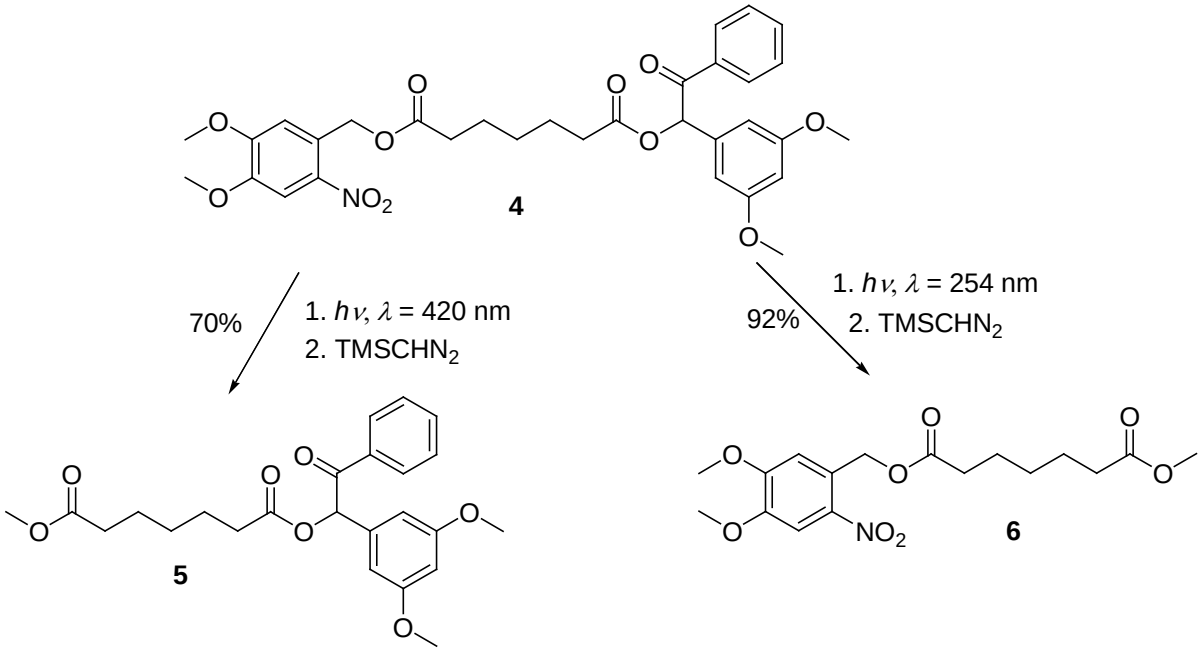
The first photoremovable protecting group was developed by Barltrop and Schofield in early 1960's.[10] It was observed that irradiation of aqueous solution of benzyloxycarbonylglycine (CbzGly) **1** by UV light (λ = 254 nm) results in a mixture of benzylalcohol **2** and glycine **3** (Scheme 1). Benzyloxycarbonyl group, usually called Cbz or Z, is one of the most common classical protecting groups for amines. Upon excitation by UV light, the benzylic bond cleaves heterolyticaly to give a benzyl cation, which is attacked by nucleophilic solvent, and carbamate which expels carbon dioxide. The quantum yield of this process is 0.15.



Scheme 1: Photodeprotection of CbzGly

Orthogonality

PPG cleavage is in principle orthogonal to all other protecting groups and the orthogonal PPGs which are cleaved autonomously by light of different wavelength[11] have also been demonstrated. A mixed diester of pimelic acid (**4**) was protected with a 3',5'-dimethoxybenzoin group at one carboxylic functional groups and an *o*-nitrobenzyl group at the other end. Despite the possibility that intramolecular energy transfer or equilibration could occur between the two chromophores, selective photolysis led to the sequential removal of each of them with high chemical yields (Scheme 2). Upon photolysis of **4** at 254 nm for 5 minutes, 92 % of **6** was released whereas irradiation at 420 nm for 24 hours released 70 % of **5** as determined by ^{1}H NMR.



Scheme 2: An Example of Orthogonal PPGs

Nomenclature and Theoretical Requirements

PPGs are sometimes called “photolabile protecting groups” or “caging compounds”. Protected substrates are therefore called “caged compounds”. The term “caging” was for the first time used by J. F. Kaplan and coworkers[12] in 1978 is their work. The described protected adenosine-3-phosphate (caged ATP) is deliberated from the cage and thus activated by absorption of UV light.

An ideal photoremovable protecting group should possess properties which have been postulated.[9,13]

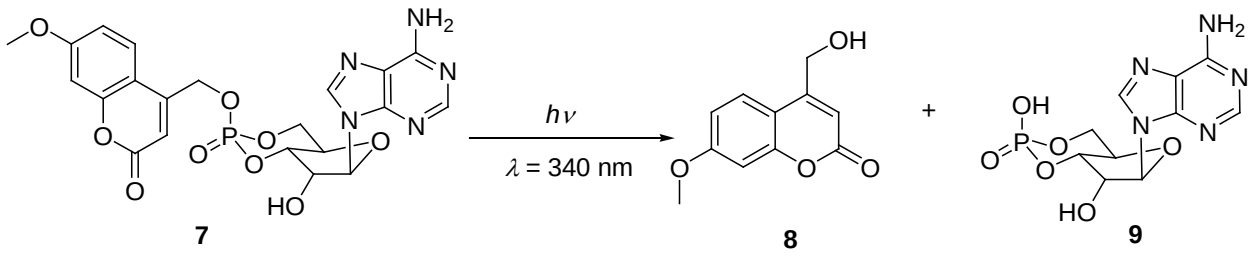
- The substrate, caged substrate, and photoproducts should have good aqueous solubility for biological studies. For synthetic applications, this requirement is relaxed.
- The photochemical release must be efficient ($\Phi > 0.10$).
- The departure of the protecting group from the substrate should be a primary photochemical process (i.e., occurring directly from the excited state of the cage chromophore).
- All photoproducts should be stable to photolysis.
- Excitation wavelengths should be longer than 300 nm and must not be absorbed by the (biological) media, photoproducts, or substrate.
- The chromophore should have a reasonable molar absorption coeffitient to capture the incident light efficiently.
- The caged compounds, as well as the photoproducts of the caging moiety, should be inert or at least benign with respect to the media, other reagents, and products.
- A high-yielding synthetic procedure for attachment of the cage to the substrate must be available.
- In the synthesis of a caged substrate, the separation of caged and uncaged derivatives must be quantitative.

Applications of PPGs

Caged Compounds: Second Messengers

Photoremovable protecting group are used not only in chemical synthesis. They have been used widely in physiology and molecular biology because their photorelease can be precisely spatially and temporally controlled by a laser beam. This enables to investigate topology of samples (for example of a tissue). Caged compounds whose biological activity is blocked by PPG are called

"photobiological triggers" and they can be reactivated by light absorption. An interesting example of a photobiological trigger has been studied by Furuta and coworkers.[14] Cyclic adenosine monophosphate (cAMP) serves in cells as a second messenger for hormones such as adrenaline, glucagon and luteinizing hormone.[15] When a hormone binds to its glycoprotein receptor outside the cell membrane, G-protein is activated (first messenger) and it activates the enzyme adenylyl cyclase which starts to synthetize cAMP from ATP in cytosol. The increase of concentration of cAMP causes changes in gene transcription or (and) changes enzyme activities of the cell. The coumarinylmethyl PPG can be used for caging of phosphates (Scheme 3). Protected cAMP **7** has been applied to a cell culture and activity of their cyclic nucleotide gated ion channels has been measured.[16] In absence of cyclic nucleotides in cytosol the ion channels are closed which can be determined by measuring potentiometric measurement. After a two photon activated solvolysis[17] the free cAMP **9** is released which causes opening of ion channels and thus change of measured potential.[18]

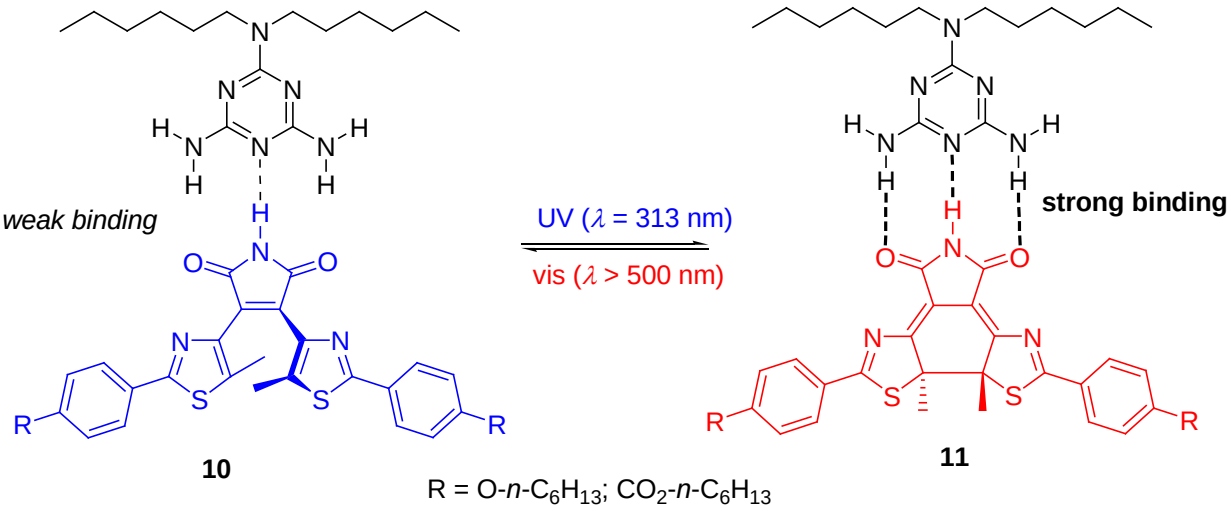


Scheme 3: Photorelease of Protected cAMP

Photochemical Switches

On the contrary, photobiological switches are covalently or non-covalently bonded internal parts of biologically molecules (enzymes, ion channels[19]), which change their conformation or configuration upon irradiation (for example *(E) – (Z)* isomerization of azobenzene); therefore, they activate or deactivate the biological function of the entire molecule. By irradiation at different wavelength, the activity can be either turned on or off. This reversible process is called photochromism and has been known since Alexander the Great who used photochromic paintings on shields of his army officers which changed colour in an appropriate day period, so he could synchronize different battalions without communication devices.[20] A photoswitchable melamine receptor has been introduced by Hecht and coworkers.[21] Photochromic bis(thiazol-4-yl)maleimides **10** (Scheme 4) displays photocyclization by irradiation with UV light. A bent structure of maleinimide becomes planar (except the six membered ring with vicinal methyls in

trans position) and the conjugation is extended and therefore, the absorption is red shifted (**11**). The association constant K_a is enhanced by the factor of 3 due to cyclization. This factor is quite high considering a typical strength of a N-H ···· (O=) hydrogen bond (1.9 kcal mol^{-1}) .[22]



Scheme 4: Photoswitchable Compound

Caged Neurotransmitters

Photoremovable protecting groups have been widely used in neurosciences for investigation of topology of neural pathways because classical methods cannot be used to activate or deactivate specific neuronal populations using electrical stimulation within a single stimulation site. The most common technique is to induce or block a neural impulse and measure the local field potential on neural tissue. This measurement could also be accomplished *in vivo* as it was demonstrated by Lopes-dos-Santos and coworkers.[23] The local field potential from the cerebral cortex of anesthetized female mice before and after infusion of caged γ-aminobutyric acid (GABA) has been recorded. GABA has been caged as (*bis*(2,2'-bipyridine-*N*,*N*')trimethylphosphine)-4-aminobutyric acid ruthenium hexafluorophosphate complex (derivative of RuBi-GABA) **12** (Figure 2). It was found that caged GABA has no measurable activity and the local field potential reached standard values. After irradiation of the tissue by a pulse of blue light (λ = 473 nm), the neural activity was completely blocked by deliberated GABA, and the local field potential reached zero values. The use of inorganic ruthenium-based PPG had an advantage of absorption in the visible region, fast photorelease of GABA (precision of few miliseconds) and no apparent toxicity.

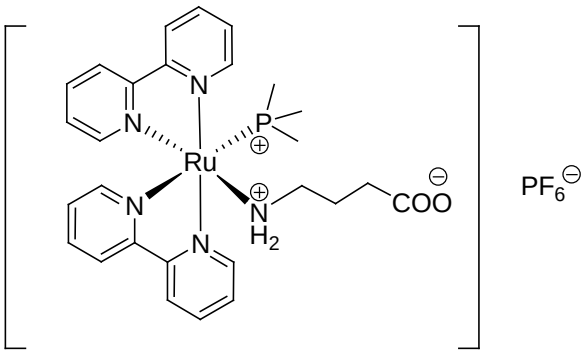


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Figure 2: (*bis*(2,2'-Bipyridine-*N*,*N*')trimethylphosphine)-4-aminobutyric Acid Ruthenium Hexafluorophosphate Complex

Caged Peptides and Proteins

Peptides and proteins have also been modified as phototriggers and photoswitchable compounds.[9] In principle there are two approaches how to synthetize a caged peptide or protein. The first is to use amino acid protected with a PPG at the C- or N- terminus or at a side chain for solid phase synthesis of a peptide. This approach is limited by the number of amino acids in the peptide chain and also provides only primary structure of the biopolymer. The second possibility is to modify natural protein by binding of PPG on an accessible functional group (reaction with thiol group of Cys[24], or phenolic group of Tyr[25]). This process is non-specific and the characterization of the product might be complicated but can be used as well for switching on/off the biological function.

Caged Fluorescent Compounds

Photoremovable protecting groups also have been used for caging of fluorescent compounds. Photolysis restores absorption and emission properties of fluorophores (shift to visible region). The most common examples of such caged fluorescent probes[26] are depicted in the Figure 3. Fluorescein **13** is one of the earliest synthetized fluorescent dyes. In general, it exists in two forms, the opened **13a** and lactone **13b** modifications. The lactone form preferred in methanol, acetone, DMSO and acetonitrile[27] is not fluorescent but is always in equilibrium with an opened fluorescent form. By blocking phenolic groups by 5-carboxymethoxy-2-nitrobenzyl PPG, the lactone form **14** (CMNB-caged fluorescein) is the only possible and, therefore, the fluorophore is caged. Other examples are 8-hydroxypyrene-1,3,6-*tris*-sulfonic acid protected by (2-nitrophenyl)ethyl group **15** (NPE-HPTS) and (2-nitrophenyl)ethyl-caged 6-chloro-7-hydroxycoumarin-3-carboxamide of dimethyl-D-glutamate **16** (NPE-HCCC1/Me)[26].

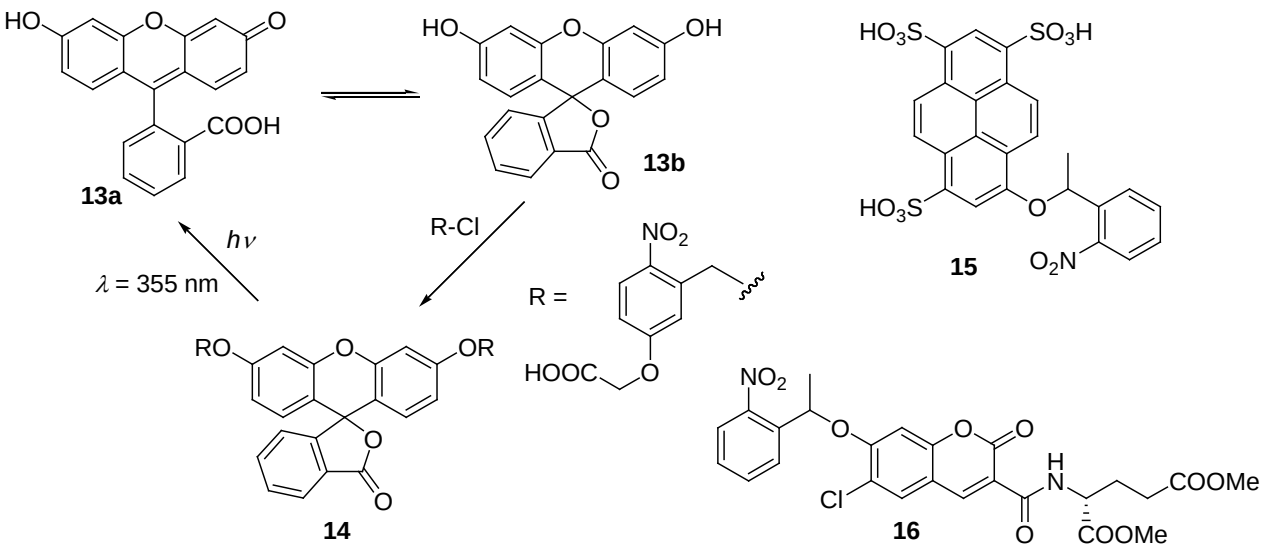


Figure 3: Examples of Caged Fluorophores

Two Photon Absorption

Almost all common PPGs are cleaved by irradiation with UV light. This is not practical for biological *in vivo* studies as the high energy photons can cause serious damages to tissues and cells. In organic chemistry applications, it can also initiate unwanted reactions. The logical solution of this problem is to shift the absorption maximum of the PPG chromophore to visible region. The advantage of this approach is that a long wavelength light sources can be used (such as visible light emitting LEDs) and most of photoproducts absorb only in the UV region and, therefore, do not compete for incident light. The main drawback is low stability in the daylight and a need of careful treatment when synthetizing and using the chromophore or caged compound.

This problematic issue can be avoided by two-photon absorption. This phenomenon has been theoretically suggested by Maria Göppert-Mayer in 1931[28] and experimentally confirmed immediately after invention of laser in 1961 by Kaiser and coworkers.[29] In two-photon photolysis UV, excitation is replaced by the almost simultaneous absorption of two IR photons of equivalent total energy. The molecule of the chromophore is excited form its ground state to an excited state. The probability of excitation is proportional to I^2 and to δ, where I is the light intensity and δ the two-photon absorption cross section measured in GM (1 Göppert-Mayer = 10^{-50} cm^4 s $photon^{-1}$) .[7] The event is restricted to a small volume (smaller than one femtolitre[30]) near the laser focus. The greatest advantage of using the two photon absorption is therefore the possibility of three-dimensional control of the localization of substrate release[31] as the laser beam

can be precisely focused in corresponding area. The two photon absorption can occur only when two photons are absorbed almost simultaneously. This requires a very high laser intensity (the probability is proportional to the square value of *I*) and such pulse lasers are much more expensive than normal ones and are available with limited variety of emission wavelengths. The sensitivity of the caged compound to two photon induced photolysis is determined by two photon action cross section δ_μ, which is defined as $\delta_\mu = \phi \times \delta$ where δ is two photon absorption cross section and ϕ is the quantum yield of photochemical reaction. The value of δ_μ should exceed 0.1 GM to be useful.[32]

Nature of the chromophores with high values of δ has been intensively investigated.[33] The systems with structure D-π-A-π-D (D is an electron donor, A is an electron acceptor, π is a π-system linker) showed the highest δ values. The more electron poor the A moiety, the higher the δ value is. The values have been determined[33] for the squaraine dyes **17** and **18** depicted in Figure 4.

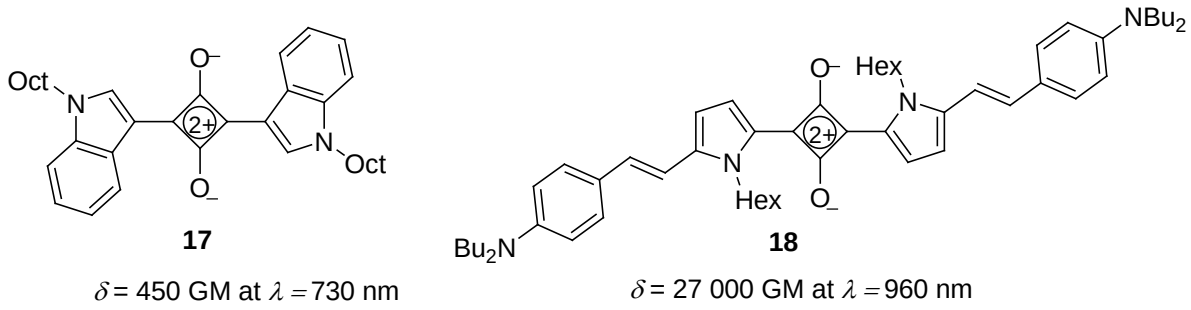


Figure 4: Squaraine Dyes

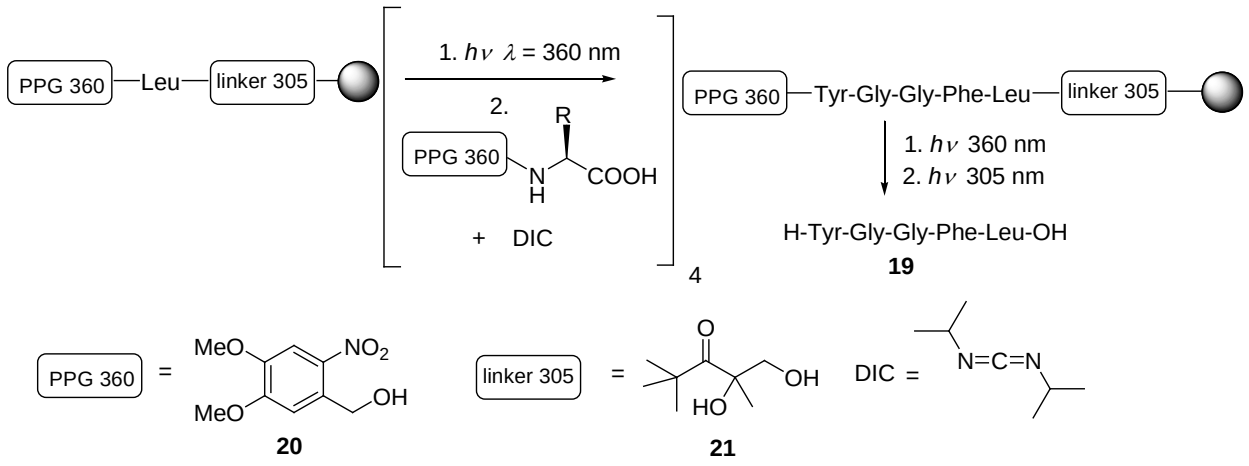
Solid Phase Synthesis

In general the solid phase synthesis (SPS) is a synthesis in solution using a solid support. A corresponding derivative is attached to a solid bead and the system is purified from excess of reagents and free byproducts by simple washing and filtering of the solid beads (usually done in a small column similar to size exclusion chromatography columns). The principle was successfully introduced in 1960's by Merrifield[34] for a synthesis of tetrapeptide. SPS has been used to synthetize peptides with up to 100 amino acids and to synthetize strands of nucleic acids. It is the method of choice when molecules need to be synthetized in a certain alignment.

The usual routine of SPS of proteins is to attach a diaryl amine linker to a solid polymer bead with free benzyl halide groups through an ether bond. The free amino group then reacts with *N*-acylisourea generated *in situ* from *N*-protected amino acid (usually a Fmoc protecting group) with carbamate to create an amide bond (usually a nucleophile organocatalyst like HOBT (1-

hydroxybenzotriazole) is added accelerate the reaction and therefore to suppress the competing reaction – a rearrangement of *N*-acylisourea by intramolecular acyl transfer to *O*-acylurea). With the first amino acid attached to the bead, the system is washed to remove unused reagents. After this purification the protection of amino group is removed (by mild basic conditions *i.e.*, piperidine[1,2] in the case of Fmoc) and subsequently the system is washed again. This cycle repeats with high coupling efficiency and deprotection yield to get the polypeptide. After a final deprotection, the linker is cleaved (*e.g.*, by TFA) to release the product with the desired order of amino acids.

Bochet and coworkers[35] has developed a methodology using orthogonal PPGs for solid phase synthesis of pentapeptide Leu-Enkephalin **19** (Scheme 5). The nitroveratryloxycarbonyl PPG **20** absorbing at 360 nm was used for protection of the *N*-terminus of amino acids. A *tert*-butyl ketone-derived linker **21** sensitive to irradiation at 305 nm was used to connect peptide chain and a resin bead. The peptide coupling has been performed by a common methodology with di*iso*propyl carbodiimide as an activating agent (Scheme 5). The pentapeptide **19** has been synthetized in 55 % yield. The synthesis was accomplished without using any acid or base treatment which is the biggest advantage of using photoactivable compounds as a linker and protecting group.



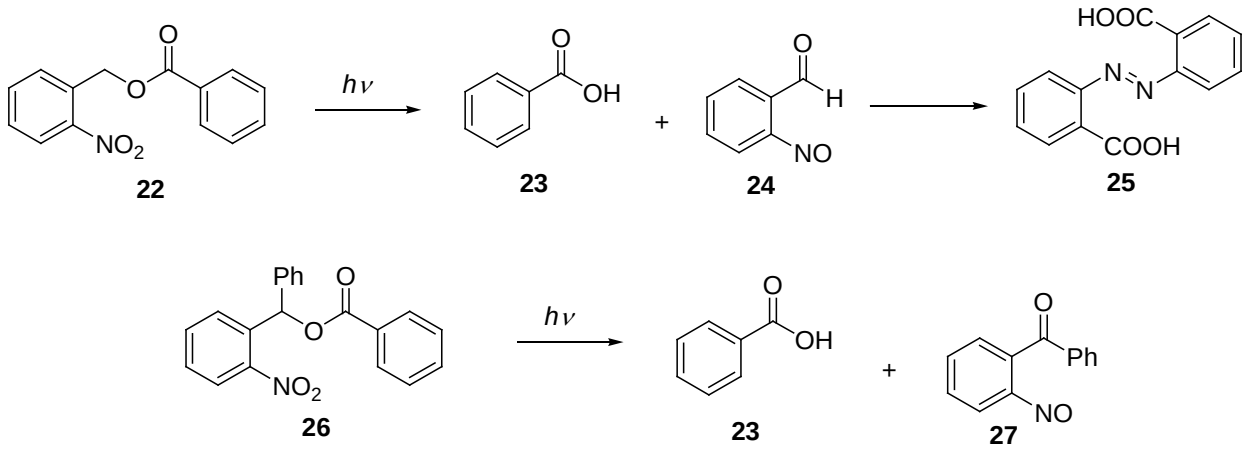
Scheme 5: Use of Orthogonal PPGs in SPS

Common PPGs

2-Nitrobenzyl PPG

2-Nitrobenzyl photoremovable protecting group (*o*NB) is one of the earliest and still the most common PPGs.[7] The parent compound of *o*NB, the 2-nitrobenzaldehyde has been studied since the beginning of 20th century.[36–38] *o*NB has been used as a PPG for benzoic acid **22** (Scheme 6)

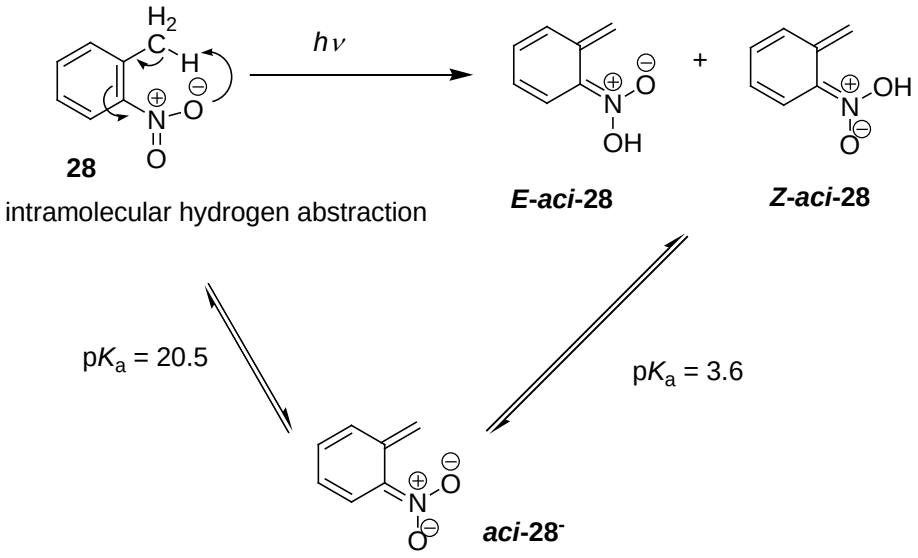
by Barltrop and coworkers.[39] The yield of benzoic acid (**23**) released was only 17 %, because the released highly reactive 2-nitrosobenzaldehyde **24** creates a secondary photoproduct, azobenzene dicarboxylic acid **25**, which competes for incident light (internal filter effect). Much better yields were obtained when the α-substituted nitrobenzyl esters **26** were used. The resulting 2'-nitrosobenzophenone **27** does not react with the released acid.



Scheme 6: Mechanism of Photodeprotection of *o*NB Benzoate

The main advantages of *o*NB photoremovable protecting group are the high yield of deprotection in various solvents and good absorption properties in the UV region. It is widely used for protection of alcohols, diols, carboxylic acids, amines, phosphates and thiols[40].

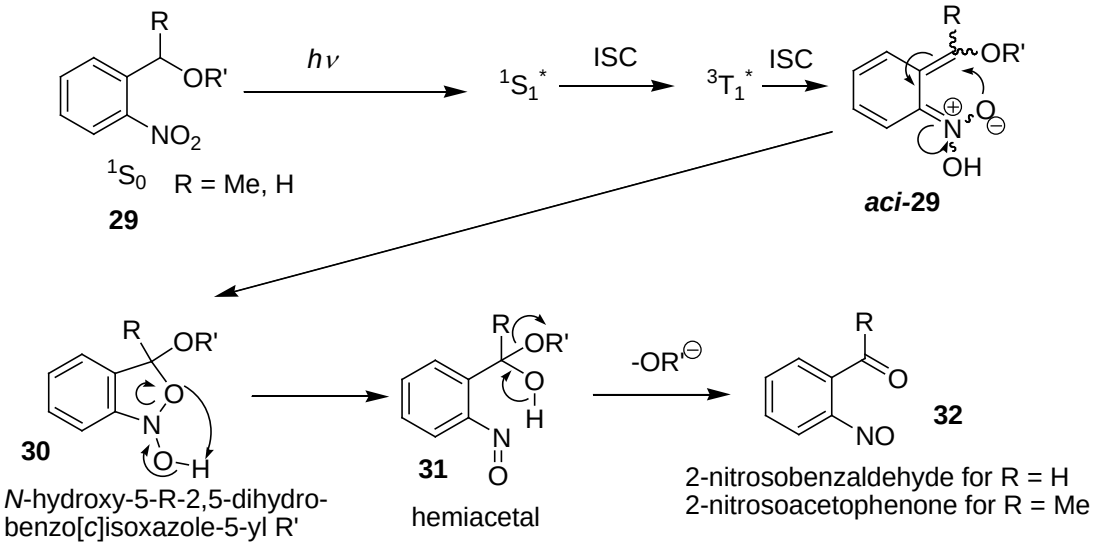
The reactivity of 2-nitrotoluene **28** has been studied[41] as a model compound to unravel the mechanism of *o*NB deprotection (Scheme 7). The primary photoproduct is a mixture of ***E*-*aci*-28** and ***Z*-*aci*-28** isomers. These are products of intramolecular hydrogen abstraction from the methyl group to the nitro group. The *aci*-nitro moieties are moderately acidic and in equilibrium with the anion ***aci*-28**$^-$ and thus with each other. The reketonization back to **28** by protonation of methylene carbon is possible but slow.



Scheme 7: Photobehavior of 2-Nitrotoluene

The mechanism of photodeprotection of *o*NB has been intensively studied[42,43] and its dependence on different conditions (pH, solvent, character of the leaving group LG) has been observed. The general mechanism of deprotection is shown in Scheme 8.

Upon excitation the *o*NB protected compound R' **29** gives the triplet *via* singlet state. Intramolecular hydrogen abstraction leads to the *aci*-nitro form ***aci*-29**. It cyclizes to the benzo[*c*]isoxazole derivative **30** and is subsequently opened to the 2-nitroso hemiacetal **31**. The cleavage of the leaving group results in the final photoproduct 2-nitrosobenzaldehyde **32**.

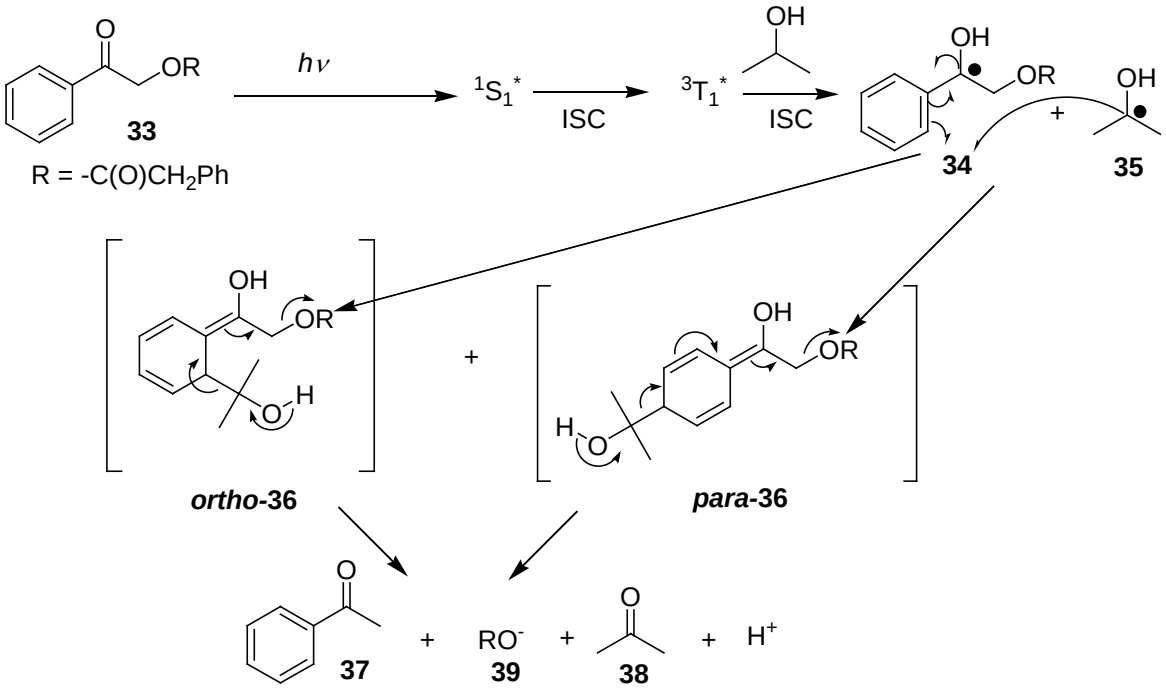


Scheme 8: Mechanism of Deprotection of oNB PPG

Phenacyl PPG

The phenacyl photoremovable protecting group has been developed by Hendrickson and Kandall in 1970 as a protecting group for carboxylates.[44] Deprotection *via* hydrolysis or hydrogenation

has been suggested.[45] Sheehan and coworkers has suggested[46] its use as a PPG thanks to its good absorption properties, rapid photocleavage of the substrate and non-toxic photoproduct. The mechanism of deprotection has been studied Falvey and coworkers[47] by laser flash photolysis technique. The Scheme 9 shows the simplified version of deprotection mechanism. The phenacyl protected 2-phenylacetic acid **33** reacts after excitation from its triplet state with hydrogen donating solvent 2-propanol *via* intermolecular hydrogen abstraction to acetophenon-like ketyl radical **34**. Due to its lifetime, it cannot escape from the generated acetone ketyl radical **35** and recombinates with it to create two regioisomeric adducts ***ortho*-36** and ***para*-36**. Its decomposition is facilitated by rearomatization of the system and releasing of the leaving group. The resultant products are acetophenone (**37**, product of photoreduction), acetone (**38**, product of photooxidation) and released 2-phenylacetic acid (**39**).

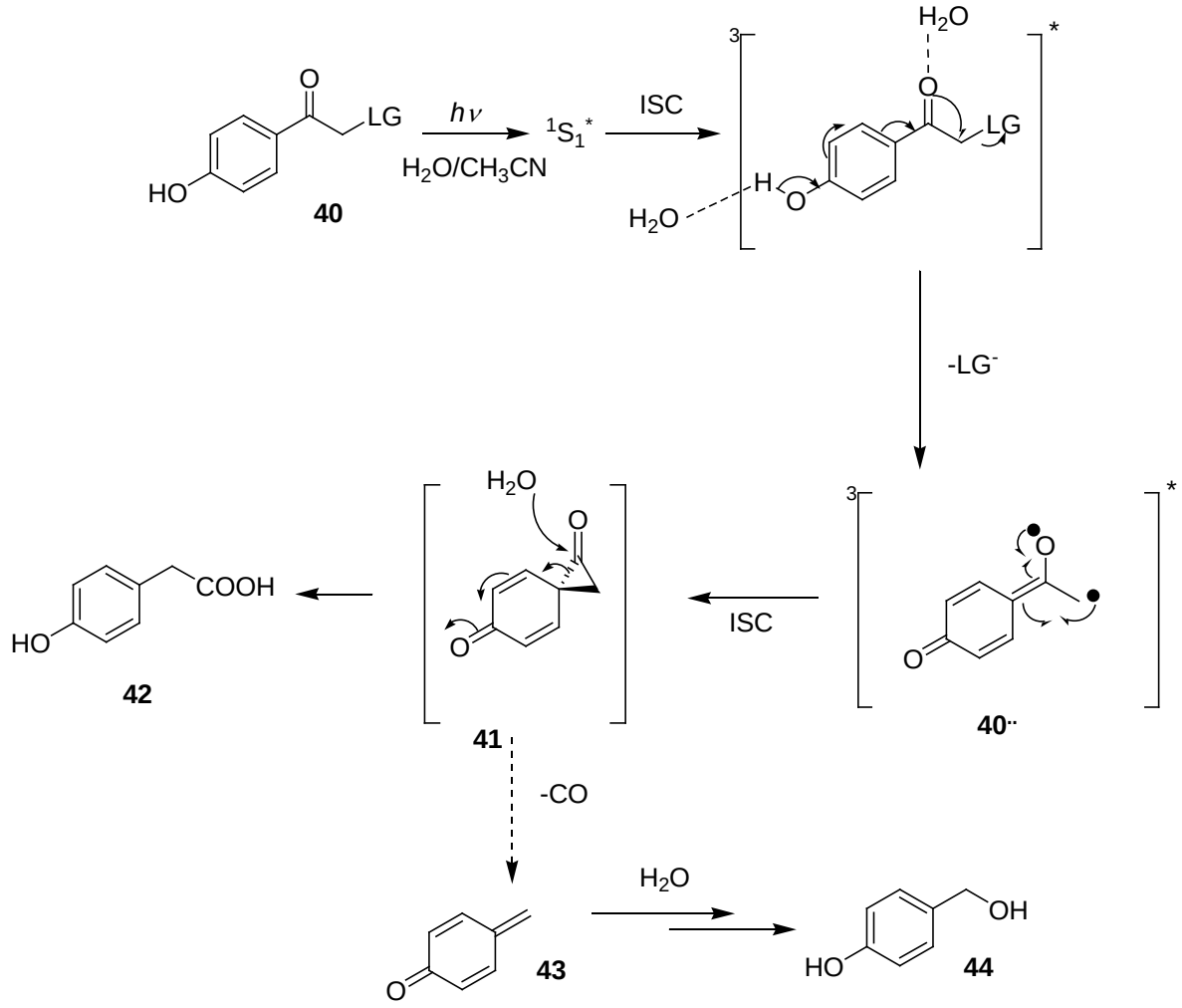


Scheme 9: Mechanism of Photodeprotection of Phenacyl PPG

Sheehan and coworkers[46] has improved the structure of the chromophore by attaching the methoxy group to the phenylic *para* position. The 4-methoxyphenacyl PPG introduced for protection of glycine has a higher molar absorption coefficient and faster release of leaving group. Analogous to the phenacyl photoremovable protecting group, the mechanism proceeds *via* photoreduction by the solvent (2-propanol, MeOH, EtOH *etc.*). The photocleavage in a polar solvent might also lead to *photo*-Favorski-like photoproduct analogically to the chemistry of *p*-hydroxyphenacyl PPG discussed below.

4-Hydroxyphenacyl PPG

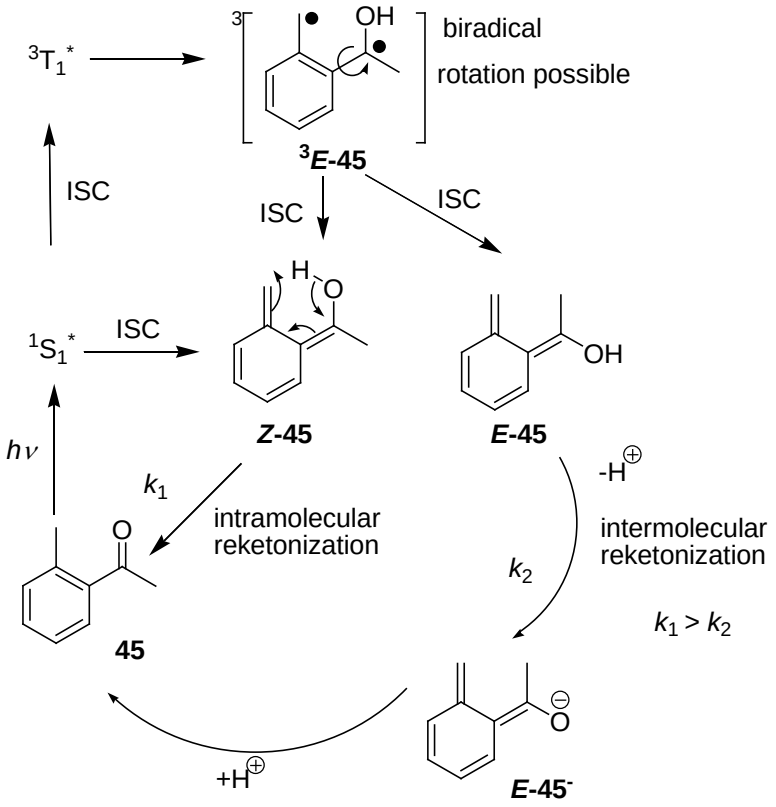
In late 1990's Givens and Park[48,49] have developed the new phenacyl based photoremovable protecting group – *para*-hydroxyphenacyl (*p*HP). It is soluble in aq. solution and stable under physiological conditions. The quantum yield of photorelease is high ($\Phi \sim 0.35$)[13] and the photoproduct is poorly absorbing. All these aspects make it a possible candidate for biological applications.[50] The mechanism of photodeprotection and the photophysical properties have been studied extensively[51–56] and are depicted in the Scheme 10. The *p*HP attached to a leaving group (LG) **40** after excitation in acetonitrile/water mixture rapidly transforms to triplet state, and with assistance of several molecules of the nucleophilic solvent, it fragmentizes to biradical **40··**. It undergoes intersystem crossing back to the ground state and cyclizes to the spirodiendione intermediate **41**. In the presence of excess of nucleophilic solvent[57], it is opened in Favorski-fashion to give the rearrangement product, *p*-hydroxyphenyl acetic acid **42**. With low amount of water in the solvent mixture, the main reaction pathway proceeds through decarbonylation to the methylidene quinone derivative **43** which is then attacked by nucleophile to give *p*-hydroxybenzyl alcohol **44**.



Scheme 10: Mechanism of Photodeprotection of *p*HP PPG

2-Alkylphenacyl and 2,5-Dimethylphenacyl PPG

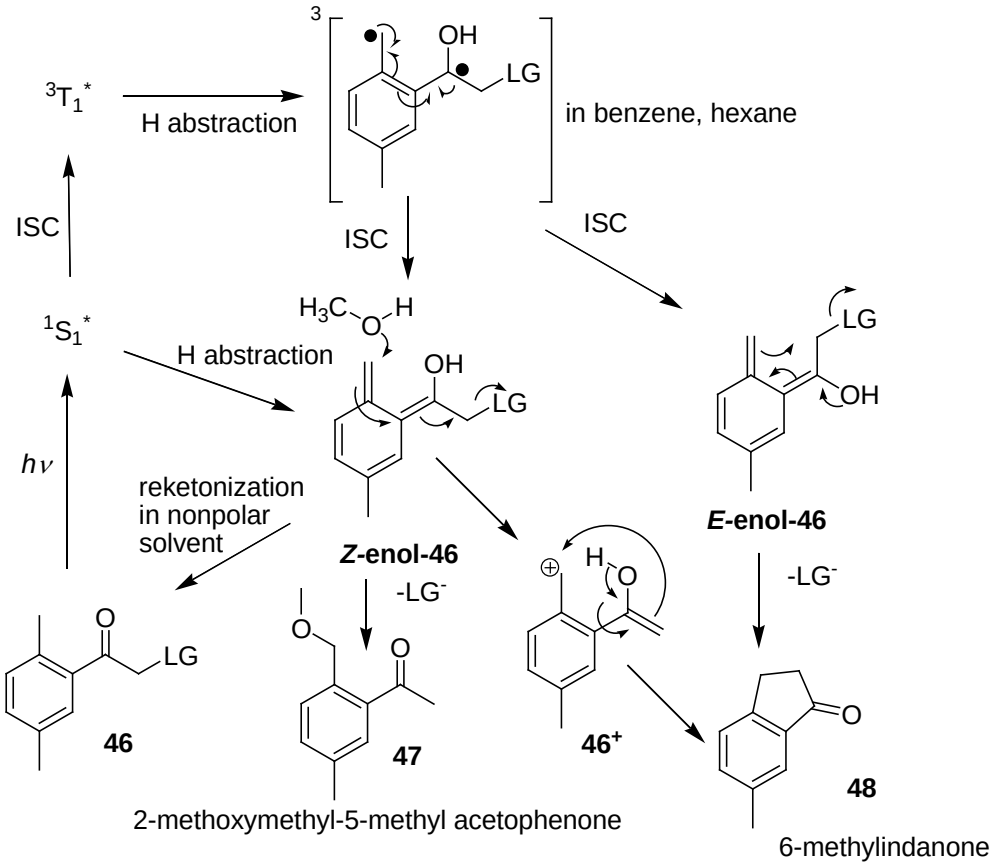
An 2-alkylphenacyl photoremovable protecting group contains a hydrogen donating alkyl group attached in *ortho*-position to the acyl moiety. The photobehavior of such chromophores is analogous to that of the model compound 2-methylacetophenone (**45**). Similar to the 2-nitrobenzaldehyde, 2-alkylacetophenones undergo photochemical enolization *via* intramolecular hydrogen transfer[58] as depicted in the Scheme 11. The enol formed is a mixture of two isomers, ***Z*-45** and ***E*-45**. The *Z*-isomer is created both from directly the singlet state and from the triplet state. Its reketonization to the starting ketone is rapid due to the favourable geometry for a reversed hydrogen transfer. The *E*-isomer is created solely from the excited triplet state by rotation of the α-ketonic bond. It is reketonized back to **45** by a much slower intermolecular hydrogen transfer.[59]



Scheme 11: Photoenolization of the 2-Methylacetophenone

2-Alkylphenacyl group (2-substituted 2'-ethylbenzophenone) has been used for carboxylic acid protection.[60] The deprotection product is *ortho*-acyl styrene.

2,5-Dimehtylphenacyl PPG (DMP) has been first described as a molecule capable release of HCl upon irradiation.[61,62] It is commonly used PPG for carboxylic acids,[63,64] sulfonates, phosphates,[65] alcohols[66] and amines[67] and as a precursor for indanone synthesis.[68] The mechanism of photocleavage is strongly solvent dependent.[69,70] After the excitation of the DMP protected substrate **46** to its singlet state (Scheme 12) in a polar solvent such as methanol the molecule undergoes photoenolization *via* intermolecular hydrogen transfer to the ***Z*-enol-46**. The rate of ***Z*-enol-46** generation is not influenced by triplet quenchers (oxygen, naphthalene) and therefore does not proceed through the triplet state in polar solvent. The molecules of the solvent stabilize ***Z*-enol-46** and prevent it partially from reketonization to the **46**. The main product of photocleavage is a substituted acetophenone **47**. By the heterolytic fission of LG-DMP bond, the cation $\mathbf{46}^+$ is formed which can cyclize to 6-methylindanone (**48**). In the nonpolar solvent (benzene, hexane), the triplet state also plays a role; ***E*-enol-46** is created which cyclizes to **48**. ***Z*-enol-46** is not stabilized and reketonizes back to the starting molecule **46**.



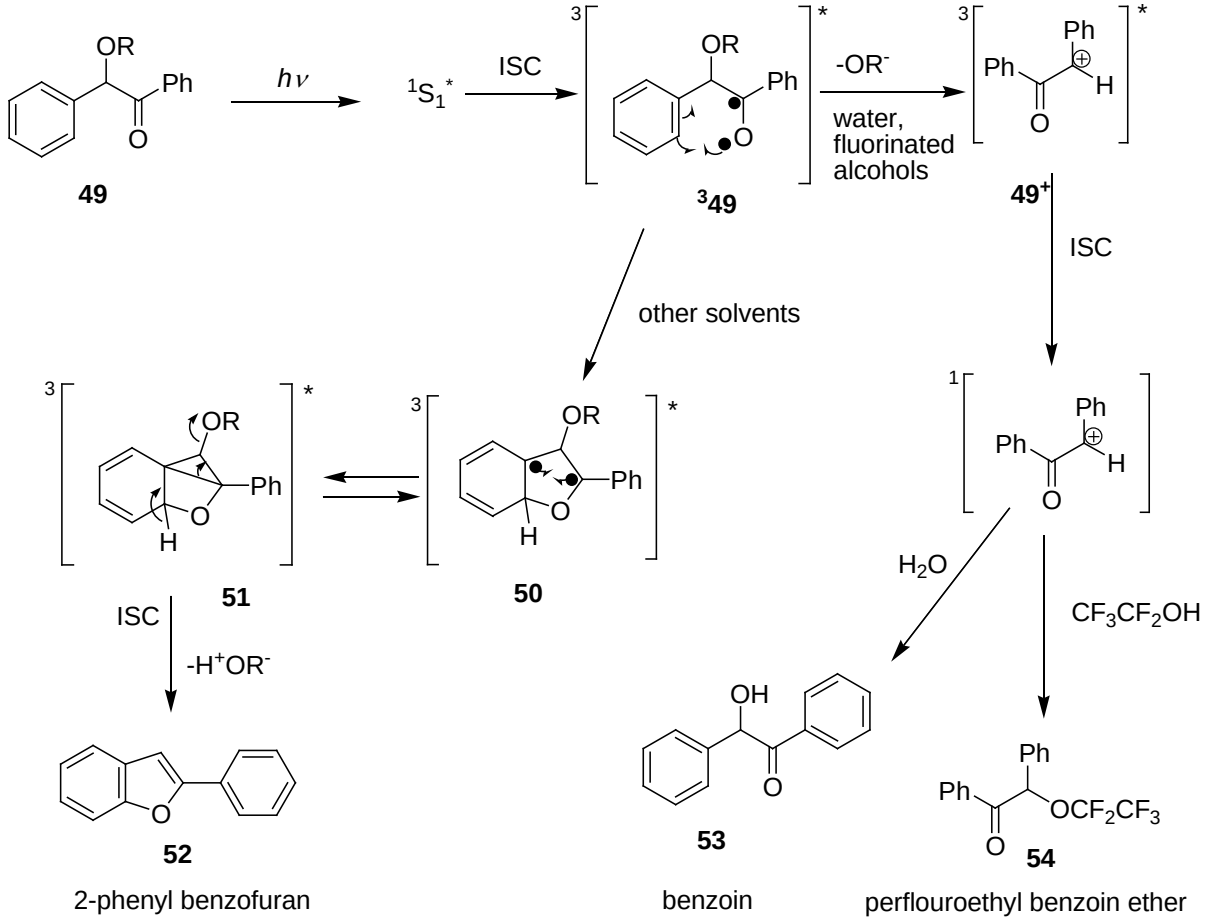
Scheme 12: Mechanism of Deprotection of DMP PPG

Benzoin PPG

Benzoin (Bnz, desyl) photoremovable protecting group has been introduced by Sheehan and Wilson in 1960's.[71] To improve its absorption properties and the yield of photorelease, a *β*-phenyl derivative was substituted by two methoxy groups in the *meta* position.[72] Benzoin PPG is commonly used for protecting carboxylic acids,[71,73] phosphates,[74] alcohols and thiols[75] and amines.[76] It has also been used as a photocleavable linker in solid phase synthesis[77] and for caging biomolecules like ATP[78] and cAMP[79]. The main disadvantage of benzoin PPG is a high absorption of the primary photoproduct, 2-phenylbenzofurane.

The mechanism of photodeprotection of the benzoin protected substrates **49** in different solvents has been studied by Wirz and coworkers[81]. The rapid intersystem crossing ($k_{ISC} \sim 2 \times 10^{10}$ s^{-1}) from the first excited singlet leads to reactive triplet state (Scheme 13). In water or fluorinated alcohols, the biradical ^{3}**49** releases the leaving group RO^- adiabatically[82] to give the triplet cation **49**$^+$. After intersystem crossing back to the singlet state, the solvent attacks it to create the benzoin **53** or the benzoin ether **54**. The mechanism of deprotection in other solvents leads to 2-phenylbenzofuran **52** *via* cyclization of triplet state to biradical **50**. The release of the leaving

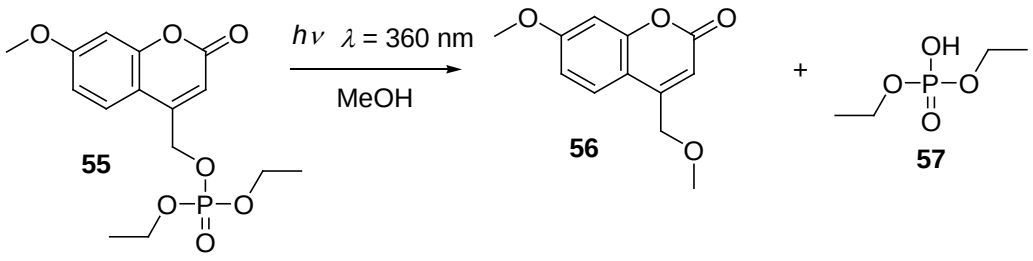
group accomplished by electron transfer mechanism with **51** as a possible intermediate is the rate determining step.



Scheme 13: Mechanism of Deprotection of Benzoin PPG

Coumarinyl PPG

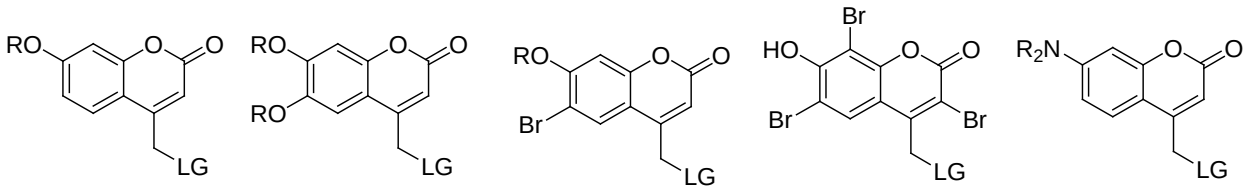
The coumarinyl group was first described as a potentially photoactivable compound in 1984 by Givens and Matuszewski,[83] when a (7-methoxycoumarin-4-yl)phosphate ester **55** was found to be photoreactive. By irradiation with UV light in methanol (Scheme 14), it released the free diethylphosphoric acid **57** and reacted with a nucleophilic solvent (or different nucleophiles present in non-nuleophilic solvent) to the corresponding methoxymethyl coumarin **56**. In 1993, Furuta and coworkers[84] has compared the photobehavior of the ester **55** with 2-NB protected and desyl-protected diethyl phosphate evaluating coumarinyl as an alternative photoremovable protecting group.



Scheme 14: Photodeprotection of (7-Methoxycoumarin-4-yl)phosphate Ester

In the following work,[14] Furuta have extended the usage of 7-methoxycoumarin-4-yl (MCM) PPG also for carboxylic esters. Irradiation of caged esters in aqueous media leads to release of the protected carboxylic acid and formation of the corresponding hydroxymethyl coumarin analogous to the compound **56**.

Coumarin PPGs possess strong absorption (ε_{max} typically from 4 000 to 20 000 M^{-1} cm^{-1}) extended to the visible region which can be tuned by proper substitution. Large two photon absorption cross section and high yields of fluorescence emission[17] are other advantages of the chromophore. The rate of photorelease is high but its quantum yield is moderate (up to 0.25).[9] A large number of coumarin derivatives have been developed when searching for the longest wavelength, strongest absorbing candidate. Structures and acronyms of described coumarinyl PPGs are shown in the Figure 5.



7-alkoxy group
MCM (R = CH_3)
HCM (R = H)
ACM (R = CH_3CO)
PCM (R = CH_3CH_2CO)
CMCM (R = CH_2COOH)

6,7-dialkoxy group
DMDM (R = CH_3)
BCMCM (R = CH_2COOH)
BECMCM (R = CH_2COOEt)

6-bromo-7-alkoxy group
BHc (R = H)
BHc/Ac (R = CH_3CO)

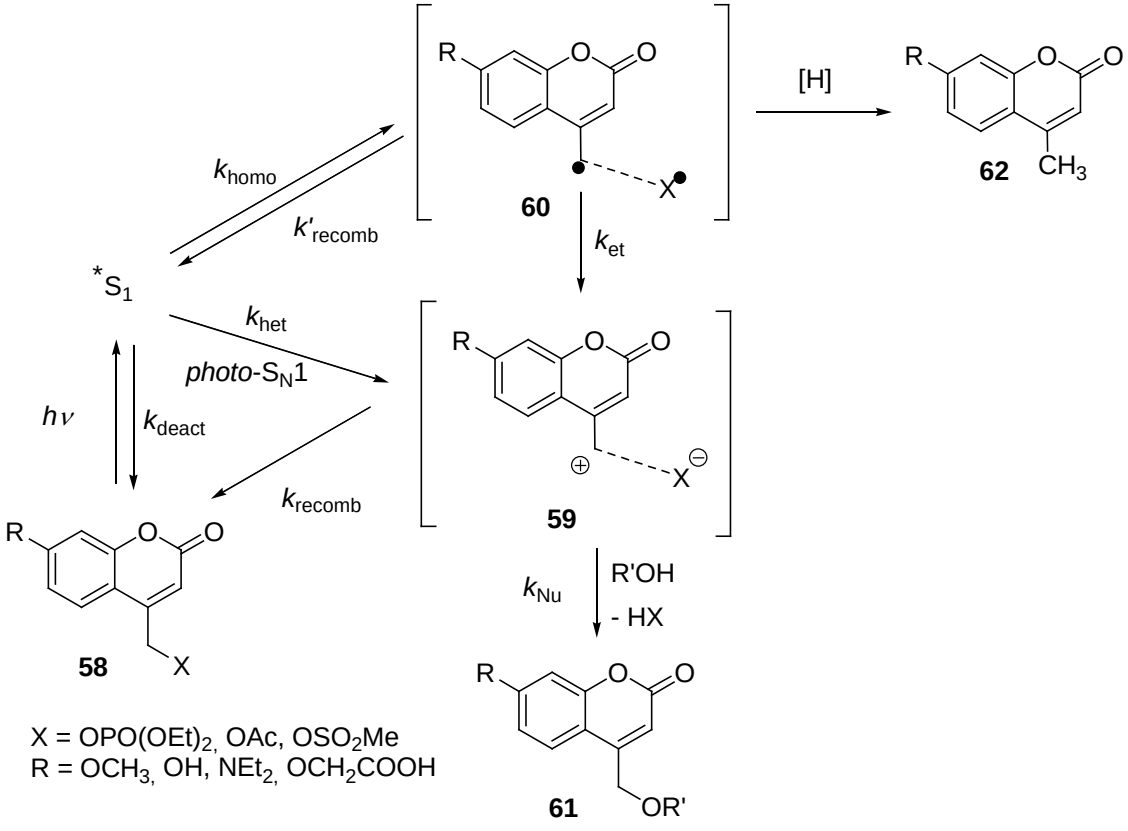
TBHCM

7-dialkylamino group
DEACM (R = CH_3CH_2)
DMACM (R = CH_3)

Figure 5: Structures and Acronyms for Coumarin Based PPGs

The coumarinyl PPG has been successfully used for protecting phosphates, carboxylates, carbonyls, alcohols, diols, amines, and sulfonates.[13] It has also been used for caging second messengers[14] and several biomacromolecules.[85] One of the first examples of a coumarinyl-caged macromolecule used in a biological system was BHc-caged mRNA for photoinduction of gene expression in zebrafish embryos.[85]

The mechanism of photorelease of different coumarinyl PPGs (Scheme 15) has been intensively studied by Bendig and coworkers.[86-88] The photoexcitation of the coumarinyl cage compound **58** leads to the first excited singlet state $^{*}S_1$ according to the Kasha rule. After a photo-S_N1 heterolytic fission (most likely the rate-determining step of photorelease; $k_{het} \sim 10^9\ s^{-1}$) of the PPG-substrate bond, the intimate ion pair **59** is created. An alternative mechanism through the radical pair **60** has also been suggested (traces of hydrogen abstraction product **62** were observed) but it seems to be a minor pathway. **60** would be very short living because k'_{recomb} and k_{et} ought to be large in comparison with k_{homo}. The reaction is facilitated in solvents with a higher polarity which supports the heterolytical mechanism. The ion pair **59** can recombine back to give the starting molecule (the value of k_{recomb} comparable to k_{Nu} is the main factor lowering the quantum yield of deprotection besides the fluorescence quenching k_{deact}) or it can be destroyed by solvent to give the product of solvolysis **61**. Photolysis of a phosphate ester in a 3 : 7 (v/v) mixture of acetonitrile and ^{18}O-labeled water showed no isotope incorporation in the free phosphate and exclusive incorporation of ^{18}O to the solvolysis product **61**. The quantum yields of deprotection of esters are low (Φ for MCM around 0.05), for deprotection of phosphates are acceptable ($\Phi \sim 0.25$). The chemical yields of deprotection are higher than 90 % which indicates that no side reactions compete with the deprotection process.

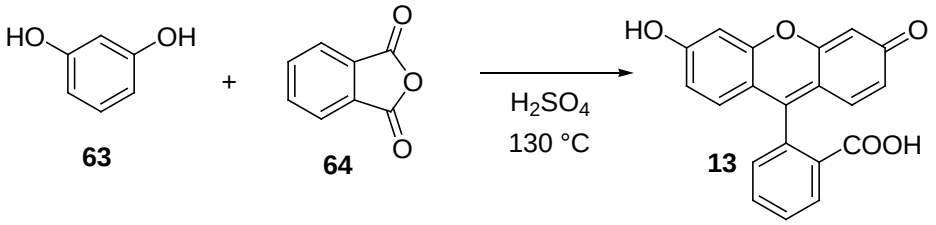


Scheme 15: The mechanism of Photorelease of Different Coumarinyl PPGs

Fluorescein and Its Derivatives

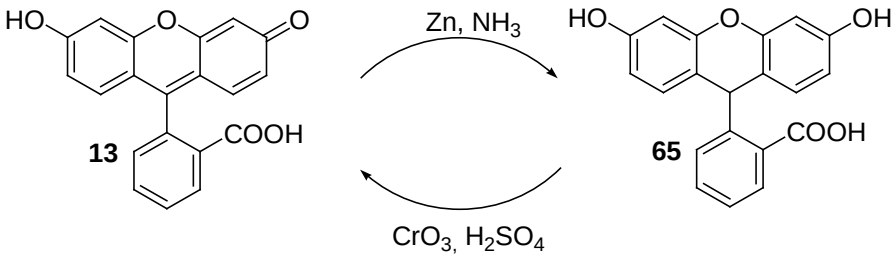
Discovery

Fluorescein **13** has been first synthetized by Adolf von Baeyer (1905 Nobel Prize for Chemistry) in 1871.[89] He reported the structure of the molecule erroneously. The classical synthesis uses condensation of resorcinol **63** and phthalic anhydride **64** in concentrated sulfuric acid (Scheme 16). The reaction can be catalyzed by zinc chloride which enables to use lower temperatures in higher yield.[90] Fluorescein is soluble in methanol and can be precipitated by addition of water.



Scheme 16: Synthesis of Fluorescein According to Baeyer

Baeyer also reported on the change in absorption and enhancement of solubility and fluorescence by addition of aqueous ammonium. The fluorescein can be reduced (Scheme 17) with zinc powder and ammonia to fluorescin (**65**) with no significant absorption in the visible region and no fluorescence. Fluorescin can be reoxidized by chromic acid.



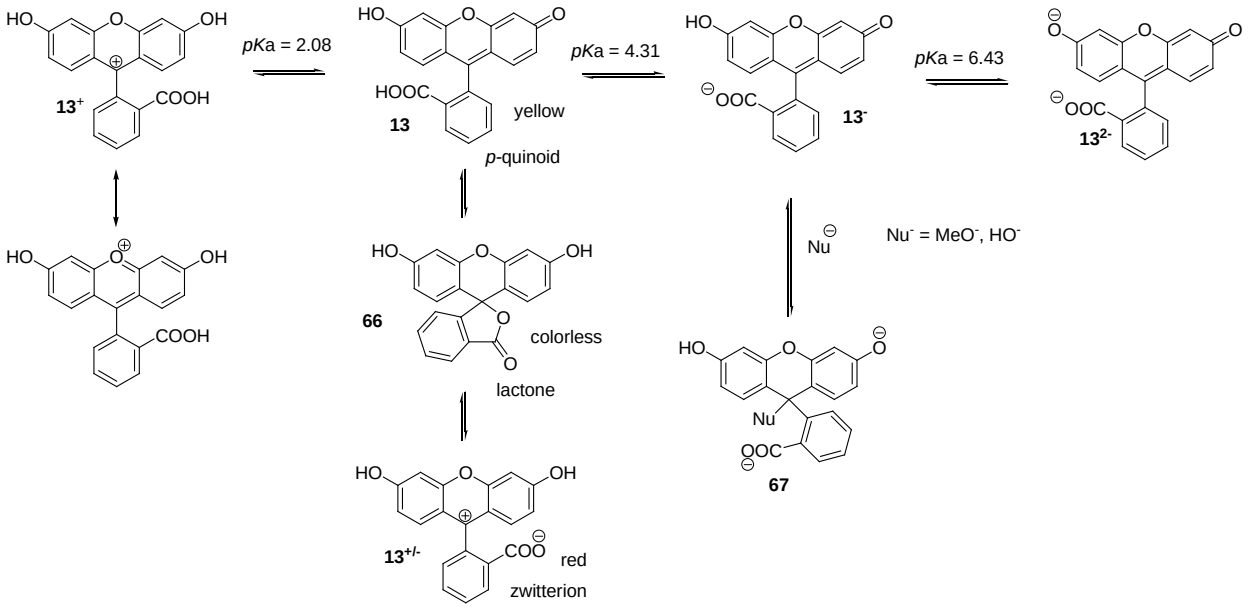
Scheme 17: Redox Behavior of Fluorescein

Physical Properties and Behaviour

Fluorescein (2-(3-hydroxy-6-oxo-6*H*-xanthen-9-yl)benzoic acid) is a highly conjugated aromatic molecule with a xanthene core. Despite the fact it is a commonly used biological probe[91,92] and the oldest synthetic fluorescent dye,[89] its photophysical properties, reactivity and solvent effects have not been fully understood yet. It can exist in five forms in a solution – cationic $\mathbf{13}^{+}$, neutral **13** and **66**, anionic $\mathbf{13}^{-}$ and dianionic $\mathbf{13}^{2-}$ forms (Scheme 18) which are distributed according to the pH and the nature of solvent. The lactone form is preferred in methanol, acetone, DMSO and

acetonitrile.[27] Fluorescein absorption and fluorescence properties are strongly pH dependent. The protolytic constants relating the concentrations of the protolytic forms have been determined by sophisticated chemometric measurements,[91] because their spectra overlap substantially and the different pK_a values are quite close.

Fluorescein can be reversibly attacked by a nucleophile at C-9 creating a non-fluorescent adduct **67**[27] which quenches its fluorescence in basic solutions (pH > 9).



Scheme 18: Different Forms of Fluorescein

The calculated absorption spectra of different forms of fluorescein[91] are shown in Figure 6. The real spectrum is always a superposition of a mixture of different species according to the distribution diagram shown in Figure 7. The dianion $\mathbf{13^{2-}}$ (——) has its main absorption peak at 490 nm (ε = 76 900 M^{-1} cm^{-1}) with a shoulder at 475 nm. It has a very weak absorption in the region 350 – 440 nm, and distinct absorption peaks in the UV region at 322 nm (ε = 9 500 M^{-1} cm^{-1}), 283 nm (ε = 14 400 M^{-1} cm^{-1}) and 239 nm (ε = 43 000 M^{-1} cm^{-1}). The anion $\mathbf{13^-}$ (– –) has a weaker absorption in the visible region with peaks at 472 nm and 453 nm of roughly the same molar absorptivity (ε = 29 000 M^{-1} cm^{-1}). It absorbs weakly in the near UV region, and has peaks at 310 nm (ε = 7 000 M^{-1} cm^{-1}) and 273 nm (ε = 17 000 M^{-1} cm^{-1}). The neutral species **13** (· · · ·) has by far the lowest absorption in the visible region with a maximum at 434 nm (ε = 11 000 M^{-1} cm^{-1}) and a side maximum at 475 nm (ε = 3 600 M^{-1} cm^{-1}). The cation $\mathbf{13^+}$ (– · · –) has the maximum absorption at 437 nm (ε = 53 000 M^{-1} cm^{-1}), and two additional peaks at 297 nm (ε = 7100 M^{-1} cm^{-1}) and 250 nm (ε = 33 000 M^{-1} cm^{-1}).

The distribution diagram of different species of fluorescein present in aqueous solution with varying pH is shown in the Figure 7.

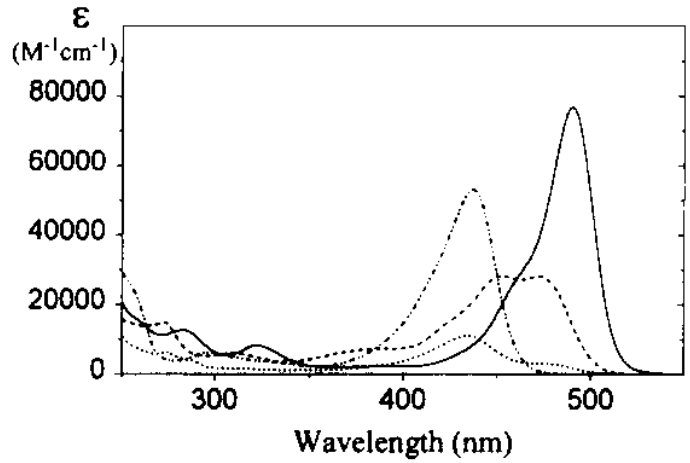


Figure 6: Spectra of Different Forms of Fluorescein in Water Solution. Cation (– · · –), neutral species (· · · ·), anion (– – – –), dianion (———); adapted from Sjöback *et al.*[91], edited.

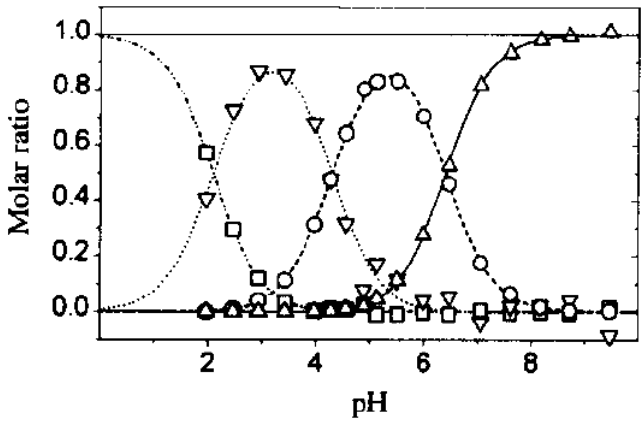


Figure 7: Distribution Diagram of Different Forms of Fluorescein in Different pH in Water Solution. Cation (□), neutral species (▽), anion (○), dianion (△); adapted from Sjöback *et al.*[91], edited.

The fluorescence of anion (- - - -) and dianion forms (———) of fluorescein is shown in Figure 8. The dianion has the most intense fluorescence with a quantum yield of 0.93 but also the anion shows considerable fluorescence with a quantum yield of 0.37. The neutral and cationic species are converted into the anion upon excitation by a loss of one and two protons, respectively, and both species fluoresce with quantum yields of about 0.30 and 0.18, respectively.

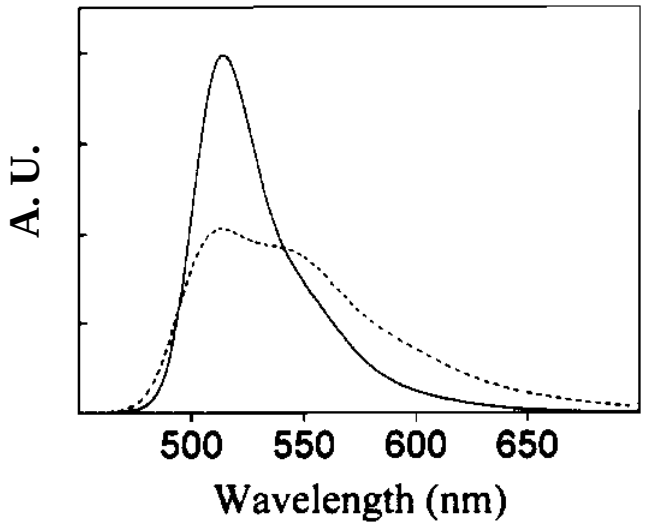


Figure 8: Emission Spectra of Anion (- - - -) and Dianion Forms (———) of Fluorescein in Aqueous Solution. The ordinate shows arbitrary quantitative property. Adapted from Sjöback *et al.*[91], edited.

The excitation maximum of fluorescein dianion $\mathbf{13}^{2-}$ is close to the 488 nm spectral line of argon ion laser which makes it suitable fluorophore for confocal laser scanning microscopy.[93] Its advantage is a relatively good water solubility (rises with increasing pH) and excellent fluorescence quantum yields (up to 93 %). The limitations of fluorescein usage are following: a relatively high rate of photobleaching (problematic quantitative measurements), pH sensitive fluorescence (limited use in bioassays) and broad emission spectrum (low utilization in multicolor applications).

Derivatives

Many derivatives of fluorescein have been developed to overcome the drawbacks of fluorescein. A lot of information about synthesis and structures of fluorescent dyes is not available to public and is protected by patents and registered trademarks (such as Alexa® dyes or DyLight® dyes) and, therefore, the presented survey is a selection of the most used derivatives. There are two basic approaches for fluorescein structure modification. The first is adding a reactive group to the phenyl ring attached to C-9 of xanthene structure. This group (for example isothiocyanate or maleinimide) reacts with nucleophilic groups of peptides (lysine amino group, cysteine thiol group of serine hydroxyl group) and binds covalently to the biomolecule. The particular biomolecule is fluorescently labeled which enables to determine its qualitative and quantitative

properties in a particular region. The second approach is to improve the properties (absorption, emission, solubility) of the fluorophore by attaching halogen atoms to the xanthene moiety.

Carboxyfluorescein and Its Derivatives

Carboxyfluorescein (CF, **68**) is produced as a mixture of isomers, 5-carboxyfluorescein **68a** and 6-carboxyfluorescein **68b** (Figure 9). This numbering is used for all fluorescein derivatives. 5-R-fluorescein is modified in the *para* position to the xanthene moiety and in the *meta* position to the carboxylic functionality. 6-R-fluorescein is substituted in the *meta* position to the xanthene moiety and in the *para* position to the carboxylic functionality. The additional carboxylic group increases its solubility in polar solvents. The λ_{max} of the chromophore is 492 nm and it emits light at 517 nm.[94] The absorption or emission properties of both isomers are identical (which is a general behavior for all the derivatives). Biomolecules labeled with different isomers have different properties such as migratory time in capillary zone electrophoresis. Isomers can be separated by crystallization or chromatography techniques. **68** is not permeable for biomembranes and its usage for fluorescence labeling *in vivo* is limited to mechanical injection of dye to the cell or incorporation into liposomes. Therefore a highly membrane permeabile dye carboxyfluorescein diacetate succinimidyl ester (CFDA-SE, **70**) has been introduced. The diacetate **70** is not fluorescent and after penetration to the cytosol it is converted to a fluorescent carboxyfluorescein succinimidyl ester (NHS-fluorescein, **69**).

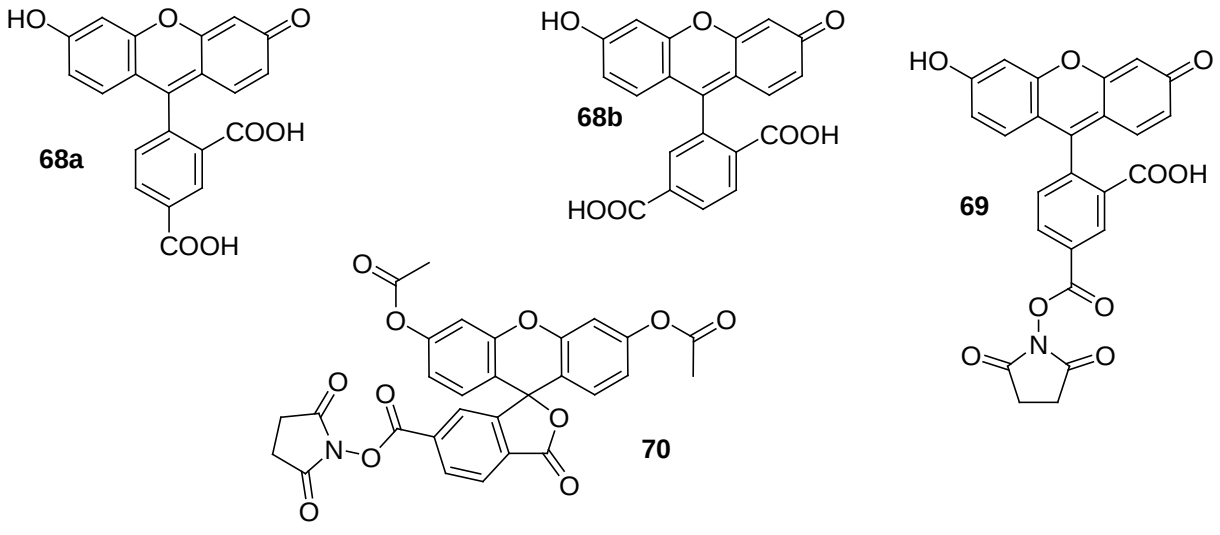


Figure 9: Isomers of Carboxyfluorescein and Its Derivatives

Fluorescein Aminoderivatives

Fluorescein derivatives with a reactive group attached to the fluorescein phenyl ring in the 5 or 6 position through nitrogen atom (Figure 10) are the most used fluorescent labeling compounds for

N- and S-nucleophiles. The best known is fluorescein isothiocyanate (FITC, **71**) which has approximately same absorption (λ_{max}^{abs} = 495 nm) and emission (λ_{max}^{em} = 521 nm) properties as fluorescein. FITC is reactive towards free amino groups and sulfhydryl groups in proteins and is used in antigene – antibody analysis (enzyme-linked immunosorbent assay, ELISA) and in flow cytometry.[95] Sulfhydryl labeling dyes such as fluorescein maleimide (**72**) or iodoacetamido-fluorescein (IAF, **73**) have similar properties and application as FITC.

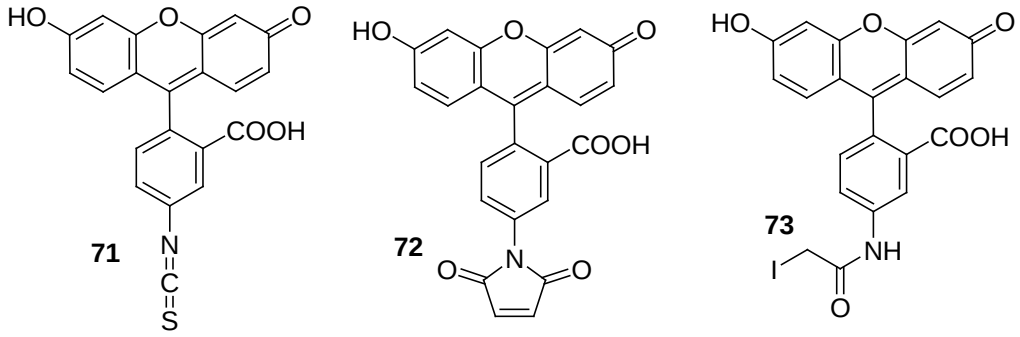


Figure 10: Fluorescein Aminoderivatives

Fluorescein Derivatives with Extended Conjugated System

The extension of xanthene conjugated system (Figure 11) causes the red shift in both absorption and emission. The most used fluorescence dyes with annelated benzene rings are carboxyseminaphthofluorescein (SNAFL, **74**) and carboxynaphthofluorescein (**75**). The excitation of **74** in biological matrices is commonly performed with λ = 490 nm which avoids autofluorescence effects of biomolecules (NADPH, melamine, chlorophyll).[96] The emission spectrum has maximum at λ = 545 nm and a pH dependent maximum in range from 587 nm (pH = 7.25) to 605 nm (pH = 6.3). The carboxynaphthofluorescein **75** has its absorption maximum at λ = 590 nm and emission maximum at λ = 662 nm at pH = 9. The quantum yield of fluorescence is diminished to 0.05[97]. The tautomerism of these molecules is the same as that of fluorescein; the cyclic lactone form is in equilibrium with the opened fluorescent form.

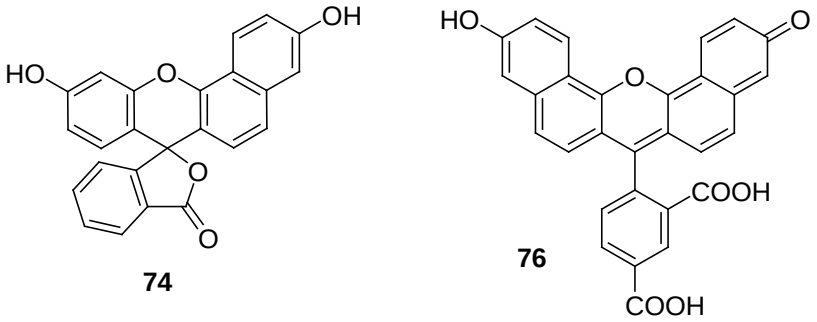


Figure 11: Extended Fluorescein-type Dyes

Tokyo Green

The discovery of Tokyo green family of fluorescent dyes (**76**, Figure 12, R = 2-methylphenyl) has been motivated by physical investigations of fluorescence mechanism of fluorescein. Fluorescein molecule can be described as a directly linked donor acceptor system. The photoinduced electron transfer (PET) between carboxyphenyl donor and xanthene acceptor is a pathway of excited singlet quenching.[98] The carboxylic group acts as a steric barrier which keeps both moieties orthogonal to each other to limit the rate of PET. Unsubstituted molecule on the phenyl ring shows no fluorescence. Any substitution (methyl, methoxy group) in the *ortho* position of the phenyl ring causes loss of planarity, thus reduces the rate of PET and increases the fluorescence quantum yield. Table 1 shows that the quantum yield of fluorescence is diminished with decreasing oxidation potential of phenyl ring. The lower the value of oxidation potential; the higher the HOMO orbital of the donor is. The energy gap between HOMO of the donor and LUMO of the acceptor is therefore lower and the rate of PET is higher.[99]

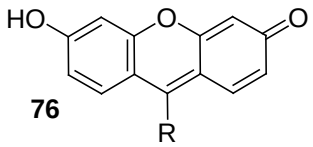
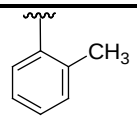
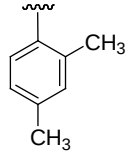
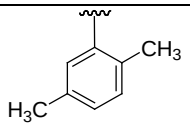
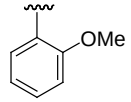
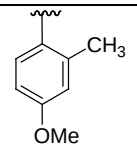
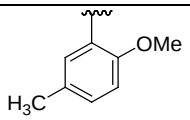
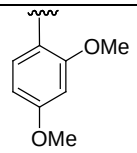
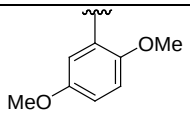


Figure 12: General Structure of Tokyo Green Derivatives

Table 1: Fluorescence Properties of Tokyo Green Derivatives[99]

R	λ excitation max [nm]	λ emission max [nm]	oxidation potential [V] vs SCE	Φ_{fl} (pH = 13)	Φ_{fl} (pH = 3.4)
	491	510	2.19	0.847	0.319
	491	510	2.08	0.865	0.307
	491	510	1.98	0.887	0.319
	494	515	1.75	0.860	0.076
	492	509	1.66	0.840	0.010
	494	514	1.57	0.500	0.004
	494	513	1.44	0.200	0.001
	494	512	1.26	0.010	0.000

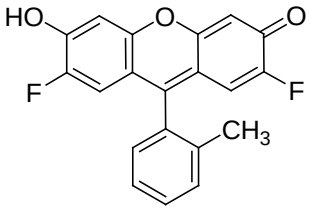
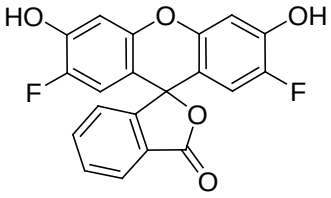
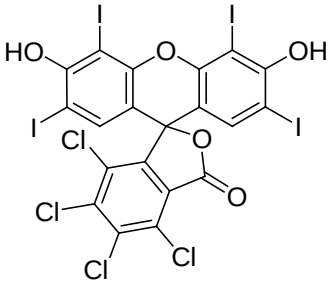
Halogen Substituted Xanthene Dyes

Substitution of aromatic hydrogen atoms by halogen changes significantly the electron density of the aromatic system. The substitution of fluorine atoms at xanthene moiety (Pennsylvania green, Oregon green, Table 2) causes larger lowering of the LUMO level localized more on the xanthene moiety than HOMO localized more on the substituted benzene ring. It results in red shift of both absorption and emission. Substitution by heavier halogen atoms enhances the rate of

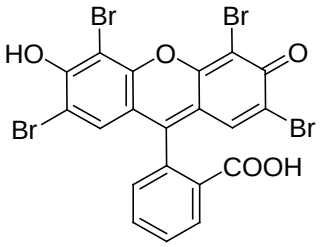
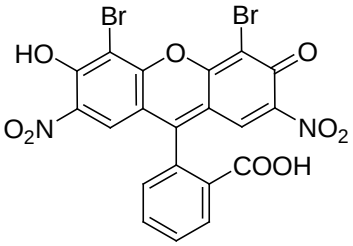
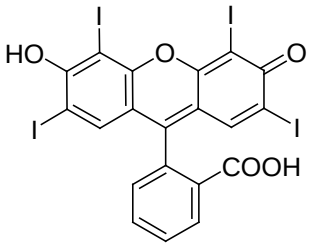
intersystem crossing by heavy atom effect. Therefore the quantum yield of fluorescence is diminished for eosins and erythrosine. Dyes of eosin type display so called E-type delayed fluorescence which is the process in which the first excited singlet state becomes populated by a thermally activated radiationless transition from the first excited triplet state. Since the populations of the singlet and triplet states are in thermal equilibrium in this case, the lifetimes of delayed fluorescence and the concomitant phosphorescence are equal.[20]

The halogen substituted xanthene dyes has been very successful and they are not assessed as fluorescein derivatives. They are used for dyeing textile fibers, coloring paper, as fluorescent labels.[100] Eosin dyes have been successfully used in visible light photocatalysis.[101]

Table 2: Halogen-substituted Fluorescein Derivatives

name	structure	λ_{max}^{abs} [nm]	λ_{max}^{em} [nm]	Φ_{fl}^{max} [a]
Pennsylvania green[102]		496	517	0.93
Oregon green[102]		490	514	0.97
Rose Bengal[103]		559	571	0.11

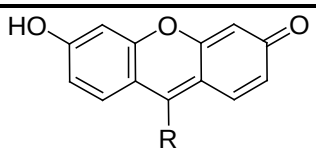
[a] maximum fluorescence quantum yield, aqueous solution

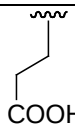
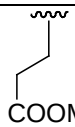
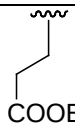
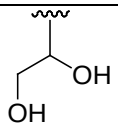
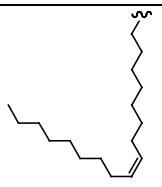
Eosin Y[104]		525	543	0.20
Eosin B[105]		520	580	0.045
Erythrosine[106]		526	550	0.05

Other Molecules with Xanthene Core

There are 2088 known substances (April 2012, Reaxys® structure search) with xanthene core substituted on C-9. 2078 of them has a substituted phenyl ring attached to xanthene. The structures, year of publishing, and the characterization of ten other molecules (besides the elementary analysis "confirming" their structure) are shown in the Table 3. This suggests that derivatizing of xanthene with a chemically non-inert group (hydroxymethyl, carbonyl or carboxyl) may be complicated.

Table 3: Structure and Characterization of Non-aromatic Xanthene Derivatives



R	published in	Characterization
	2005[107]	m.p.; NMR; IR; HRMS; UV/vis
	1993[108]	UV/vis
	1923[109]	m.p.
	1988[110]	m.p.; NMR; IR; UV/vis
	1988[110]	m.p.; NMR; IR; MS; UV/vis
	1923[111]	m.p.
	1925[112]	m.p.
	1893[113]	m.p.
	1923[114]	-
	1929[115]	m.p.

Flavin photocatalysis

Introduction

Photocatalysis using visible light is a strategy leading to more sustainable chemical processes and to the chemical storage of the light energy. The second aim has its best example in the nature, the highly efficient photosynthesis. Many homogenous and heterogenous photocatalytic systems for redox reactions have been investigated,[116–121] but so far there are severe limitations (high rate of photobleaching, low turnover number, low quantum yield of the process, low selectivity and low yields of the chemical reaction).

The redox photocatalysis is based on a light induced electron transfer. An electron is inserted or removed to from the excited molecule of the photocatalyst. Excited species are much better electron acceptors and donors than molecules in the ground state. The redox potential of excited state can be estimated by the Rehm-Weller equation (Eq. 1, 2).[122] As it is shown in Figure 13 for molecules in the gas phase with neglecting of reorganization energy, the electron affinity (EA) is higher and the ionization potential (IP) is lower exactly by the HOMO-LUMO energy gap (ΔE^{0-0}). ΔG^0 is the standard Gibbs energy, E^0 is the redox potential of the particular compound. In the solution, interactions with the solvent molecules play a role and, therefore, the enthalpy of solvation (ΔH_{solv}) and the coulombic energy of the ion pair ($\frac{e^2}{4\pi\varepsilon r}$) should be added ($e$ is elementary charge, ε is permittivity of the environment and r is distance of ions).

$$\Delta G^0 = E^0_{D+./D} - E^0_{A/A-.} - \Delta E^{0-0}_D - \Delta H_{solv} - \frac{e^2}{4\pi\varepsilon r} \quad \text{(Eq. 1)}$$

$$\Delta G^0 = E^0_{D+./D} - E^0_{A/A-.} - \Delta E^{0-0}_A - \Delta H_{solv} - \frac{e^2}{4\pi\varepsilon r} \quad \text{(Eq. 2)}$$

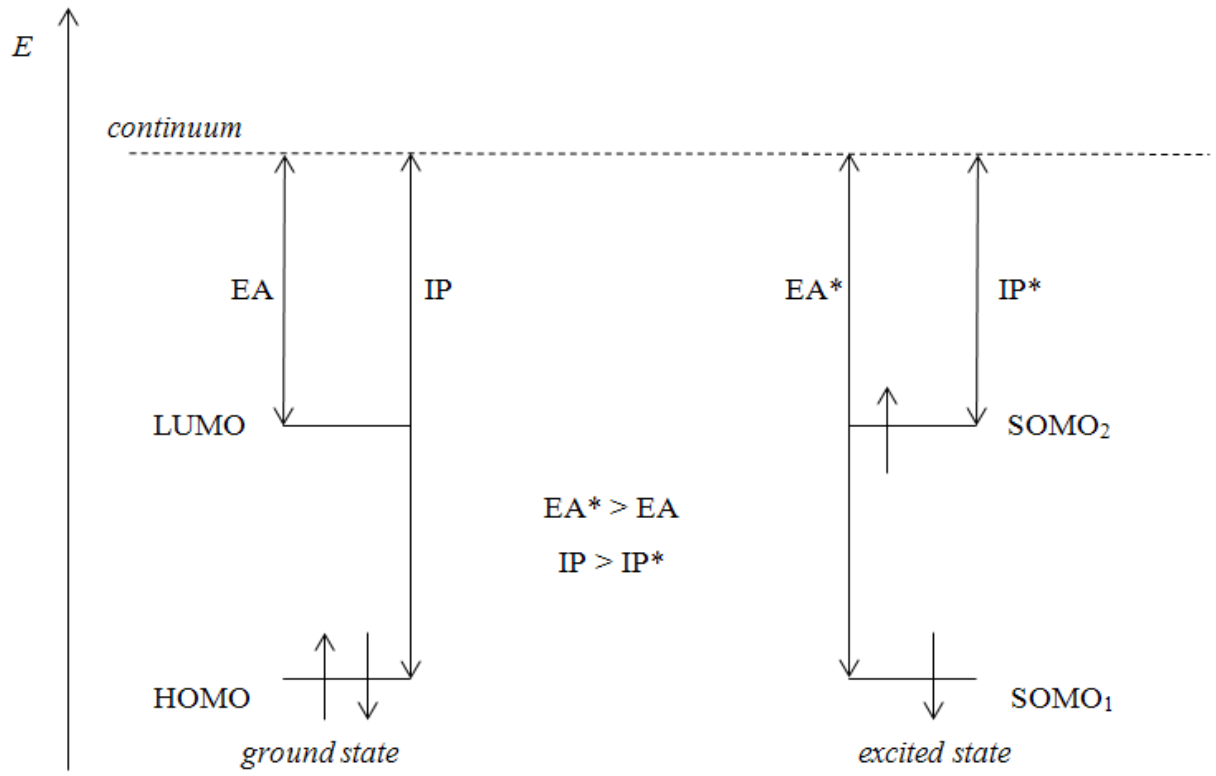


Figure 13: Ionization Potential (IP) and Electron Affinity (EA) for the Ground and Excited State

As described by Marcus[123], the inner-sphere electron transfer (eT) between donor and acceptor is much faster than that of outer-sphere eT. The rate of electron transfer is strongly dependent on the distance, mutual orientation of the donor and acceptor, and the difference in IP of the donor and EA of the acceptor. With a very high energy difference of EA and IP, the reaction rate is slowed down (Marcus inverted region). The most efficient mode of photocatalysis is when substrate reversibly binds to the chromophore by non-bonding interactions (π-π stacking, hydrogen bonding, and dative bonding). Systems for photocatalysis are often designed with the catalyst placed in confined media or various templates.[124,125]

Flavins

Flavins are common natural redox active compounds which serve as chromophores, fluorophores and prosthetic groups in oxidoreductases. The most important biologically active species are riboflavin **77** (vitamin B2), flavin mononucleotide **78** (FMN) and flavin adenine dinucleotide **79** (FAD) (Figure 14).

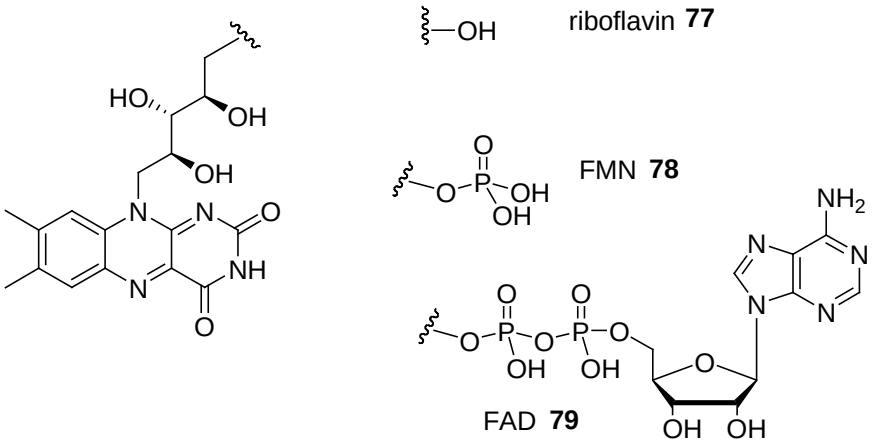


Figure 14: Biological Flavins

The flavin structure is based on the isoalloxazine conjugated core (benzo[*g*]pteridine) which serves as a chromophore and fluorophore. The numbering of atoms in the flavin core is shown in Figure 15 and it will be used throughout this thesis. Flavin conjugated system has λ_{max} = 450 nm[125] and it can be easily excited by a blue emitting LED. The strong fluorescence is emitted in the green region of light. The properties of flavins (redox potentials, absorption maximum and chemical reactivity and resistance against photo-bleaching) are influenced by its substitution, complexation or hydrogen bonding.[127–129]

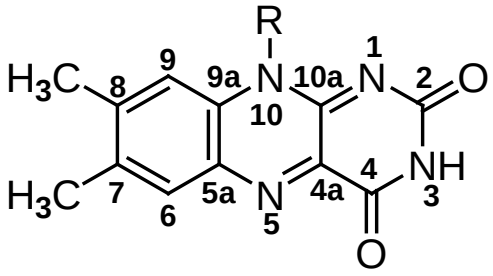
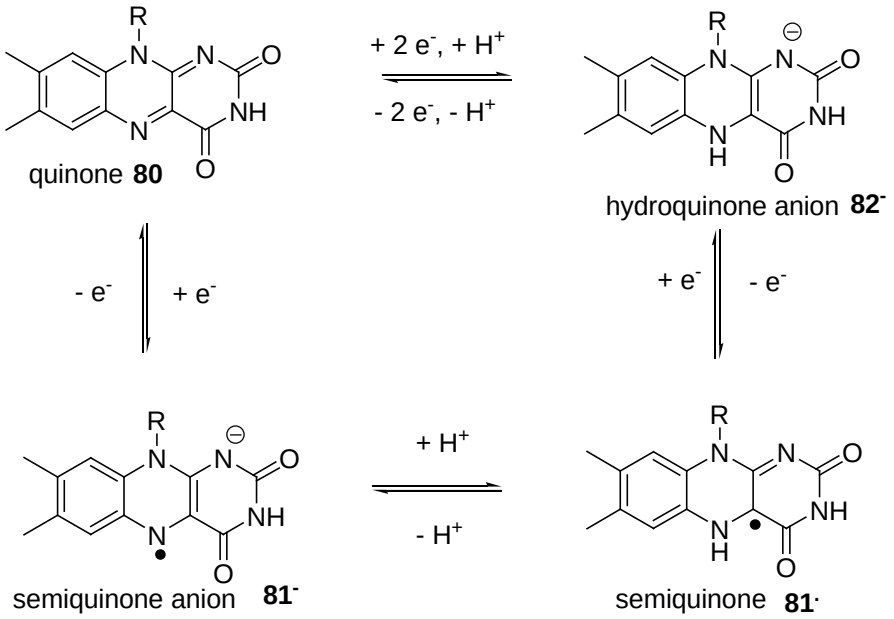


Figure 15: Numbering of Atoms in the Flavin Molecule[b]

Flavins can be reduced by a sequential electron uptake to three different oxidation states (Scheme 19). The oxidized quinone structure **80** is stable in an oxygen containing atmosphere. After one-electron reduction the semiquinone radical, anion **81**$^{-}$ is formed. It is protonated on the nitrogen atom N-5 to give semiquinone **81**. This can accept one electron to reach the fully reduced hydroquinone anion **82**$^{-}$. The semiquinone species **81**$\cdot$ and **81**$^{-}$ are not stable and usually the redox process ends up with a fully reduced/oxidized flavin. All oxidation states are easily distinguished by UV/vis spectrometry.

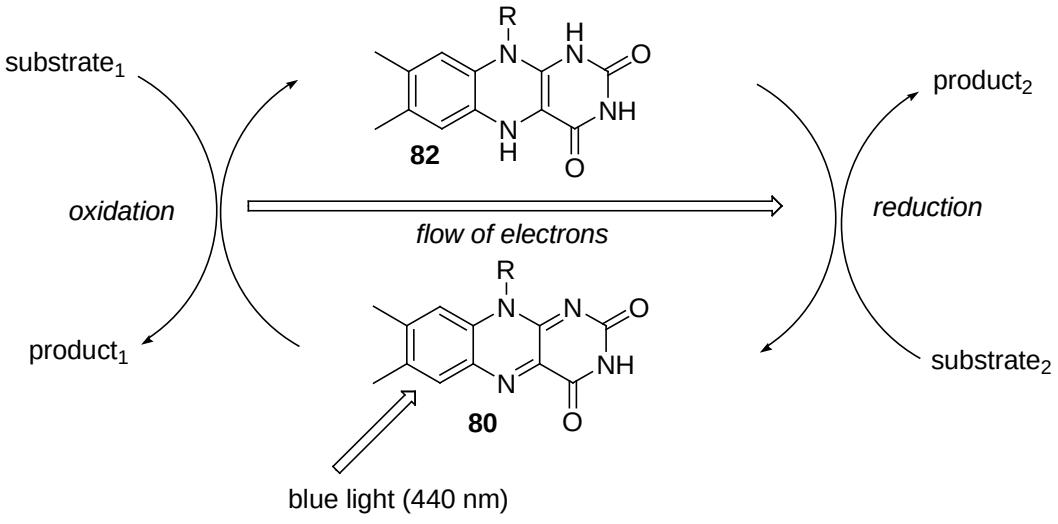
[b] adapted from: Satoh A. *et al.*[130]



Scheme 19: Oxidation States of Flavins

Photocatalytic Cycle

Because of the favorable change of electron affinity and ionization potential of excited species, the flavin molecule can be used as a redox photocatalyst in both halves of the flavin redox cycle. In the Scheme 20 the general principle of the catalysis is shown.



Scheme 20: General Scheme of Flavin Photocatalysis

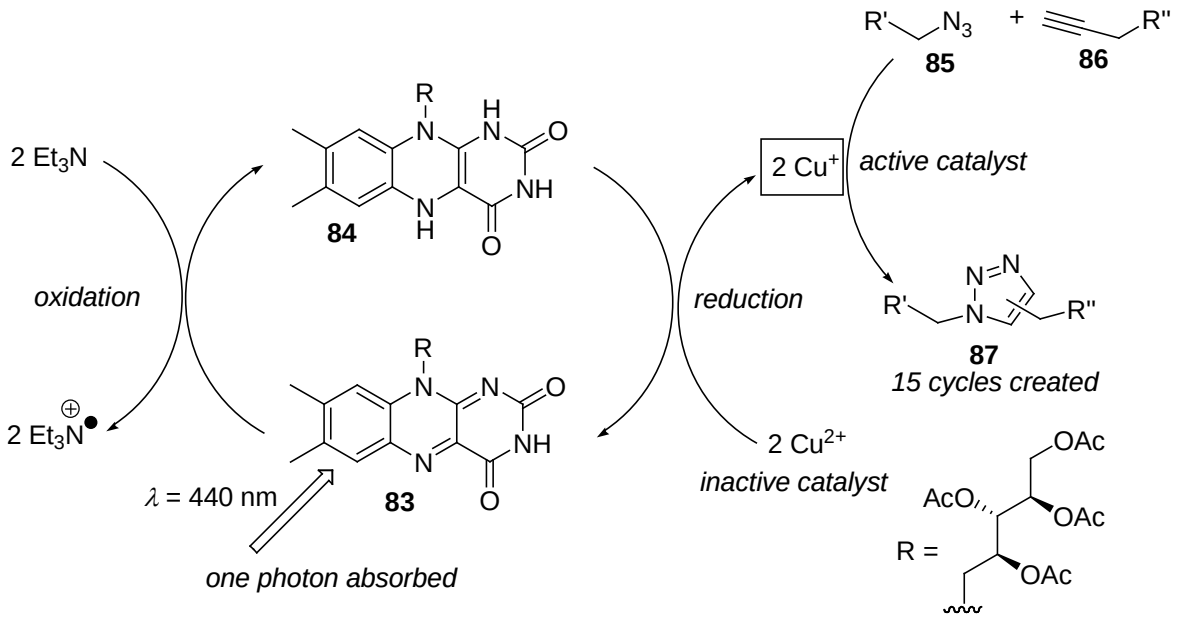
The excitation of the flavin **80** leads to the electron transfer from the substrate$_1$ to the flavin core. The resulting highly reducing flavin hydroquinone **82** is oxidized back very easily to the flavin

80 by the substrate$_2$. The substrate$_1$ is thus oxidized and the substrate$_2$ is reduced. In the most cases, **82** is reoxidized back by oxygen (substrate$_2$) present in the system generating hydrogen peroxide (product$_2$) as a reduction photoproduct. In this mode, flavins are used for photooxidation of substrates (oxidative catalytic cycle).

For photocatalytic reduction of the substrates, a sacrificial electron donor is added to the system. Typical donors are electron rich compounds with soft electron pairs such as triethyl amine or EDTA.[131,132] In this "reductive catalytic cycle", the reoxidation by oxygen must be suppressed. Oxygen can be either excluded from the apparatus which is complicated (even traces of oxygen decrease the catalytic activity) or the desired reduction reaction must be much faster than the diffusion controlled reoxidation by oxygen.

Use of Flavins as Redox Photocatalysts

An interesting example of using flavin in the reductive catalytic cycle has been demonstrated by König and coworkers[133]. The riboflavin tetraacetate **83** (RFT) was reduced with triethylamine (Scheme 21) to its hydroquinone form **84** which *in situ* reduced copper(II) ions present in the system to Cu(I).



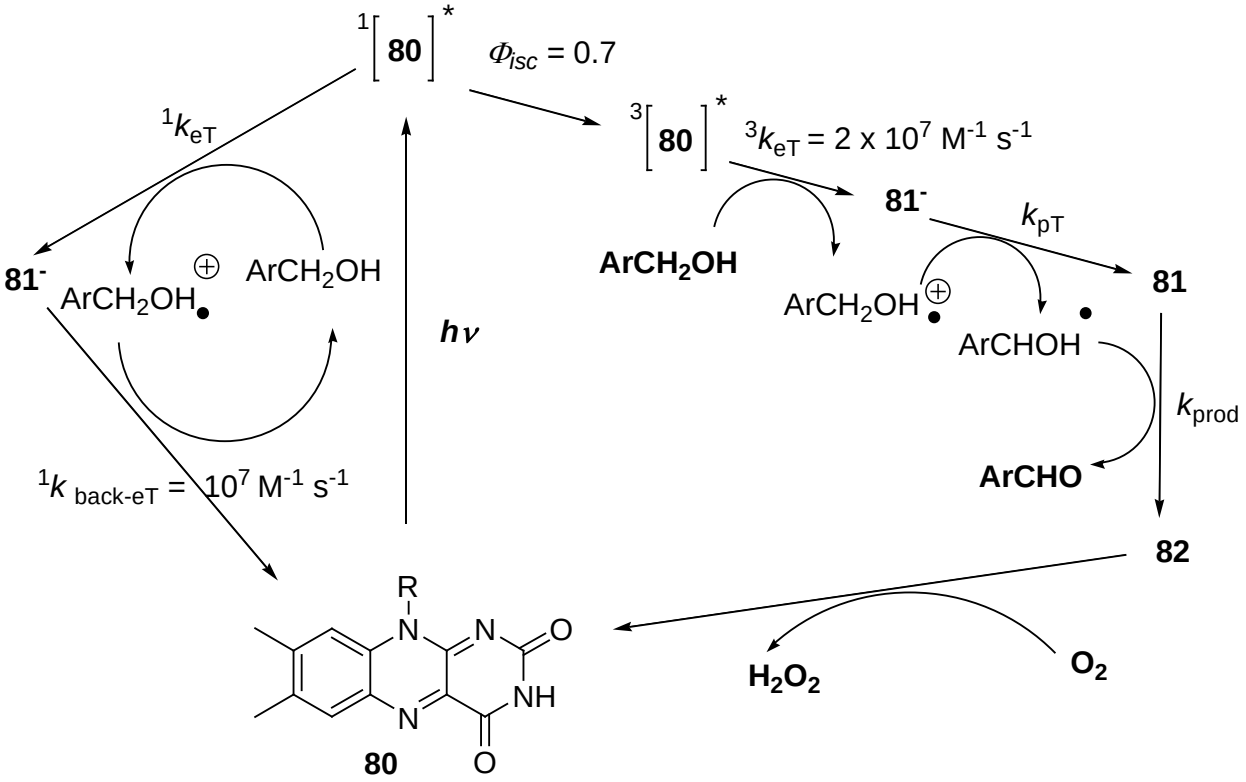
Scheme 21: Flavin-initiated Huisgen Reaction

Copper(I) ions served as a catalyst for a subsequent Huisgen reaction ("click reaction", [3+2] cycloaddition). Copper is reduced by **84** instead of oxygen because the metal ions coordinate to

the heteroatoms of the flavin[134] and the eT is therefore faster between **84** and Cu(II) than O_2. Absorption of one photon by the system resulted into a generation of 15 triazole rings **87** (as a mixture of two stereoizomers) from azide **85** and terminal alkyne **86** present in the system.

Riboflavin tetraacetate (RFT) **83** as the most stable flavin for photocatalysis has been used in oxidative catalytic cycle for oxidation of benzyl alcohols,[135] benzyl amines,[136] oxidative cleavage of stilbenes and oxidation of electron rich toluenes.[137] The product of oxidation is in most cases α-aryl-aldehyde. The hydroquinone **84** has been reoxidized back by oxygen in all cases.

The mechanism of photooxidation of benzyl alcohols has been studied by a pump-probe femtosecond laser flash spectroscopy.[138] The simplified version is shown in the Scheme 22.



Scheme 22: Mechanism of Photooxidation of Benzyl Alcohols[c]

Upon excitation the majority of the molecules (70 %) undergo intersystem crossing to the triplet state and abstracts electron from the benzyl alcohol. After cascade of subsequent steps the aldehyde ArCHO is formed beside the hydroquinone **82** which is oxidized back by oxygen. Electron transfer from singlet state is not productive because the back electron transfer rate $^1k_{\text{back-eT}}$ is high and the charge recombination leads in the starting material **80**.

[c] **81**$^-$ is flavin semiquinone anion; **81** is flavin semiquinone; **82** is flavin hydroquinone

A paraboloid dependence of the reaction quantum yield (in order of 1 – 3 %) on the concentration of the substrate with the optimum at c_{sub} ~ 10 mM has been found. With a low concentration of the substrate (benzyl alcohol), the reaction rate is slow (bimolecular reaction). With a high concentration of the substrate the reaction rate is high but the triplet yield is low because of the competing inefficient electron transfer from singlet (singlet and triplet quenching).

Aims

Introduction

This thesis consists of 3 different projects in synthetic photochemistry. They are based on synthesis and investigation of photoreactivity of photoactivatable compounds.

The first project, Photoremovable Chiral Auxiliary, is introduced in my bachelor thesis[139] and therefore it will not be discussed here in details. The results of the project have been published.[80]

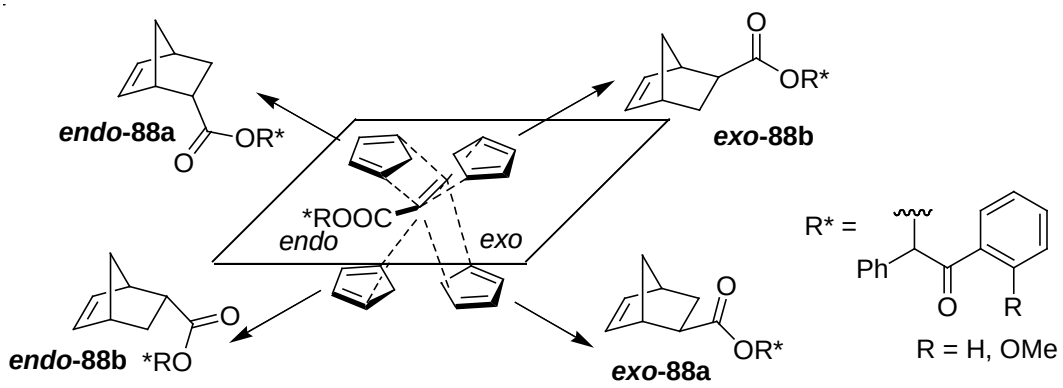
The second project, Phenanthroline-flavin for Photocatalysis, was done at the University of Regensburg, Germany in the group of prof. Burkhard König. The results will be published soon.

The final project, (6-Hydroxy-3-oxo-*3H*-xanthen-9-yl)methyl: A Photoremovable Protecting Group, was accomplished with the collaboration with prof. Wirz from University of Basel, Switzerland. The results of the project are going to be submitted for publication.

Photoremovable Chiral Auxiliary

Chiral auxiliaries[140–142] are chiral molecules attached to an achiral substrate with a prochiral center. The auxiliary dictates the stereochemistry of the particular reaction of the prochiral center and afterwards is cleaved to deliberate the enantiomericaly pure product of the reaction. The cleavage is accomplished usually by solvolysis of the auxiliary.

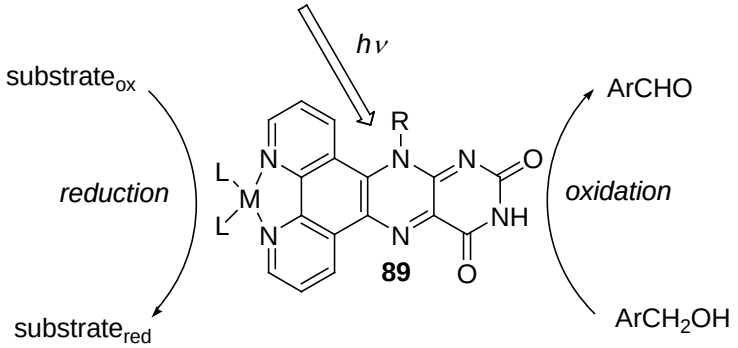
Benzoin PPG contains a chiral center and therefore it can be in principle used as a chiral auxiliary. The aim of the this project was to prove the hypothesis of chiral induction by benzoin PPG for Diels-Alder reaction of benzoin acrylate with cyclopentadiene (Scheme 23) (partially covered in bachelor thesis[139]) and to optimize the reaction conditions (solvent, temperature, catalyst) and the structure of the chiral auxiliary to result in the highest enantioselectivity.



Scheme 23: Diels-Alder Reaction of Cyclopentadiene and Benzoin Acrylate Leading to Four Different Diastereomers of Benzoin 5-Norbornene-2-carboxylates **88**

Phenanthroline-flavin For Photocatalysis

The flavin photocatalysis has been performed in only either oxidative or reductive catalytic cycle. For oxidations, the sacrificial electron donor must be present and, for reduction, either complexation of metal ions to the chromophore or exclusion of oxygen is needed. To couple the oxidative and reductive cycle together, a new system **89** for photocatalysis has been proposed (Scheme 24). The project deals with the total synthesis of the system **89**.



Scheme 24: Phenanthroline-flavin for Coupled Redox Process

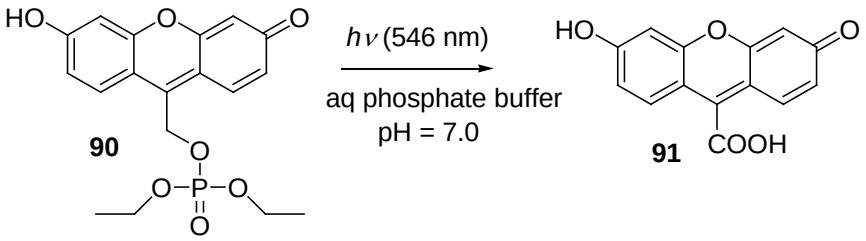
The conjugated system of the flavin is extended by two pyridine rings which should improve its absorption properties and should enable the complexation of transition metals M (M = Pd, Pt, Ni, Cr).[143] After excitation, the photoinduced electron transfer from the benzyl alcohol generates a reduced hydroquinone structure. The accepted electrons reduce the metal ions coordinated to the phenanthroline part. The reduced metal may reduce another substrate present in the reaction. Substrate$_{ox}$ should be an inert molecule which will activated by reduction similarly to the activation of moderately reactive benzyl alcohol to benzaldehyde. In an ideal case, the benzaldehyde could react with the substrate$_{red}$ to create final product of the reaction.

(6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl Moiety: A Photoremovable Protecting Group

Photoremovable protecting groups (PPGs) are frequently used as versatile tools allowing for the temporally and spatially controlled release of various bioagents in order to study the kinetics of chemical processes in living cells.[9,17] Attractive features of coumarin-derived PPGs are their strong absorption extending to the visible range and appearance rate constants of the free substrates that are on the order of 10^9 s^{-1} following excitation with a short light pulse.[144] In effort

to extend the wavelength range of coumarin PPGs, the (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl system (Scheme 25) was designed as a novel PPG absorbing over 500 nm.

The aim of the project was to synthetize the phosphate **90** and its photoproduct **91** (Scheme 25) and to optimize and reinvestigate their syntheses.

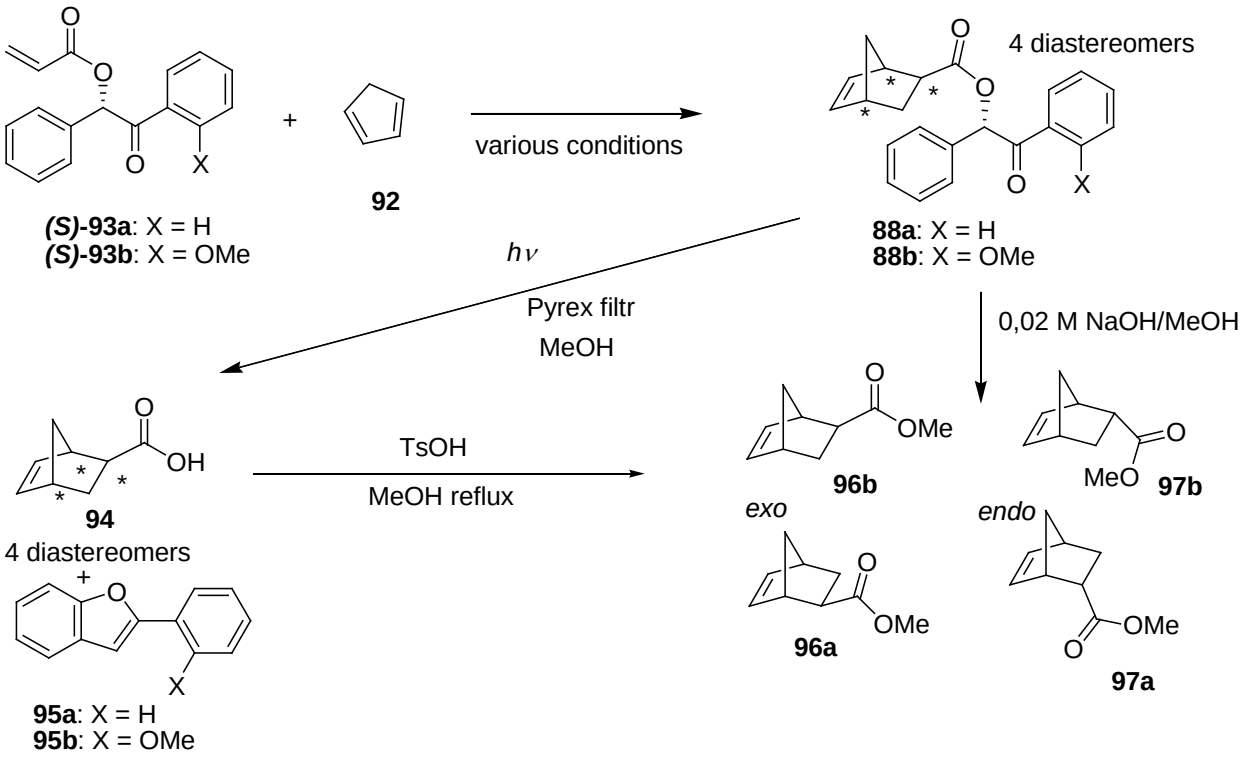


Scheme 25: (6-Hydroxy-3-oxo-*3H*-xanthen-9-yl)methyl PPG and Its Photoproduct

Results and discussion

Photoremovable Chiral Auxiliary

The project was based on investigation of the Diels-Alder [4+2] cycloaddition of cyclopentadiene **92** with acrylate with attached *(S)*-benzoin or *(S)*-2-methoxybenzoin chiral auxiliary ***(S)*-93** (Scheme 26). The product **88** created as a mixture of 4 diastereomers was irradiated in methanol to release free norbornenic acid **94** and corresponding substituted 2-phenylbenzofuran **95**. The mixture of stereoisomers of norbornenic acid was further esterified to corresponding methyl esters **96** and **97** with acid catalyst to simplify the determination of the diastereomeric composition of the mixture.

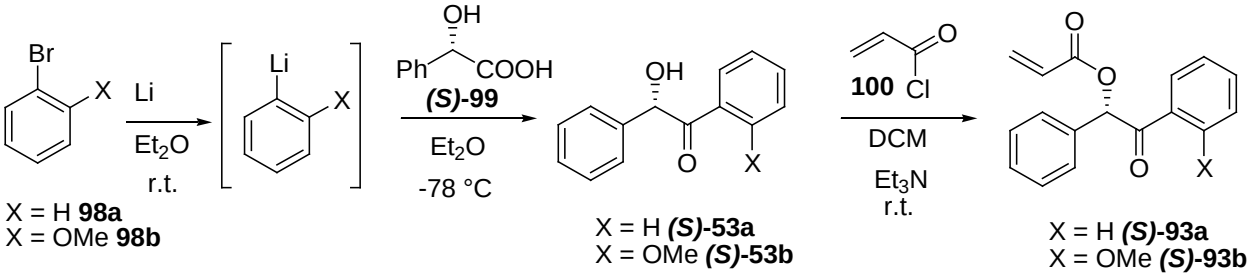


Scheme 26: Diels-Alder Reaction and Subsequent Photolysis and Esterification

The synthesis of the 2-R-benzoin acrylate (R = H, OMe) ***(S)*-93** was accomplished in a two-step procedure (Scheme 27). The enantiomericaly pure benzoins ***(S)*-53** were prepared from the corresponding bromoarene (bromobenzene **98a**, 2-bromoanisole **98b**) by preparation of aryllithium and subsequent reaction with *(S)*-mandelic acid ***(S)*-99** according to a known procedure.[145] As there are two acidic protons in the molecule of the mandelic acid not

compatible with the organometalic reagent, 3 equivalents of the aryl lithium were used. To amount the amount of aryllithium, disodium salt of mandelic acid created by addition of 2 equivalents of NaH to the solution of ***(S)*-99** in THF was also used.

The subsequent esterification[71] with the acryloyl chloride **100** and a stoichiometric amount of triethylamine in dichloromethane (DCM) resulted in pure ***(S)*-93** in overall yield 85 – 90 %.



X = H **98a**
X = OMe **98b**

X = H ***(S)*-53a**
X = OMe ***(S)*-53b**

X = H ***(S)*-93a**
X = OMe ***(S)*-93b**

Scheme 27: Synthesis of 2-R-Benzoin Acrylate (R = H, OMe) ***(S)*-93**

The reaction conditions of the Diels–Alder reaction were modified to give the highest enantioselectivity induced by the PCA. The effects of solvent (toluene, dichloromethane, water emulsion), catalyst (different molar ratios of $EtAlCl_2$ or $SnCl_4$ vs. non-catalyzed reaction), reaction temperature (–78 °C to 50 °C) and the structure of benzoin auxiliary (*(S)*-benzoin, *(R)*-benzoin, *(S)*-2-hydroxybenzoin, *(S)*-2-methoxybenzoin, *(S)*-2-acetoxybenzoin, *(S)*-2'-chlorobenzoin) on the enantioselectivity of the cycloaddition were investigated.

Table 4: Cycloaddition of ***(S)*-93** with Cyclopentadiene and Subsequent PCA Release from **88**

Entry	Ester	Cycloaddition reaction conditions			Photorelease of the PCA		
		Catalyst	*T*/°C	Time/h	de (%)	ee (*endo*) (%)	ee (*exo*) (%)
1	***(S)*-93a**	-[d]	20	36	46	35	–36
2	***(S)*-93a**	$EtAlCl_2$ (1 eq)[e]	–20	2	85	12	9
3	***(S)*-93a**	$SnCl_4$ (2 eq)[d]	–78	6	62	46	58
4	***(S)*-93b**	-[d]	20	72	46	31	–42
5	***(S)*-93b**	$EtAlCl_2$ (1 eq)[e]	–20	2	73	54	50
6	***(S)*-93b**	$SnCl_4$ (2 eq)[d]	–78	5	53	–43	96

[d] solvent: toluene
[e] solvent: dichloromethane

In the Table 4 a few examples of conditions of the cycloaddition are shown. The complete set of results was recently reported[80]. The diastereoselectivity and enantioselectivity of the cycloaddition are shown as de and ee, respectively. Enantiomeric and diastereomeric excesses were calculated according to Equations 3, 4 and 5.

$$\text{de} = \frac{([\mathbf{97a}]+[\mathbf{97b}])-([\mathbf{96a}]+[\mathbf{96b}])}{[\mathbf{97a}]+[\mathbf{97b}]+[\mathbf{96a}]+[\mathbf{96b}]} \times 100 \qquad \text{(Eq. 3)}$$

$$\text{ee}\,(endo) = \frac{[\mathbf{97a}]-[\mathbf{97b}]}{[\mathbf{97a}]+[\mathbf{97b}]} \times 100 \qquad \text{(Eq. 4)}$$

$$\text{ee}\,(exo) = \frac{[\mathbf{96a}]-[\mathbf{96b}]}{[\mathbf{96a}]+[\mathbf{96b}]} \times 100 \qquad \text{(Eq. 5)}$$

Lewis acid as a catalyst was found to enhance the reaction rate significantly (Table 4). $EtAlCl_2$ induces the highest diastereoselectivity (highest excess of *endo* products) in comparison to $SnCl_4$ and non-catalyzed reaction. The non-catalyzed reaction has moderate diastereoselectivity and a negative value of *exo* ee ([**96b**] is higher than [**96a**]). The catalysis with 2 eq. of $SnCl_4$ showed the highest enantioselectivity (ee (*exo*) = 96 %) for 2-methoxybenzoin as a chiral auxiliary. The *endo* enantioselectivity for this case was found to be highly influenced by the reaction temperature and varied from –43 % at –78 °C to 11 % at 20 °C.

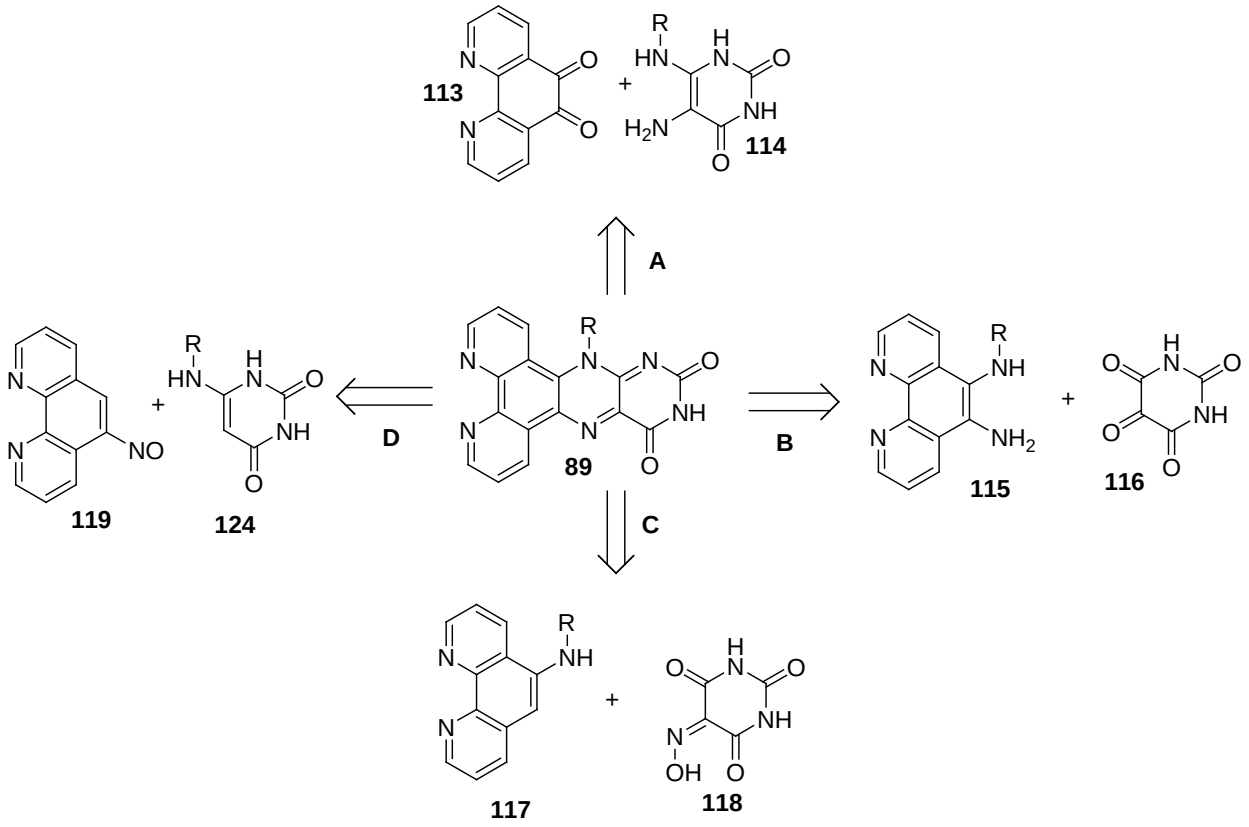
The photorelease of PCA from cycloaddition products was accomplished with a high chemical yield >85 % by irradiation at λ = 313 nm in MeOH. The enantiomeric purity of the auxiliary and the isomeric ratio of the cycloaddion products were not influenced by reaction conditions of the synthetic procedure.

Beside the work published in my bachelor thesis[139] I contributed to the project by the synthesis of acrylates ***(S)*-93a** and ***(S)*-93b**, examination of the Diels-Alder reaction of both acrylates in various conditions (besides the reactions in water), determination of the isomeric ratio of the methyl norbornenates **96a**, **96b**, **97a** and **97b** with chiral GC and chiral HPLC determination of the enantiomeric purity of all other chiral compounds synthetized.

Phenanthroline-flavin for Photocatalysis

General Synthetic Strategy

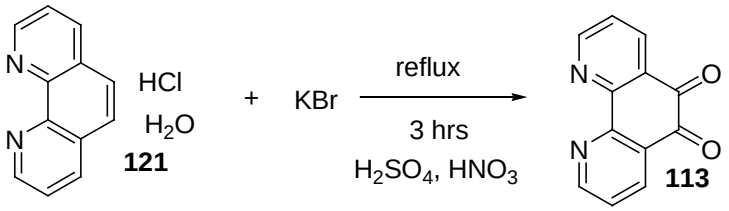
The aim of this project was to synthetize the phenantroline flavin **89**. As there are no similar structures known, the total synthesis of the system has been developed. The retrosynthetic analysis resulted in four different routes of the system construction (Scheme 28). The route A is a condensation of 1,10-phenanthroline-5,6-dione **113** and 5,6-diaminouracil **114**. This approach has been used in the synthesis of lumazine.[142] The same symmetrical disconnection with the reversal of heteroatom placement (route B) was applied in flavin synthesis[147] and leads to 1,10-phenanthroline-5,6-diamine **115** and a commercially available alloxane **116**. The route C is another common way in flavine synthesis.[148] It is a condensation of 1,10-phenanthroline-5-amine **117** and a commercially available violuric acid **118**. Both alloxane and violuric acid have two planes of symmetry which simplifies the retrosynthetic analysis. After the condensation the substituent R is always attached to the N-10 and unstable structures with substitution on N-5 are cleaved back to the starting material. The last possibility of flavin construction[149] is the route D starting from 5-nitroso-1,10-phenanthroline **119** and 6-aminouracil **124**.



Scheme 28: Retrosynthetic Analysis of Phenanthroline Flavin **89**

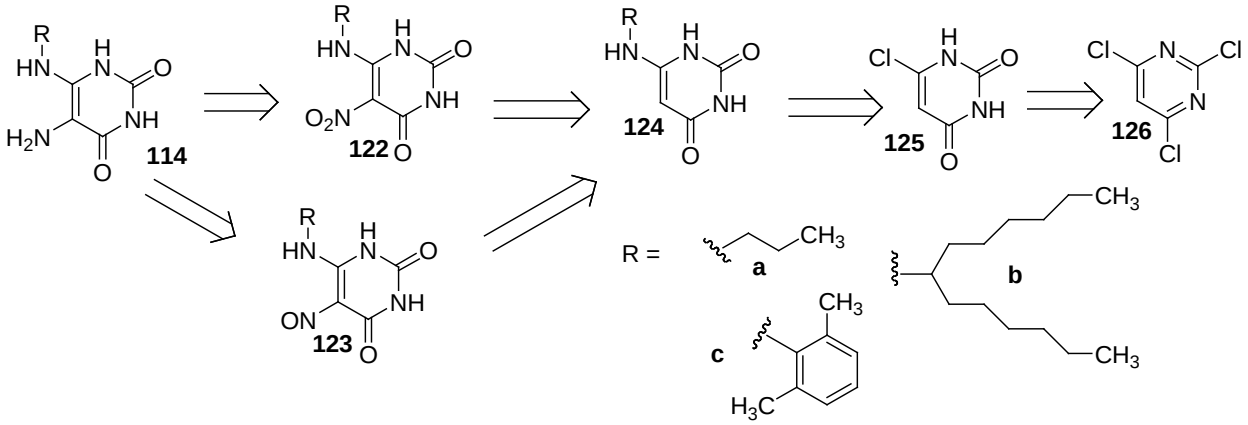
Route A

Neither **113** nor **114** are commercially available and therefore both of them should be prepared. The 1,10-phenanthroline-5,6-dione **113** was synthetized (Scheme 29) according to the known procedure[150] by oxidation of 1,10-phenanthroline hydrochloride monohydrate **121** by bromine generated *in situ*. The reaction is sensitive to the amount of bromine generated. The conditions and the recrystallization of the product were optimized to the final yield 88 %.



Scheme 29: Synthesis of 1,10-Phenanthroline-5,6-dione **113**

All attempts to synthetize the 6-substituted 5,6-diaminouracil **114** were based on the retrosynthetic Scheme 30.

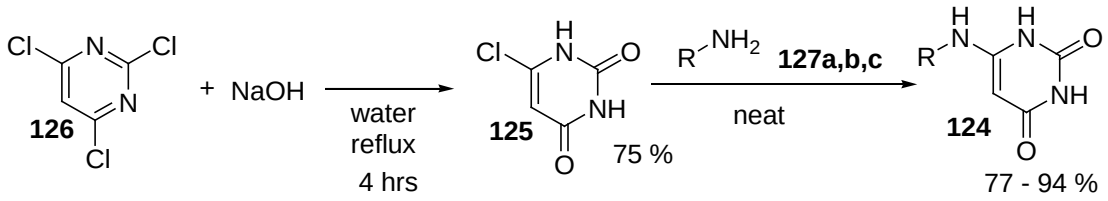


Scheme 30: Retrosynthetic Analysis of 6-Substituted 5,6-Diaminouracil **114**

The 6-substituted 5,6-diaminouracil **114** cannot be prepared by N-alkylation of one of nitrogen atom of 5,6-diaminouracil because of the similar reactivity of both amino groups. The nitration of 6-chlorouracil **125** resulted in a mixture of products, which is very hard to separate. These issues determined the final order of steps in the synthetic pathway.

The 6-alkylaminouracil **124** has been synthetized from a commercially available 2,4,6-trichloropyrimidine **126** in two steps according to the Scheme 31. The nucleophilic displacement of the chlorine atoms in **126** by a hydroxide ion was accomplished according to the known

procedure[147] in 75 % yield. The resulting 6-chlorouracil **125** precipitates from acidic water as a white solid in very high purity.



Scheme 31: Synthesis of 6-Alkylaminouracil **124**

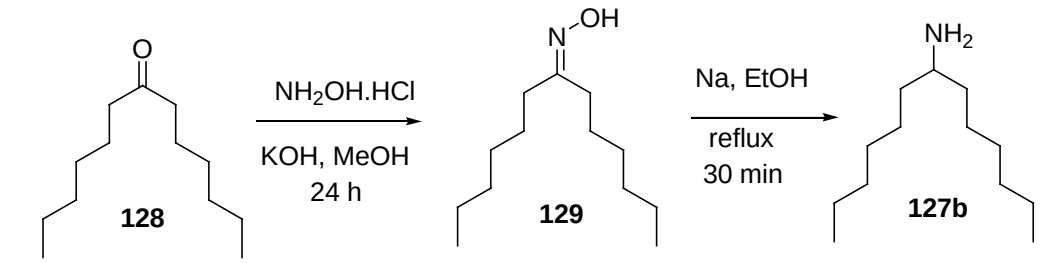
The subsequent nucleophilic substitution of **125** by alkylamine (propylamine **127a**, tridecan-7-amine **127b**) or aniline[151] (**127c** 2,6-dimethylaniline) was accomplished in the corresponding amine as a solvent under nitrogen atmosphere (all efforts to use a common organic solvent to spare the amine led to low conversion). The reaction conditions and the isolated yields are shown in Table 5.

Table 5: Reaction Conditions and Yields for Substitution of 6-Chlorouracil Alkylamines

code	№ of equivalents of amine	temperature [°C]	reaction time [min]	yield [%]
124a	6	60	180	94
124b	2	140	240	62
124c	3	180	40	77

Both **124a** and **124c** precipitate from methanol in sufficient purity, but **124b** was contaminated with the amine. The long alkyl chain decreased the polarity of the molecule and, therefore, unlike other uracil derivatives it could be purified by a column chromatography.

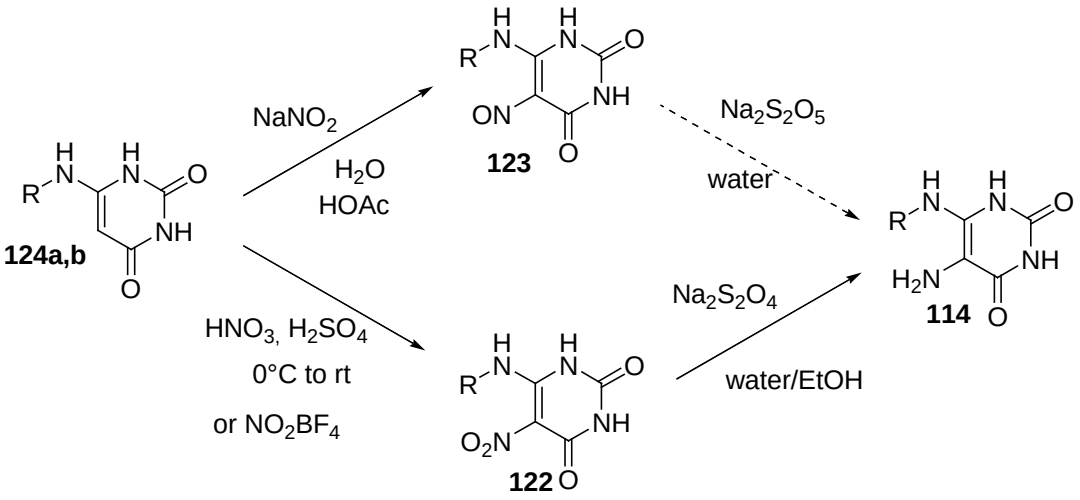
Propylamine **127a** and 2,6-dimethylaniline **127c** are commercially available and were used as purchased. The tridecan-7-amine **127b** was synthetized (Scheme 32) in two steps from the commercially available dihexylketone **128** according to the known procedure[152].



Scheme 32: Synthesis of Tridecan-7-amine **127b**

The base catalysed formation of dihexyloxime **129** was executed in almost quantitative yield (98 %) and no purification was needed. The reaction is heterogeneous, starting material and product create an oily phase on the surface, potassium hydroxide does not dissolve completely. The subsequent reduction of the oxime **129** by sodium in ethanol resulted in 94 % yield of pure tridecan-7-amine **127b**. The reduction is sensitive to the reaction temperature and time. With a higher reaction temperature and prolonged time the inseparable coloured byproducts are formed. The amine **127b** is prepared as colourless oil but easily crystallizes from hexane.

The introduction of amino group at C-5 in 6-aminouracil **124a,b** was attempted by different approaches summarized in the Scheme 33.



Scheme 33: Synthesis of 6-Alkyl-5,6-aminouracil **114**

The classical nitration of 6-(propylamino)uracil **124a** provided the product **122a** in a good conversion with no side-products. The only complication was the high polarity of the product preventing extraction of the product from water solution to any organic solvent. The water phase must have been evaporated to dryness and the product was extracted from its mixture with salts present in the reaction mixture (Na_2SO_4, $NaNO_3$). The product **122a** was obtained in 88 % yield.

The nitration of **124b** was more complicated and the tridecan-7-amine partially cleaved from uracil core which decreased the yield. Despite the TLC showed full consumption of the starting material, the product **122b** was isolated only in low yield and as a mixture with salts.

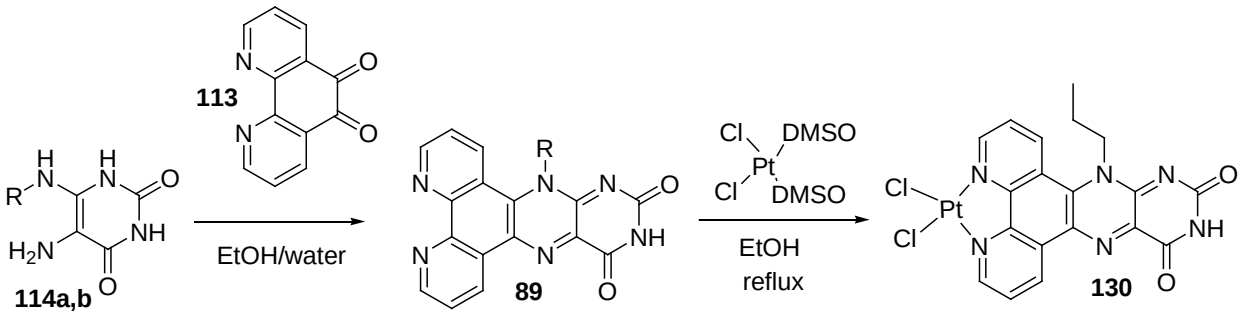
All attempts of develop the nitration of **124a,b** using a non-acidic nitration agent nitronium tetrafluoroborate (NO_2BF_4) in non-aqueous organic solvent failed. The most common solvents used for similar nitrations are chloroform, dichloromethane and acetonitrile.[153] Unfortunately, none of these solvents dissolve the starting material **124a,b**. Methanol as a nucleophilic solvent destroyed the nitration agent and use of DMSO or DMF did not result in any product and after a week of heating all the starting material was isolated.

The nitrosation of **124a** by sodium nitrite with acetic acid as a catalyst was also investigated. Three different procedures were examined. The nitrosation in methanol/water mixture[154] resulted in cleavage of the propyl chain. The use of water solution and slightly modified procedure[155] did not lead to any reaction. The last procedure according to Pfleiderer and coworkers[156] resulted in a low yield of product (25 %).

The 5-nitro-6-(alkylamino)uracil **124** was reduced to the target (6-alkylamino)-5-aminouracil **114** in the same fashion as the reduction of alkyllumazines by sodium dithionite in ethanol/water mixture.[157] The 6-substituted 5,6-diaminouracil is considered to be very unstable towards oxidation (analogy with non-substituted 5,6-diaminouracil[158] and therefore was not isolated and the 1,10-phenanthroline-5,6-dione **113** was added to the reaction mixture after no starting material **124** was observed (one pot reaction). The conversion was monitored by TLC.

The reduction of 5-nitroso-6-(propylaminouracil) **123a** by sodium disulfite in water[159] did not lead to the desired product **114a**.

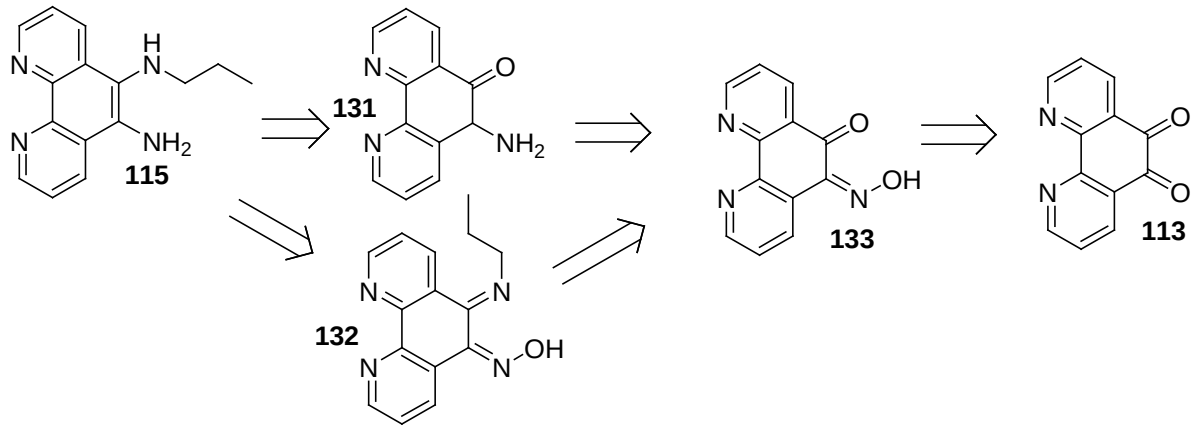
The condensation of **113** and **114** (Scheme 34) was accomplished parallel to the lumazine synthesis of Eugster and coworkers[146]. In case of propyl derivative **114a** the product precipitated from the solution and it was found to be insoluble, which prevents any further analysis. Therefore it was complexed with *cis*-dichlorobis-(DMSO)platinum(II) prepared by Susanne Kümmel to compound **130** to provide better solubility. The analogous reaction with the tridecan-7-yl substituted 5-nitrouracil **122a** did not lead to any detectable product. The structure of **112** and **130** was not proved and the chemical identity of the low soluble products was not determined.



Scheme 34: Condensation *via* Route A and Complexation of the Product

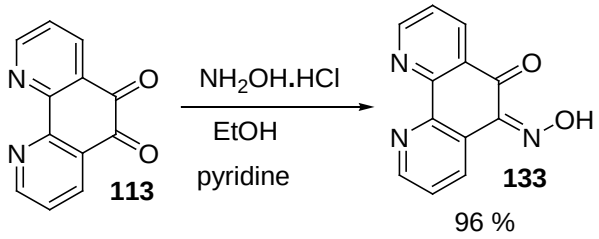
Route B

The condensation of the commercially available alloxan **116** with 5-(alkylamino)-6-amino-1,10-phenanthroline **115** should lead to the target molecule **89** in analogy with the flavin synthesis[147]. The synthesis of the mono-alkylated diamine **115** was accomplished according to the retrosynthetic Scheme 35.



Scheme 35: Retrosynthetic Analysis of 5-(Propylamino)-6-amino-1,10-phenanthroline **115**

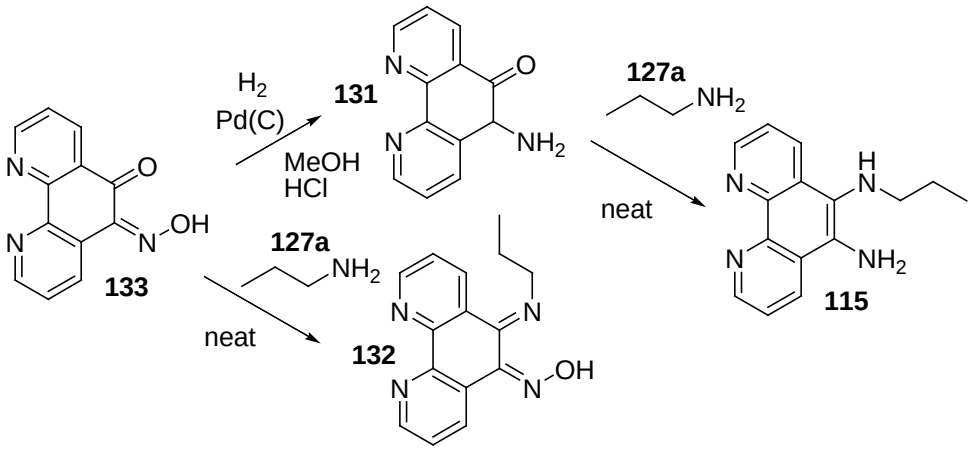
The 1,10-phenanthroline monooxime **133** has been prepared according to the procedure for analogic phenanthrene[160] in 96 % yield (Scheme 36).



Scheme 36: Preparation of Phenanthroline Monooxime **133**

The reaction is sensitive to the amount of added hydroxylamine hydrochloride. The excess of hydroxylamine leads to the creation of dioxime. The crude reaction product can be carefully recrystallized from ethanol to remove the dioxime and non-reacted starting material.

The synthesis of the mono-alkylated 1,10-phenanthroline-5,6-diamine **115** is depicted in the Scheme 37.



Scheme 37: Synthesis of the N^5-Propyl-1,10-phenanthroline-5,6-diamine **115**

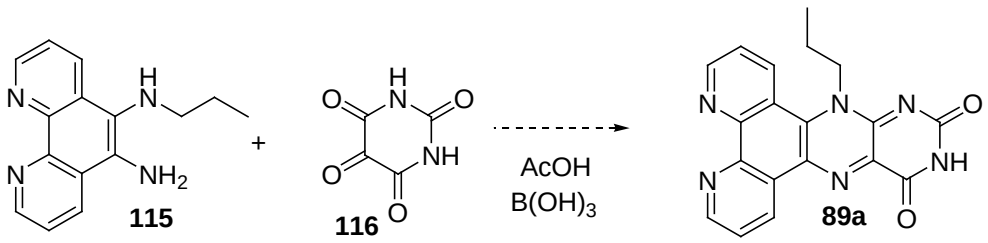
The monooxime **133** was reduced in hydrogen atmosphere (15 bars) with 5 % palladium on charcoal heterogenous catalyst. The product was prepared in 67 % yield. The hydrochloric acid was added to dehydrate the hydroxylamine created by reduction of oxime and to protonate the created amino group to prevent **131** from self-condensation. The compound shows interesting absorption behavior with the change of pH. The solid hydrochloride is red, so is its acidic aqueous solution (λ_{max} = 390 nm). After addition of a base to the solution the colour changes to intense green (two maxima at 405 nm and 675 nm).

The spectrum of basic aqueous solution is not stable and in order of a few minutes it bleaches to no significant absorption in the visible region.

The 1,10-phenanthroline-5,6-diamine **115** has been synthetized in a quantitative yield (assumed by TLC) by a condensation reaction with propylamine **127a** as a solvent under nitrogen atmosphere. It is assumed that **115** is unstable towards oxidation[160] and therefore it was used directly after a careful evaporation of the non-reacted propylamine for condensation with alloxane **116**.

Another pathway of preparing **115** has been suggested and investigated. The almost quantitative (yield 97 %) condensation of monooxime **133** with propylamine **127a** as a solvent results in 1,10-phenanthroline-5-(propylimine)-6-oxime **132**. The subsequent reduction of the oxime in presence of imine needs to be chemoselective and the solvolysis of the imine must be avoided. This reduction procedure has not been developed yet.

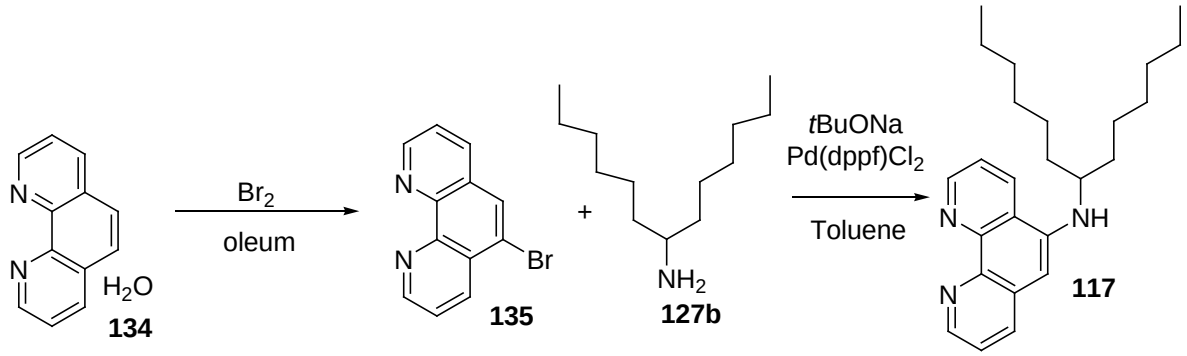
The condensation of **115** and **116** (Scheme 38) was accomplished according to the Gaertner and coworkers[147] in acetic acid with 10 equivalents of boronic acid. The reaction gave a deep red product, which cannot be extracted to any organic solvent from the water phase. The structure of the product was not determined.



Scheme 38: Condensation *via* Route B

Route C

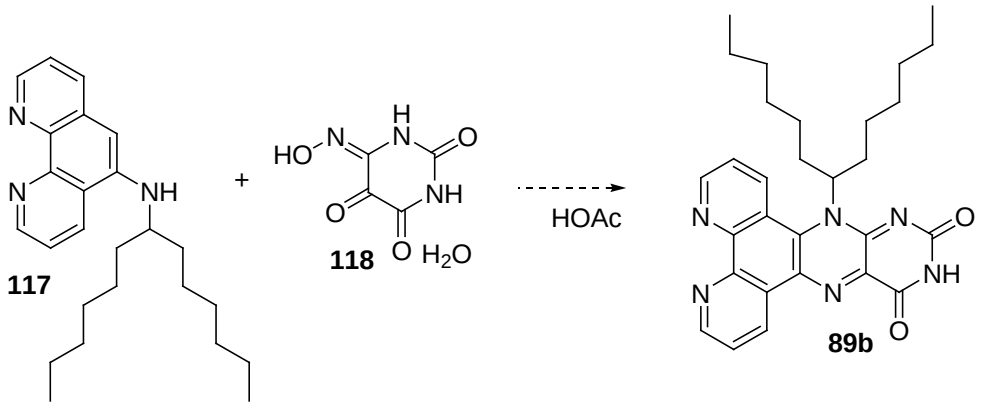
The condensation of commercially available violuric acid **118** with 5-(tridecan-7-ylamino)-1,10-phenanthroline **117** was accomplished according to the flavin synthesis.[148] The phenanthroline amine **117** has been synthetized in two steps shown in the Scheme 39.



Scheme 39: Synthesis of the 5-(Tridecan-7-ylamino)-1,10-phenanthroline **117**

The bromination of 1,10-phenanthroline hydrate **134** was initially tested with bromine in dichloromethane according to Näther and coworkers,[161] but only the starting material was isolated back. The bromination was accomplished by heating **134** to 135 °C for 23 hours in 20 % oleum with bromine according to Eisenberg and coworkers[162]. The yield of the reaction and the purity of the product (dibromide and **113** are formed as byproducts) are very sensitive to the reaction temperature, the amount of bromine added, the reaction time and the rate of heating of the reaction mixture to the maximum temperature. The conditions were optimized together with recrystallization to the final yield of 84 %. The product **135** exists in two polymorphic modifications, one is created in chloroform and is soluble in diethylether and the other, insoluble in ether, is formed from the first one in a few minutes when standing in the ether solution. The product **135** does not crystallize when contaminated by toluene.

The subsequent Buchwald coupling of **135** with **117b** was accomplished in degased toluene with $Pd(dppf)Cl_2$ catalyst and sodium *tert*-butoxide as a base. The reaction mixture after workup (crude yield 79 %) was used for the condensation with violuric acid **118** without purification. The condensation reaction (Scheme 40) according to Matsui and coworkers[148] did not lead to any product. Starting material was isolated.



Scheme 40: Condensation *via* Route C

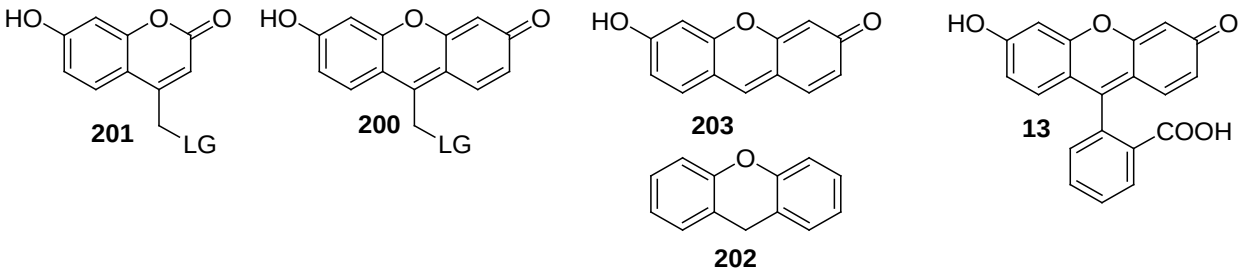
Route D

The condensation of 6-(alkylamino)uracil **124** with 5-nitroso-1,10-phenanthroline **119** was not investigated because **119** or any similar structure has not been described in literature.

(6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl: A Photoremovable Protecting Group

Introduction

The main goal of this project was to synthetize and investigate the usage of (6-hydroxy-3-oxo-*3H*-xanthen-9-yl)methyl moiety **200** attached to a proper leaving group (LG) as a photoremovable protecting group. This work concerns with the synthesis and photobehavior of the diethyl phosphate derivative of **90** (LG = $OP(O)(OEt)_2$). The structure is based on 7-hydroxycoumarin PPG **201**; the extension of conjugation by one additional aromatic ring promised a large red shift of the absorption maximum. It resembles xanthene dyes like fluorescein **13** derived from xanthene molecule **202** and is a derivative of 3-hydroxy-6-fluorone **203** (Scheme 41).

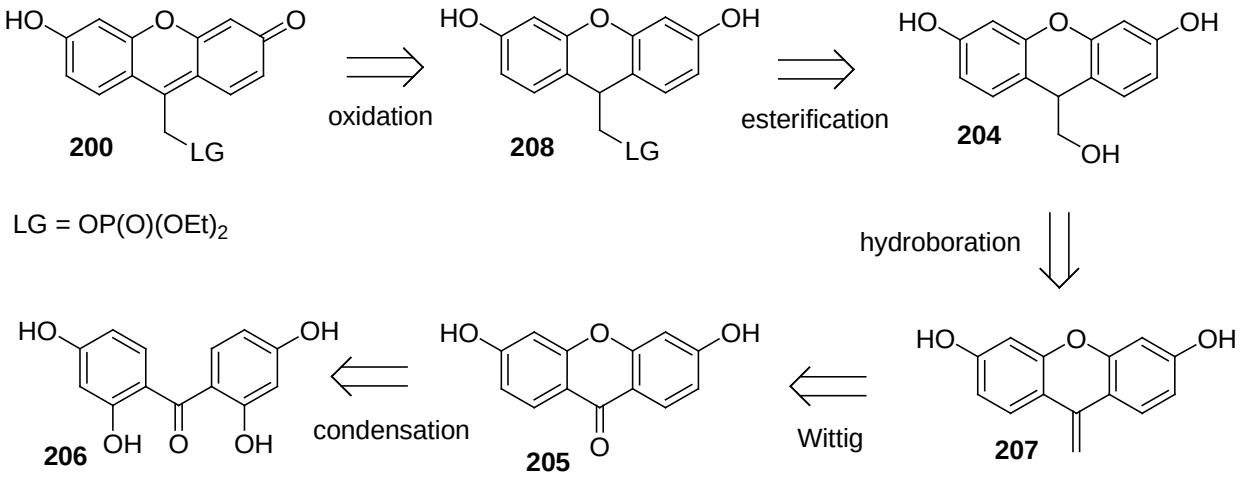


Scheme 41: Structures of 7-Hydroxycoumarin and Xanthene Derivatives

Synthesis of the (6-Hydroxy-3-oxo-3H-xanthen-9-yl)methyl Derivative

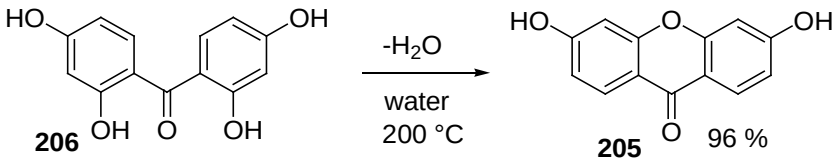
After a few preliminary experiments and a literature search, the retrosynthetic scheme (Scheme 42) for preparation of (6-hydroxy-3-oxo-*3H*-xanthen-9-yl)methyl LG **200** was proposed. The derivatization of the trihydroxy compound **204** might not have been fully chemoselective (despite the 5 orders of magnitude difference of p*K*a of phenolic and aliphatic alcohol) and therefore the phenolic hydroxyl groups needed to be protected before introduction of the third hydroxyl. The Wittig reaction cannot be accomplished in the system containing acidic protons which destroy the non-stabilized ylide. The possible deprotonation of phenols before addition to the ylide solution deactivates the carbonyl group of the compound **205** and the protection of phenolic hydroxyls was proposed prior to the Wittig reaction. The protection of phenols is slightly more complicated in comparison to aliphatic alcohols because several protection groups used commonly for aliphatic alcohols (*e. g.*, TMS) are easily cleaved when attached to phenols.

Therefore, the methoxy group, the most rigid protective group for phenols, was used for protection.



Scheme 42: Retrosynthesis of **200**

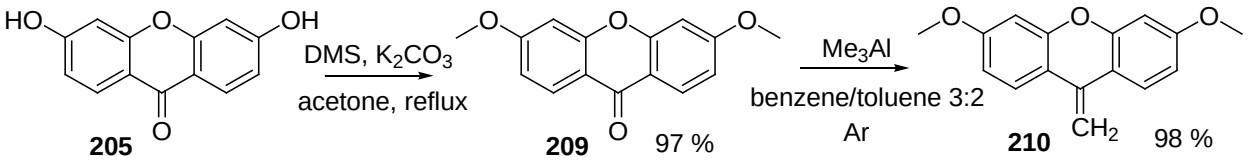
The 3,6-dihydroxy-9*H*-xanthen-9-one **205** was prepared from 2,2',4,4'-tetrahydroxybenzophenone **206** by cyclizing condensation in water in an autoclave at 200 °C according to a known procedure[163] (Scheme 43). The loading of the 100 mL autoclave PTFE cylindrical vessel was optimized to shorten the reaction time with maximum yield of the pure product **205**. The best ratio between 2,2',4,4'-tetrahydroxybenzophenone **206** and water was found to be 1:6 with the reaction time of 6 hours and temperature not exceeding 220 °C. The reaction needs no catalysis and has almost quantitative conversion. The cyclization proceeds probably by an addition-elimination mechanism (the carbonyl group in the right orientation supports the suggestion) and the product **205** is not water soluble which shifts the equilibrium of the reaction.



Scheme 43: Synthesis of 3,6-Dihydroxy-9*H*-xanthen-9-one **205**

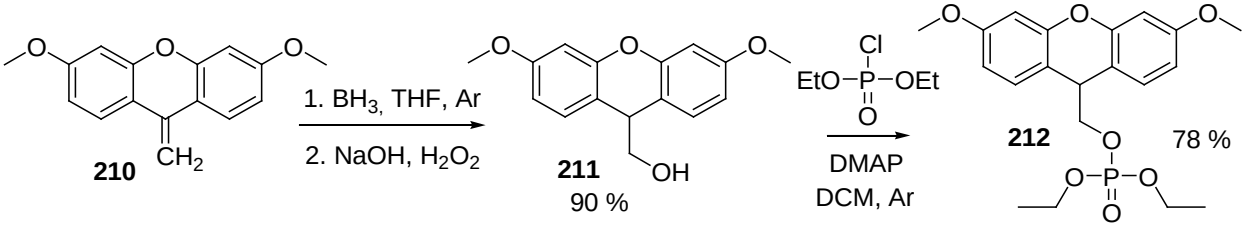
The 3,6-dimethoxy-9-methylene-9*H*-xanthene **210** was synthetized from 3,6-dihydroxy-9*H*-xanthen-9-one **205** in two steps and overall yield 95 % (Scheme 44). The protection of phenolic hydroxyls was accomplished by a known procedure[164] using 18 eq. of dimethyl sulfate (DMS) as a methylating agent and potassium carbonate as a base. The subsequent modification of the carbonyl group to an exocyclic double bond can be done by Wittig procedure with triphenyl

phosphine, methyl iodide and BuLi in THF, but a reaction with trimethyl aluminum[165] in a non-polar aromatic solvent worked with a higher yield and without a necessity of purification. The mixture benzene/toluene 3:2 (v/v) was used instead of pure benzene because of better solubility of the starting material **209**. Analogous to the phosphorus atom in triphenyl phosphine, the aluminum atom is also oxophilic and creates an Al-O bond with the carbonyl oxygen. After the cleavage of the intermediate, methane is generated and exocyclic double bond is created. The reaction is sensitive to air and moisture and also the yellow product **210** is easily being oxidized to an unidentified green impurity with a very high molar absorption coefficient. The 3,6-dimethoxy-9-methylene-9*H*-xanthene **210** can be purified easily by recrystallization with *n*-hexane.



Scheme 44: Synthesis of 3,6-Dimethoxy-9-methylene-9*H*-xanthene **210**

The (3,6-dimethoxy-9*H*-xanthen-9-yl)methyl diethyl phosphate **212** was synthetized by a two-step procedure from 3,6-dimethoxy-9-methylene-9*H*-xanthene **210** starting with hydroboration-oxidation, the well-known anti-Markovnikov procedure for hydration of a double bond results to hydroxyl attached to the less substituted carbon (**211**, Scheme 45). The reaction did not lead to any byproducts and no purification was needed. Attaching of the diethyl phosphate group which was used as a model leaving group for biological active phosphates (*e. g.* ATP, cAMP) was optimized. DMAP (4-(dimethylamino)pyridine) was used as a nucleophilic organocatalyst and a base reacting with generated HCl. The use of DMAP is more convenient in comparison with aliphatic tertiary organic amines (*e. g.*, Et_3N) because it enhances the reaction rate[166] by reaction with diethyl chlorophosphate creating 1-(diethoxyphosphoryl)-4-(dimethylamino)pyridinium, a highly reactive intermediate.



Scheme 45: Synthesis of (3,6-Dimethoxy-9*H*-xanthen-9-yl)methyl Diethyl Phosphate **212**

The deprotection of the methoxy groups (Scheme 46) was accomplished according to a widely used procedure[167] using boron tribromide (13 eq.) in dichloromethane. Use of lower amount of BBr_3 led to a mixture of mono- and double-deprotected xanthene derivative. The reaction temperature had to be –78 °C and was allowed to slowly rise to –10 °C within 24 h (temperature was controlled by a cryostat). At higher reaction temperatures the conversion was not completed and product of cleavage of phosphate was observed. The optimized procedure led to a quantitative yield of (3,6-dihydroxy-9*H*-xanthen-9-yl)methyl diethyl phosphate **213**.

The oxidation of 3,6-dihydroxyxanthene moiety to 3-hydroxy-6-fluorone was accomplished by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in acetonitrile[108] at 20 °C. The reaction proceeds very rapidly and the product precipitates from the solution as an orange-red powder. However, instead of the anticipated pure (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl derivative **90**, the precipitation appeared to be an equimolar (1:1) complex with DDQ (**90**•DDQ) created in 50 % yield (calculated with respect to the molecular weight of the complex; a 1.25-molar excess of DDQ was used for the reaction).

The complex exists in two tautomeric forms, the keto form shown in the Scheme 46 and the enol form with exocyclic double bond and symmetric aromatic core. The enol form predominates in the acidic solutions and DMSO and absorbs poorly in the visible region at around 400 nm. The keto form is a much better chromophore and has a λ_{max} at 528 nm with ε = 39100 M^{-1} cm^{-1}. It exists in aqueous conditions at neutral and basic pH and other polar protic solvents like MeOH. The UV-vis absorption spectrum of **90**•DDQ in aq. buffer (pH = 7) is shown in the Figure 16.

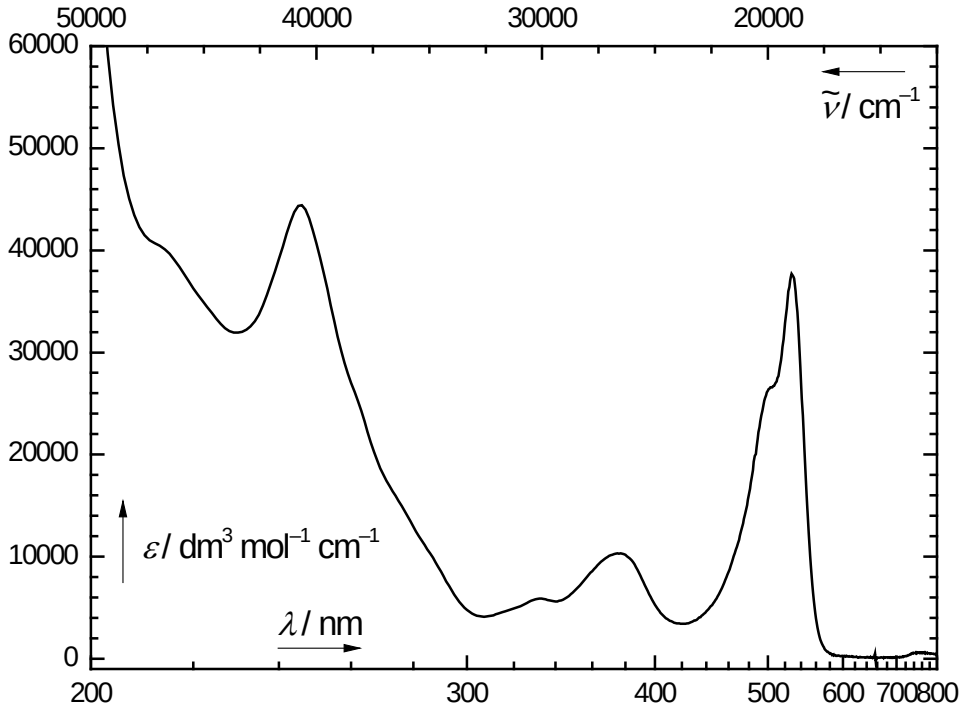
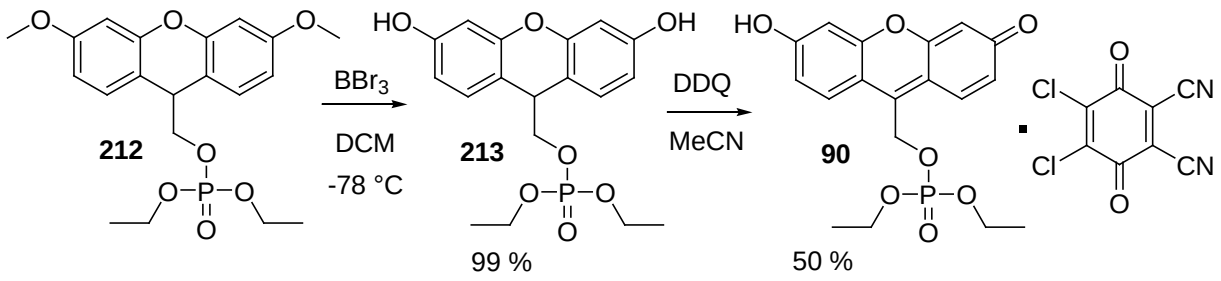


Figure 16: UV-vis (aq Phosphate Buffer, pH = 7.0, *I* = 0.1 M): Diethyl (6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl Phosphate•DDQ Complex (**90**•DDQ)

We attempted to find out the structure and behavior of the complex and to deliberate **90**. We studied the complexation of DDQ and DDQ-H_2 with 3-hydroxy-6-fluorone derivatives. Due to their scope the results of these experiments will not be discussed in this thesis and will be published soon.

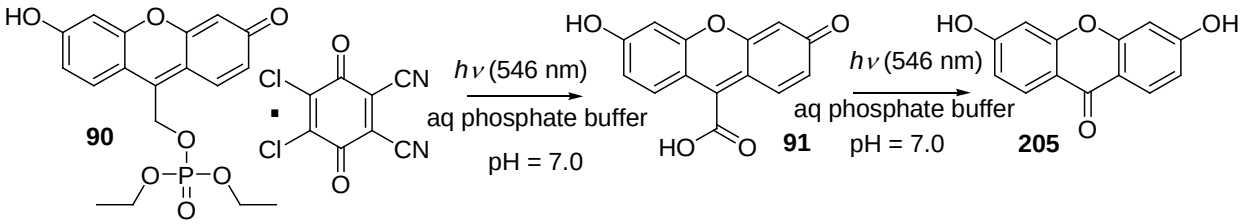
The complex **90**•DDQ is relatively well soluble (up to ~10 mM) in aqueous buffer solutions (pH ~ 7–8; ionic strength *I* ~ 0.1 M) or water. DDQ is known to decompose rapidly in water.[168] It reacts within seconds forming 2,3-dichloro-5-cyano-6-hydroxy-1,4-benzoquinone and HCN which does not associate with **90**. We conclude that compound **90** is no longer associated with DDQ when the solid complex **90**•DDQ is dissolved in aqueous solvents. Slow decomposition of **90** occurred in phosphate buffer (pH 7, *I* = 0.1 M) in the dark at room temperature (7 days half-life in 30 mM solution).



Scheme 45: Synthesis of Diethyl (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl Phosphate•DDQ Complex (**90**•DDQ)

Photoreactivity

The diethyl (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl phosphate•DDQ complex (**90**•DDQ) was irradiated to investigate its photochemistry. The green light isolated from a high-pressure mercury arc (λ = 546 nm) was used. The course of the reaction was monitored by UV-vis spectroscopy. The absorption maximum of **90**•DDQ at λ ~ 528 nm was replaced by a new maximum at λ ~ 500 nm during irradiation indicating the formation of a new product. The irradiation in methanol led to another photoproduct, 3,6-dihydroxy-9*H*-xanthen-9-one **205** (Scheme 47). In aqueous phosphate buffer (pH = 7.0) the primary photoproduct the 6-hydroxy-3-oxo-3*H*-xanthene-9-carboxylic acid **91**, was observed. The exhaustive irradiation resulted in 3,6-dihydroxy-9*H*-xanthen-9-one **205** as well.



Scheme 47: Irradiation of Diethyl (6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl Phosphate•DDQ Complex (**90**•DDQ)

The change of the oxidation state from alcohol to carboxylic acid could be explained by the presence of DDQ which is a strong oxidant.

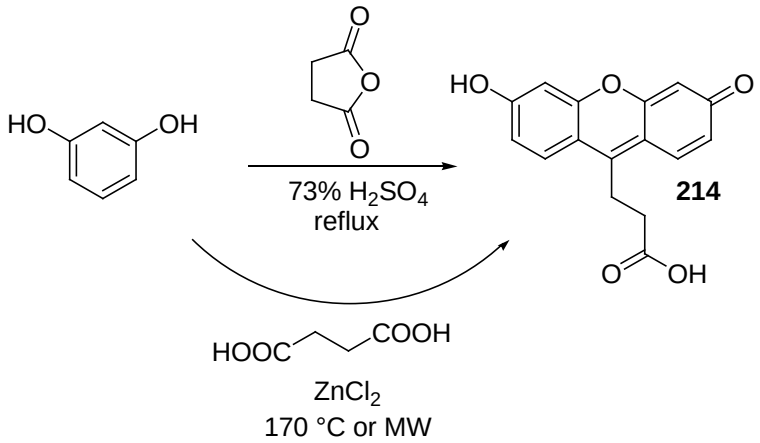
The interesting photobehavior of the (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl phosphate•DDQ complex (**90**•DDQ) was ascribed to the presence of DDQ. In order to prepare the free **90** the synthesis procedure needed to be revised.

Direct Condensation

In analogy to the synthesis of fluorescein[89] **13** the direct condensation method of the preparation of a model compound succinylfluorescein **214** (Scheme 48) was examined. In the classical procedure[110] resorcinol and succinic acid anhydride in 73 % aq. sulfuric acid were used. After reflux at 140 °C for 6 hours, cooling down and adjusting pH to 5 the product of the condensation precipitates from the solution in a high yield (80 %). The method is unfortunately limited by usage of anhydrides and harsh reaction conditions.

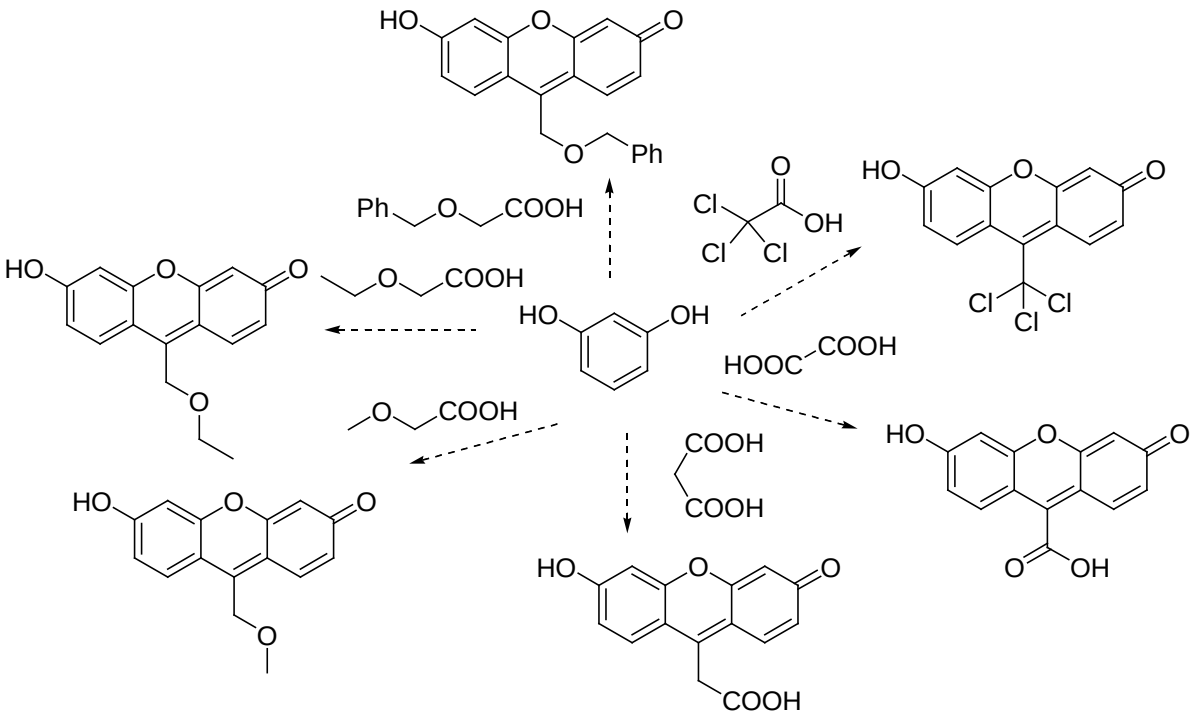
Another method according to Venkateswarlu and coworkers[90] using succinic acid and zinc chloride as a catalyst (0.09 eq.) with resorcinol as the solvent (m. p. 110 °C) was also accomplished and after 4 hours at 170 °C the succinylfluorescein **214** was isolated in a moderate yield (46 %).

To shorten the reaction time the solvent-free variant was performed in a microwave reactor. The solid mixture was heated to 130 °C for 58 minutes to give the product in 20 % yield. The prolonged heating caused generation of by-products and the conversion was held at approx. 50%. The heating of the mixture (maximal power, temperature gradient) needed to be optimized very carefully because at 110 °C the resorcinol melted and the absorption of the MW radiation rose significantly which caused overheating of the whole sample to 200 °C.



Scheme 48: Syntheses of the Succinylfluorescein **214**

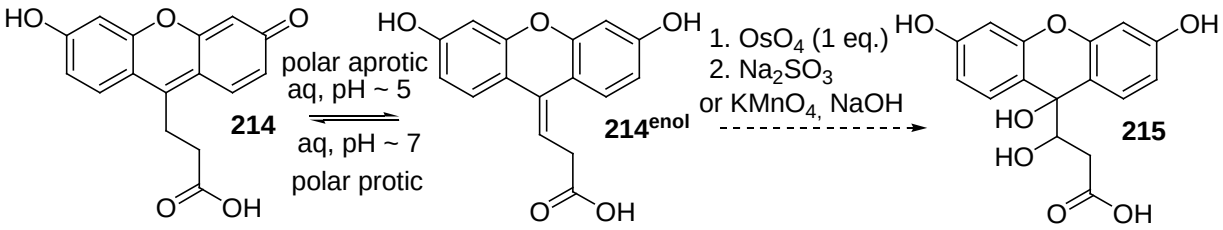
In analogy to the Venkateswarlu synthesis of succinylfluorescein we tried to use other carboxylic acids to synthetize analogous 3-hydroxy-6-fluorone derivatives. All attempts are shown in the Scheme 49.



Scheme 49: Attempts of Synthesis of 3-Hydroxy-6-fluorone Derivatives by Direct Condensation

All the attempts shown above (Scheme 49) were accomplished by both the thermal procedure by Venkateswarlu and in the MW reactor. The conditions were similar as for succinylfluorescein (2 eq. of resorcinol, 1 eq. of acid, 0.09 eq. of anhydrous zinc chloride). All of the reactions resulted in a complicated mixtures of products (more than 10 products observed on TLC for each mixture) with different colours and fluorescence. Unfortunately none of the products could be isolated (unlike **214** precipitating by pH adjustment no precipitation was observed). Some of the acids decomposed or decarboxylated by heating creating black tar.

Succinylfluorescein **214** exists in two tautomeric forms (Scheme 50) similar to the diethyl phosphate derivative **90**. The keto form exists in water above pH ~ 6 and in polar protic solvents and the enol form **214**$^{\mathbf{enol}}$ exists in water below pH ~ 6 and in polar aprotic solvents. Two dihydroxylation reactions of **214** were tested: potassium permanganate with sodium hydroxide in cold THF (0 °C), and osmium tetroxide in water at pH = 5. No product was observed in either case and the modification of the reaction conditions was limited by poor solubility of **214** in common organic solvents.

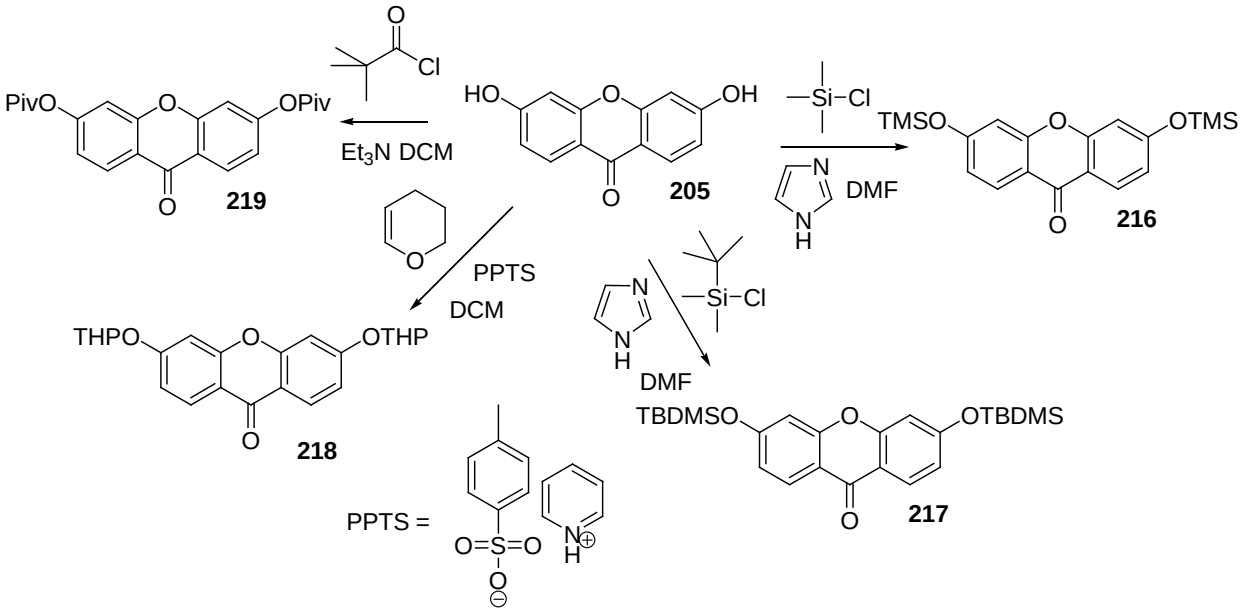


Scheme 50: Tautomeric Equilibrium of Succinylfluorescein and Attempts of its Dihydroxylation

Protection of the 3,6-Dihydroxy-9H-xanthen-9-one **205**

Because the derivatization of the exocyclic double bond did not lead to the expected products, we decided to rethink the whole synthetic strategy starting from the 3,6-dihydroxy-9*H*-xanthen-9-one **205**. The phenolic hydroxyls of the molecule were protected by different protecting groups. All protection procedures (except the methylation leading to 3,6-dimethoxy-9*H*-xanthene-9-one **209** mentioned before) are shown in Scheme 51. The protection reactions were accomplished according to classical procedures.[1,2]

The stability of the TMS protected 3,6-dihydroxy-9*H*-xanthen-9-one **216** is very low in both acidic and basic conditions and therefore a more persistent silyl protecting group (*tert*-butyldimethylsilyl TBDMS) was used. The silyl groups are electron donors and they deactivate the carbonyl group towards nucleophilic addition. Therefore, the decrease the electron density of the whole system by attaching of electron withdrawing protecting group was desirable and tetrahydropyranyl (THP) and pivaloyl (Piv) groups were suggested to be most suitable.



Scheme 51: Protection of the 3,6-Dihydroxy-9*H*-xanthen-9-one **205**

Experimental part

Instrumentation

All solvents and chemicals were purchased from commercial suppliers and used as received. Ethanol for reduction of oxime **129** was dried by a freshly flame-dried 3 Å molecular sieves (20% v/v) for 72 hours. Acetone, acetonitrile, and dichloromethane were dried by standard procedures, kept over high-temperature dried 3 Å molecular sieve (8–12 mesh) under dry N_2; they were freshly distilled for each experiment. Tetrahydrofuran was dried by sodium pellets with addition of acetophenone for monitoring the water content. Synthetic procedures were performed under ambient atmosphere unless stated otherwise. All glassware was flame-dried prior to use when water- and/or air-sensitive reagents were used. Oxygen was removed from solutions by three freeze-pump-thaw cycles or bubbling with inert gas (N_2 or Ar) for at least 15 minutes.

Reactions initiated by microwave irradiation have been accomplished in the microwave reactor PROLABO 402 Synthewave at working frequency 2450 MHz.

TLC was performed on silica gel 60 F254 aluminum sheets (Merck), with detection under 254 nm or 333 nm UV light. Flash column chromatography was carried out on silica gel (0.035–0.070 mm), obtained from Acros.

NMR spectra were recorded on 300, 400, 500, or 600 MHz spectrometers in acetonitrile-d_3, chloroform-d, dichloromethane-d_2, dimethylsulfoxide-d_6, methanol-d_4, trifluoroacetic acid-d, water-d_2, or their respective mixtures. The measurements were performed in 5-mm diameter Pyrex cuvettes at 30 °C. The signals in ^{1}H and ^{13}C NMR were referenced to the residual peak of a (major) solvent except for H_2O, while those of the ^{31}P NMR were unreferenced. The deuterated solvents (except for CF_3COOD and D_2O) were kept over high-temperature-dried 3 Å molecular sieve (8–12 mesh) under dry N_2. The signals have been assigned to atoms of the structure and the numbering of the molecules shown is according to computational algorithm used in common chemical software (ChemOffice, MestReNova) and not according to IUPAC.

Mass spectra were recorded on a GC-coupled (30 m DB-XLB column) spectrometer Hawlett-Packard 5890/5971 or a Finnigan TSQ 710 spectrometer in a positive mode with EI or FAB. Electrospray ionization (ESI) mass spectra were recorded with a ThermoQuest Finnigan MAT

9595 spectrometer. MALDI-MS analyses were performed using an automatic spectrometer with *p*-nitroaniline as a matrix. Exact masses were performed using a triple quadrupole electrospray ionization mass spectrometer in positive or negative modes or a ThermoQuest Finnigan MAT 95 spectrometer.

UV/Vis spectra were recorded on Varian Cary 50 Bio UV-Vis spectrometer, Shimadzu UV 1601 UV-VIS spectrometer, or Agilent 8453E UV-visible Spectroscopy System in 1.000 cm quartz cuvettes.

IR spectra were obtained on an FTIR ATI Matson Genesis series spectrometer in KBr tablets (weight ratio 1:100) or on a PTFE tape.

Melting points were obtained in open-end capillary tubes using a non-calibrated digital melting point apparatus or a non-calibrated Kofler's hot stage melting point apparatus VEB Wägetechnik Rapido 79/2106.

Elemental analyses were performed on an automatic analyzer.

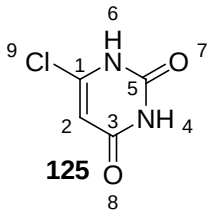
The solution pH values were determined using a WTW pH 320 pH meter with a glass electrode calibrated with certified buffer solutions at pH = 4, 7 or 10.

A 40-W medium pressure mercury arc Heraeus Noblelight ST-41 equipped with the corresponding band-pass or cut-off filters was used for irradiation.

Instrumentation used in Photoremovable Chiral Auxiliary project is mentioned in the Supporting information of the publication.[80]

Synthesis of compounds

6-Chlorouracil[147] **125**

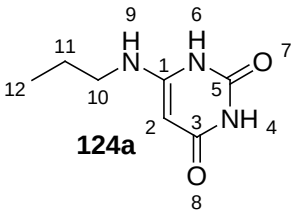


Solid NaOH pellets (8.80 g, 220 mmol, 4 eq.) were dissolved in 90 mL of distilled water. 2,4,6-Trichloropyrimidine **126** (6.3 mL, 10 g, 54.52 mmol, 1 eq.) was added dropwise in 5 minutes, while the emulsion was created. The mixture was heated on oil bath and refluxed for 4 hours. White precipitation was generated slowly in the mixture and after 2 hours it dissolved. After cooling down to 20 °C the white precipitation was observed in the whole volume. The pH of the mixture was adjusted to 3 – 4 with 37 % HCl (9 mL). The white precipitate was filtered off and washed with warm water (2 × 20 mL) and acetone (2 × 10 mL) and it was dried under vacuum. **125** was obtained (5.96 g, 75 %) as white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 12.11 (bs, 1H, N*H*), 11.13 (s, 1H, N*H*), 3.86 (s, 1H, =C*H*–)

^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) = 162.86 (C-3), 150.94 (C-5), 146.17 (C-1), 99.07 (=*C*H–).

6-(Propylamino)uracil **124a**



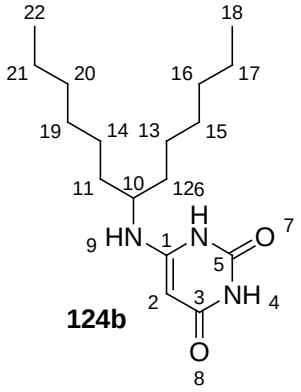
6-Chlorouracil **125** (2.93 g, 20.0 mmol, 1 eq.) was mixed with 10 mL (121.8 mmol, 6 eq.) of propylamine **127a** under nitrogen atmosphere. The reaction mixture was heated to 60 °C on oil bath and cooled by cooling finger. The color changed to yellowish after 20 min and the white flakes were observed in the reaction mixture. MeOH (20 mL) was added and the precipitate of impurity was filtered out.
The filtrate was then evaporated to dryness and mixed with 10 mL of $CHCl_3$. The formed crystals were filtered off and dried under vacuum to give 3.205 g (94 %) of **124a**; beige solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 8.17 (bs, 3H, N*H*), 5.07 (s, 1H, =C*H*-), 2.76 (t, J = 7.5 Hz, 2H, H_2C-10), 1.53 (tq, J_1 = 7.5 Hz, J_2 = 7.5 Hz, 2H, H_2C-11), 0.89 (t, J = 7.5 Hz, 3H, H_3C-12)

^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) = 165.54 (C-3), 160.31 (C-5), 158.01 (C-1), 93.23 (=*C*H-), 40.38 (C-10), 20.41 (C-11), 10.74 (C-12)

MS (ESI$^+$, m/z, %): $[M+H]^+$ 170.1 (100), $[2M+H]^+$ 339.2 (60)

6-(Tridecan-7-ylamino)uracil **124b**



124b

To a 10 mL Schlenk flask was given tridecan-7-amine **127b** (272 mg, 1.36 mmol, 2 eq.). The content of the flask was heated to 50 °C and the amine melted. 6-chlorouracil **125 (**100 mg, 0.68 mmol, 1 eq.) was added and the reaction mixture was heated to 140 °C. The reaction progress was monitored by TLC (chloroform : MeOH – 10:1, (v/v); **125**: R_f = 0,1; **124b**: R_f = 0,2). After 4 hours the slightly orange red viscous solution was observed and the conversion was almost complete, and the reaction mixture was allowed to cool down to 20 °C. The reaction mixture solidified, MeOH (3 mL) was added and the suspension was vigorously stirred for 15 minutes. The white solid precipitation of impurity appeared in the solution. It was filtered and the mother liquor was solidified by evaporation of solvent. The crude reaction mixture contained amine **127b** and therefore it was purified by column flash chromatography on silica gel (dichloromethane : MeOH – 10:1, (v/v)) to get **124b** (130 mg, 62 %) as ivory solid.

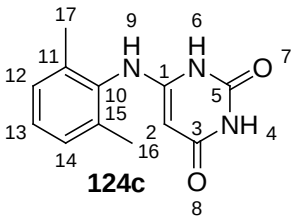
^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.15 (bs, 1H, *H*N-6), 9.62 (bs, 1H, *H*N-4), 5.91 (s, 1H, *H*N-9), 4.40 (s, 1H, =C*H*-), 3.34 (s, 1H, *H*C-10), 1.42 (m, 4H, H_2C-11, H_2C-12), 1.24 (m, 16H, H_2C-13, 14, 15, 16, 17, 18, 20, 21), 0.85 (t, J = 6.3 Hz, 6H, H_3C-18, H_3C-22).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) = 164.27 (C-3), 153.81 (C-5), 150.76 (C-1), 72.29 (C-1), 51.29 (C-10), 34.15 (C-11, 12), 31.22 (C-13, 15), 28.65 (C-14, 16), 25.11 (C-18, 20), 22.04 (C-18, 21), 13.93 (C-19, 22)

MS (ESI$^+$, m/z, %): $[M+H]^+$ 310.1 (60), $[MH+MeCN]^+$ 351.2 (100), $[2M+H]^+$ 619.5 (80)

HRMS (ESI$^+$): calcd. for $C_{17}H_{31}N_3O_2$ $[MH]^+$ 310.2489, found: 310.2497

6-(2,6-Dimethylphenylamino)uracil **124c**

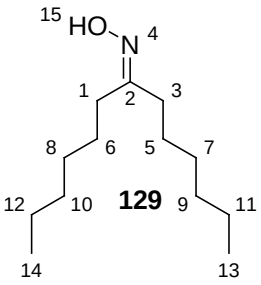


124c

A mixture of chlorouracil **125** (1.00 g, 6.82 mmol, 1 eq.) and 2,6-dimethylaniline **127c** (2,5 mL, 20.47 mmol, 3 eq.) was heated under nitrogen atmosphere to 180 °C for 40 minutes. After cooling down to 20 °C MeOH (3 mL) was added and the reaction mixture was stirred for more 15 minutes. A white precipitation was observed. It was filtered, washed with diethylether (2 × 2 mL) and methanol (2 mL) and dried under vacuum to yield **124c** (1.22 g, 77 %) as white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 10.37 (s, 1H, *H*N-4), 10.31 (s, 1H, *H*N-6), 7.82 (s, 1H, *H*N-9), 7.16 (m, 3H, *H*C-12, 13, 14), 3.69 (s, 1H, =C*H*-), 2.17 (s, 6H, H_3C-16, 17)

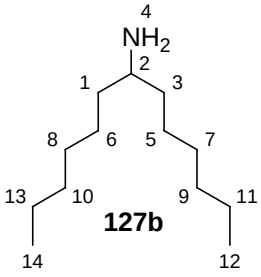
Dihexyloxime[148] **129**



A suspension of 7-tridecanone **128** (2.50 g, 12.6 mmol, 1 eq.), hydroxylamine hydrochloride (1.50 g, 21.5 mmol, 1.7 eq.) and technical KOH (2.41 g, 43 mmol, 3.4 eq.) in methanol (60 mL) was stirred at 60 °C for 24 h. After stirring overnight no starting material **128** was observable (TLC; diethyl ether : petroleum ether – 1:10, v/v). After 24 hours the heating was stopped and the suspension was filtered. After filtration the filtrate was concentrated to 25 mL under reduced pressure and acidified with 1 N HCl (50 mL) to pH ~ 2. The resulting solution was added to ether (water phase extracted to ether once) and washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated *in vacuo* to yield **129** (2.63 g, 98 %) as pale yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 2.33 (t, J = 8.0 Hz, 2H), 2.17 (t, J = 8.0 Hz, 2H, H_2C-3), 1.50 (m, 4H, H_2C-5, 6), 1.30 (m, 12H, H_2C-7, 8, 9, 10, 11, 12), 0.89 (t, J = 6.0 Hz, 6H, H_3C-13, 14).

Tridecan-7-amine[152] **127b**

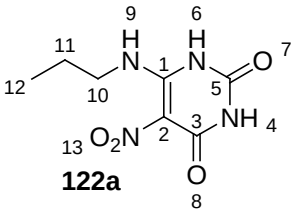


Dihexyloxime **129** (2.560 g, 12.0 mmol, 1 eq.) of was dissolved in absolute EtOH (25 mL). The solution was heated up to 60 °C. Sodium pellets (4.5 g, 195 mmol, 16 eq.) were added carefully (EtOH is evaporating) and the suspension was heated to reflux for 30 min. The suspension got viscous and white. After the heating was stopped, MeOH (35 mL) was added in

order to dissolve the remaining sodium. The solution was concentrated under reduced pressure. The residue was added to diethyl ether, brine was added and inorganic salts precipitated in the water phase. Water phase was extracted with Et_2O and combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. **127b** (2.248 g, 94 %) was obtained as colorless waxy solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 2.66 (s, 1H, *H*C-2), 1.46 (m, 4H, H_2C-1, 3), 1.35 (m, 4H, H_2C-5, 6), 1.26 (m, 12H, H_2C-7, 8, 9, 10, 11, 13), 0.86 (t, J = 6.7 Hz, 6H, H_3C-12, 14).

5-Nitro-6-(propylamino)uracil **122a**



122a

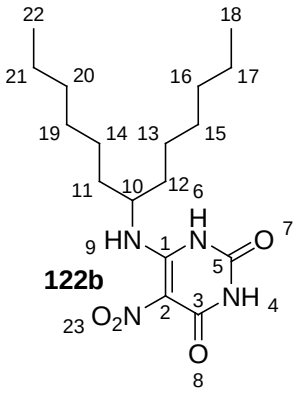
6-(Propylamino)uracil **124a** (0.508 g, 3.00 mmol, 1.0 eq.) was mixed with concentrated sulfuric acid (1.4 mL, 85.2 mmol, 28 eq.). The solution was cooled down to 0°C with ice/water cooling bath and fuming nitric acid (0.7 mL, 16.8 mmol, 5.6 eq.) was added. After 15 minutes of stirring at 0 °C the color of the solution changed to yellowish. Then it was allowed to warm to room temperature and the color changed to intense yellow. After 15 min it was poured on ice (20 g). After basification with NaOH to pH = 7 the solution was evaporated to dryness. The solid (mixture of **122a** with sodium sulfate and nitrate) was treated four times with MeOH (20 mL), the suspension was sonicated, decanted and filtrated. The solution was solidified to yield **122a** (565 mg, 88 %) as yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 9.97 (bs, 1H, *H*N-4), 7.83 (bs, 2H, *H*N-6, 9), 2.73 (t, J = 7.4 Hz, 2H, H_2C-10), 1.55 (tq, J_1 = 7.4 Hz, J_2 = 7.4 Hz, 2H, H_2C-11), 0.89 (t, J = 7.4 Hz, 3H, H_3C-12).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) = 170.89 (C-3), 159.39 (C-1), 149.68 (C-5), 40.37 (C-10), 20.29 (C-11), 10.74 (C-12).

MS (ESI$^+$, m/z, %): $[M+H]^+$ 215.0 (100), $[M+NH_4^+]$ 232.0 (7), $[MH+MeCN]^+$ 256.1 (20)

5-Nitro-6-(tridecan-7-ylamino)uracil **122b**

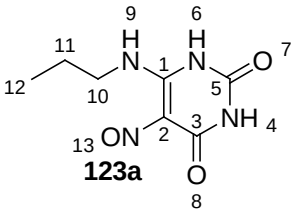


6-(Tridecan-7-ylamino)uracil **124b** (0.187 g, 0.60 mmol, 1 eq.) was mixed with concentrated sulfuric acid (1.4 mL, 85.2 mmol, 47 eq.). The solution was cooled down to 0 °C with ice/water cooling bath and fuming nitric acid (0.7 mL, 16.8 mmol, 27 eq.) was added. The colour changed to yellow and the emulsion was created. After 45 min of stirring at 0 °C the reaction mixture was poured on ice (20 g). After basification by NaOH (4.1 g) to pH = 7 the colour changed to orange red. The reaction mixture was solidified and the solid was extracted by MeOH. It was not possible to isolate the product from its mixture with salts and therefore the yield was not determined. **122b** was isolated in mixture with Na_2SO_4 and $NaNO_3$ (150 mg) as yellow solid after extraction.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 9.76 (bs, 1H, *H*N-6), 9.32 (bs, 1H, *H*N-4), 8.43 (s, 1H, *H*N-9), 3.69 (s, 1H, *H*C-10), 1.47 (m, 4H, H_2C-11, H_2C-12), 1.22 (m, 16H, H_2C-13, 14, 15, 16, 17, 18, 20, 21), 0.83 (t, J = 7.5 Hz, 6H, H_3C-18, H_3C-22).

MS (ESI$^+$, m/z): $[M+H]^+$ 355.1 (25), $[MH+MeCN]^+$ 396.3 (40), $[2M+H]^+$ 705.5 (5)

5-Nitroso-6-(propylamino)uracil **123a**

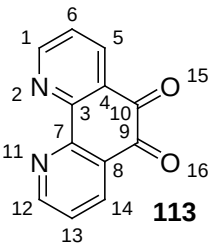


6-(Propylamino)uracil **124a** (200 mg, 0.59 mmol, 1 eq.) was mixed with sodium nitrite (160 mg, 2.32 mmol, 4 eq.) in a 7.5 mL Schlenk flask under nitrogen. Distilled water (3.6 mL) was added and the suspension was heated to 90 °C for 1 hour. The solid dissolved in 5 minutes and the solution turned yellow. After 1 hour of stirring at 90 °C the reaction was cooled down to 20 °C. To the yellow solution 100 % acetic acid (2 mL) were added and a gas evolution was observed. Immediately after addition of acid the reaction was cooled down to 0 °C. The color changed to red and after 5 minutes a brick-red precipitation appeared. The precipitation was filtered on Büchner funnel and dried over vacuum to give impure **123a** (58 mg, 25 %) as orange red solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 8.84 (s, 1H, *H*N-6), 8.13 (s, 2H, *H*N-9, 4), 2.69 (t, J = 7.6 Hz, 2H, H_2C-10), 1.58 (tq, J_1 = 7.5 Hz, J_2 = 7.6 Hz, 2H, H_2C-11), 0.89 (t, J = 7.5 Hz, 3H, H_3C-12).

MS (ESI⁻, m/z, %): $[M–H]^-$ 196.9 (40), $[M+HCOO^-]$ 242.9 (42)

1,10-Phenanthroline-5,6-dione[150] **113**



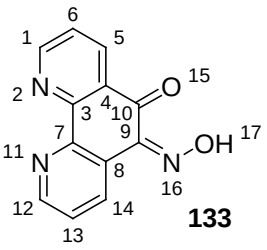
113

Phenanthroline hydrate hydrochloride **121** (5.21 g, 22.2 mmol, 1 eq.) and potassium bromide (4 g, 33.6 mmol, 1.5 eq.) of were mixed together. The ice cold mixture of concentrated sulfuric acid (40 mL) and fuming nitric acid (20 mL) was added in one portion and the mixture was heated while stirring to reflux. The heating was stopped after 3 hours and the reaction mixture was poured on ice (300 g). The color changed to green. The solution was stirred and the bromine was evolved as the ice melted. After most of the bromine was expelled, the colour changed to yellow green. The pH was adjusted with 10 % NaOH (300 mL) and then with saturated Na_2CO_3 (aq.) to pH = 5. Yellow precipitation was observed in the whole volume. The mixture was extracted with 300, 250, 200 and 150 mL of chloroform. The organic phase was dried with sodium sulfate and solvent were evaporated to give 4.85 g of orange yellow solid. The impure product **113** was recrystallized from 80 mL EtOH to give 4.078 g (88 %) of **113**; yellow needles.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 9.13 (dd, J_1 = 4.7 Hz, J_2 = 1.8 Hz, 2H, *H*C-1, 12), 8.51 (dd, J_1 = 7.9 Hz, J_2 = 1.8 Hz, 2H, *H*C-5, 14), 7.60 (dd, J_1 = 7.9 Hz, J_2 = 4.7 Hz, 2H, *H*C-6, 13)

^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm) = 178.71 (C-9, 10), 156.48 (C-1, 12), 152.95 (C-3, 7), 137.38 (C-5, 14), 128.12 (C-4, 8), 125.69 (C-6, 13).

6-(Hydroxyimino)-1,10-phenanthrolin-5-one **133**



133

Pyridine (0.408 mL, 5.07 mmol, 1.5 eq.) was added as drops to a solution of phenanthroline-5,6-dione **113** (711 mg, 3.38 mmol, 1 eq.) in ethanol (70 mL) and hydroxylamine hydrochloride (234 mg, 3.38 mmol, 1 eq.) was added in one batch. After 75 min of refluxing the reaction mixture was cooled down, poured on ice (50 g) and fine yellow crystals were formed. They were filtered on Büchner funnel to give 427 mg of off-yellow crystals. The filtrate was concentrated to volume 20 ml and was left in the fridge over weekend to give second portion of impure **133**. The

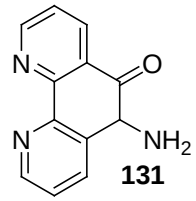
monooxime **133** was carefully recrystallized from ethanol to give pure **133** (731 mg, 96 %) as dirty yellow powder.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 9.05 (dd, J_1 = 4.6 Hz, J_2 = 1.8 Hz, 1H, *H*C-1), 8.85 (dd, J_1 = 4.6 Hz, J_2 = 1.6 Hz, 1H, *H*C-12), 8.46 (dd, J_1 = 7.9, J_2 = 1.8 Hz, 1H, *H*C-9), 8.44 (dd, J_1 = 8.2, J_2 = 1.6 Hz, 1H, *H*C-14), 7.70 (dd, J_1 = 7.9, J_2 = 4.6 Hz, 1H, *H*C-13), 7.64 (dd, J_1 = 8.2, J_2 = 4.6 Hz, 1H, *H*C-6).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) = 154.87 (C-10), 154.70 (C-1), 152.21 (C-12), 150.48 (C-7), 146.59 (C-9), 135.61 (C-3), 134.86 (C-14), 127.64 (C-5), 125.04 (C-4), 124.90 (C-13), 124.53 (C-6), 123.22 (C-8)

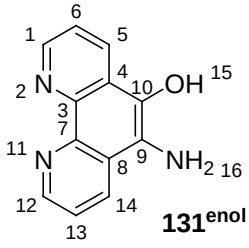
MS (ESI$^+$, m/z, %): $[M+H]^+$ 225.9 (100), $[MH+MeCN]^+$ 266.9 (20), $[2M+H]^+$ 451.0 (5)

6-Amino-1,10-phenanthrolin-5(6H)-one **131**



131

Phenanthrolinone oxime **133** (256 mg, 1.13 mmol, 1 eq.) was dissolved in MeOH (30 mL). Hydrochloric acid (1 mL, 12 M) and Pd/C (27 mg, 10 % w/w) was added. The reaction mixture was stirred overnight in autoclave under hydrogen atmosphere (15 bars). The pressure decreased to atmospheric and the suspension turned ocre. After filtration over Celite, the solution was deep red and the Celite layer was greenish. The solvent was evaporated to dryness to provide **131** (187 mg, 67 %) as orange solid. The product was formed as a hydrochloride.

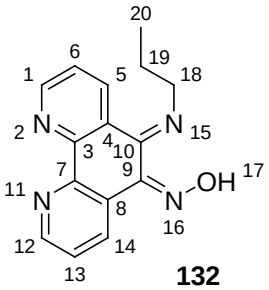


131^{enol}

^{1}H NMR (300 MHz, DMSO-d_6): *enol form* **131^{enol}** δ (ppm) = 10.69 (bs, 1H, -O*H*), 9.11 (pseudo-dd, J_1 = 4.9 Hz, J_2 = 1.4 Hz, 2H, *H*C-1, 12), 9.05 (pseudo-dd, J_1 = 8.5, J_2 = 1.4 Hz, 2H, *H*C-5, 14), 8.13 (pseudo-dd, J_1 = 8.5, J_2 = 4.9 Hz, 2H, *H*C-6, 13), 7.48 – 7.07 (m, J = 51 Hz, 3H, H_3N^+–).

UV-Vis (H_2O, pH = 2): λ_{max} / nm (rel. intensity) = 385 (100); (H_2O, pH = 9): λ_{max} / nm (rel. intensity) = 405 (100), 675 (30)

6-(Propylimino)-1,10-phenanthrolin-5(6H)-one oxime **132**



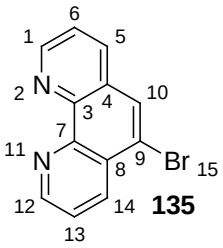
132

Phenanthroline monooxime **133** (32 mg, 0.142 mmol, 1 eq.) was mixed with propylamine **117a** (1 mL, 12.2 mmol, 86 eq.) under nitrogen atmosphere. The monooxime dissolved immediately to green solution. The reaction was stirred for 3 hours at room temperature and the precipitation was observed. MeOH (5 mL) was added, the precipitation dissolved and a deep green solution has been formed. The solvent and rests of propylamine have been evaporated to give 132 (37 mg, 97 %); green solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 9.76 (d, J = 6.7 Hz, 1H, *H*C-1), 8.99 (d, J = 2.8 Hz, 1H, *H*C-12), 8.74 (d, J = 3.0 Hz, 1H, *H*C-14), 8.43 (d, J = 7.6 Hz, 1H, *H*C-5), 7.37 (m, 2H, *H*C-6, 13), 2.96 (m, 2H, H_2C-18), 1.68 (m, 2H, H_2C-19), 0.95 (m, 3H, H_3C-20)

MS (ESI$^+$, m/z, %): $[M+H]^+$ 267.0 (80), $[2M+H]^+$ 451.0 (100)

5-Bromo-1,10-phenanthroline[162] **135**



135

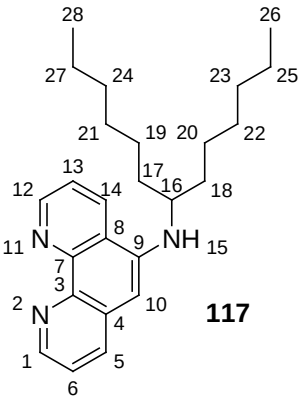
1,10-Phenanthroline monohydrate **134** (1.19 g, 6 mmol, 1 eq.) was put into a heavy-walled glass reaction tube with a screw top. The reaction vessel was placed to ice bath and approx. 20 % oleum (3.6 mL) and bromine (0.18 mL, 3.5 mmol, 1.16 eq.) was added. The solid did not dissolve completely. The reaction was sealed with a screw and it was slowly (in 90 minutes) heated to 135 °C. At this temperature the reaction mixture was stirred for 23 hours. The reaction mixture was poured on 30 g of ice to create yellow solution and it was neutralized with potassium carbonate to pH = 7. The resultant pink solution was extracted with chloroform (3 × 50 mL), washed with brine (20 mL) and dried over magnesium sulfate. The crude product **135** was mixed with diethyl ether (20 mL) and white solid dissolved and insoluble reddish solid remained on the bottom of the flask. The solution was transferred to another flask and after 5 minutes the product started to crystalize from the diethyl ether solution. The white flakes were filtrated and washed with ether to yield 135 (1.302 g, 84 %) as white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 9.22 (ddd, J_1 = 4.5 Hz, J_2 =2.9 Hz, J_3 = 1.7 Hz, 2H, *H*C-1, 12), 8.69 (dd, J_1 = 8.3 Hz, J_2 = 1.6 Hz, 1H, *H*C-14), 8.20 (dd, J_1 = 8.1 Hz, J_2 = 1.7 Hz, 1H, *H*C-5), 8.16 (s, 1H, *H*C-10), 7.76 (dd, J_1 = 8.4 Hz, J_2 = 4.3 Hz, 1H, *H*C-13), 7.66 (dd, J_1 = 8.1 Hz, J_2 = 4.4 Hz, 1H, *H*C-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ (ppm) = 150.98 (C-1), 150.76 (C-12), 146.68 (C-7), 145.70 (C-3), 136.00 (C-14), 135.19 (C-5), 129.73 (C-10), 128.87 (C-8), 127.96 (C-4), 123.91 (C-6), 123.74 (C-13), 120.87 (C-9).

MS (EI^+, m/z, %): $[M]^{\cdot+}$ 258.0 (100), 260.0 (87), $[M–Br]^{\cdot+}$ 179.1 (80)

N-(tridecan-7-yl)-1,10-phenanthrolin-5-amine **117**

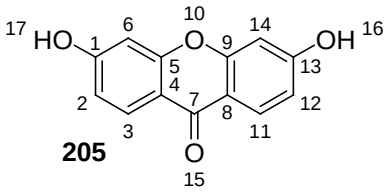


117

5-Bromo-1,10-phenanthroline **135** (50 mg, 0.193 mmol, 1 eq.), sodium *tert*-butoxide (26 mg, 0.270 mmol, 1.4 ′ eq.) and [1,1 - bis(diphenylphosphino)ferrocene]dichloropalladium(II) complex with dichloromethane (8 mg, 9.65 μmol, 0.05 eq.) were mixed in 15 mL vial. The cap with septum was sealed and the air atmosphere was displaced by nitrogen atmosphere (5 vacuum/nitrogen cycles). Degased toluene (5 mL) was added and the colour changed to dark brown. The nitrogen atmosphere was maintained with vacuum nitrogen cycles. A solution of tridecan-7-amine **127b** (154 mg, 0.772 mmol, 4 eq.) in degased toluene (5 mL) was added dropwise and the colour changed to orange red. The suspension was heated to 80 °C and was stirred for 2.5 hours. After cooling to 20 °C it was stirred overnight. The reaction mixture was evaporated to dryness, DCM (25 mL) and water (25 mL) was added, organic layer was separated, filtered over Celite and dried with $MgSO_4$. The evaporation to dryness yielded 60 mg (79 %) of brown solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 9.16 (dd, J_1 = 4.2 Hz, J_2 = 1.4 Hz, 1H, *H*C-12), 9.13 (dd, J_1 = 4.2 Hz, J_2 = 1.5 Hz, 1H, *H*C-1), 8.64 (dd, J_1 = 8.3 Hz, J_2 = 1.4 Hz, 1H, *H*C-9), 8.49 (s, 1H, *H*C-10), 8.47 (dd, J_1 = 8.1 Hz, J_2 = 1.2 Hz, 1H, *H*C-14), 7.91 (dd, J_1 = 8.3 Hz, J_2 = 4.3 Hz, 1H, *H*C-6), 7.80 (dd, J_1 = 8.1, J_2 = 4.3 Hz, 1H, *H*C-13), 3.33 (m, 1H, *H*C-16), 3.08 (bs, 1H, *H*N-15), 1.21 (m, 4H, H_2C-17, 18), 1.14 (m, 16H, H_2C-19, 20, 21, 22, 23, 24, 25, 27), 0.74 (t, J = 6.6 Hz, 6H, H_3C-26, 28)

3,6-Dihydroxy-9H-xanthen-9-one **205**



205

A stirred suspension of 2,2',4,4'-tetrahydroxybenzophenone (**206**, 4.00 g, 16.3 mmol, 1 eq.) in distilled water (24 mL) was heated in an autoclave at 200 °C for 6 hours. The mixture was cooled to 20 °C and 3,6-dihydroxy-9*H*-xanthen-9-one **205** was obtained as a cluster of needles. It was filtered off, washed with hot distilled water (3 × 10 mL), and dried under reduced pressure to give 3.55 g (96%) of pure title product; light orange needles.
This compound has also been characterized elsewhere.[163,169,170]

Mp: 330 °C (decomp.) (lit. 320 °C[171]; 347 °C[172]).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 6.82 (d, 2H, J = 2.0 Hz, *H*C-6, 14), 6.86 (dd, 2H, J_1 = 8.7 Hz, J_2 = 2.1 Hz, *H*C-2, 12), 7.98 (d, 2H, J = 8.7 Hz, *H*C-3, 11), 10.82 (s, 2H, −OH).

^{13}C NMR (100.5 MHz, DMSO-d_6): δ (ppm) = 102.0 (C-6, 14), 113.5 (C-2, 12), 113.9 (C-4, 8), 127.6 (C-3, 11), 157.3 (C-5, 9), 163.2 (C-1, 13), 173.8 (C-7).

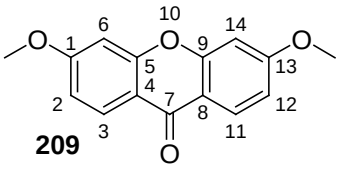
MS (ESI$^-$; CH_3OH + 2% NH_3, γ ~ 0.1 mg cm^{-3}, m/z, %): [M–H$^+$] 227.2 (100), [M$^-$] 228.25 (12.8).

FTIR (cm^{-1}): 3382, 3095, 1629, 1611, 1575, 1454, 1393, 1352, 1324, 1291, 1273, 1254, 1245, 1243, 1169, 1115, 1104, 986, 847, 831, 790, 693, 665, 635.

UV-Vis (C_2H_5OH, c = 1.46 × 10^{-6} mol dm^{-3}): λ_{max}/nm (ε/m^{-1} cm^{-1}) = 209 (29000), 239 (42500), 267 (11400), 280 (8050), 312 (24400), 321 (23600).

Anal. calcd. for $C_{13}H_8O_4$: C, 68.42; H, 3.53; O, 28.04. Found: C, 67.81; H, 3.70; O, 28.49.

3,6-Dimethoxy-9H-xanthen-9-one **209**



209

A mixture of anhydrous potassium carbonate (3.30 g, 23.9 mmol, 6.5 eq.) in acetone (75 mL) was added to a stirred suspension of 3,6-dihydroxy-9*H*-xanthen-9-one **205** (840 mg, 3.68 mmol, 1 eq.). Dimethyl sulfate (6.3 mL, 66.4 mmol, 18 eq.) was then added dropwise in 20 min. The resulting solution was stirred for 30 min at 20 °C and then refluxed for 20 h. After cooling to 0 °C using an ice-water bath, aqueous ammonia (c = 0.5 mol L^{-1}, 15 mL) was added dropwise, and the reaction

mixture was stirred for an additional 1 hour at 20 °C. A white precipitate, obtained by addition of water (100 mL) to the mixture, was filtered off, washed with distilled water (3 × 10 mL), and dried under reduced pressure to give 914 mg (97 %) of pure title product; white solid.
This compound has also been characterized elsewhere.[173,174]

Mp: 184.5–186.0 °C (lit. 187–188 °C)[173,174]

^{1}H NMR (500 MHz, CD_2Cl_2): δ (ppm) = 3.90 (s, 6H, H_3C-1, 2) 6.84 (dd, 2H, J_1 = 2.4 Hz, J_2 =1.0 Hz, *H*C-6, 14), 6.90 (ddd, 2H, J_1 = 8.8 Hz, J_2 = 2.4 Hz, J_3 = 0.7 Hz, *H*C-2, 12), 8.13 (d, 2H, J = 8.8 Hz, *H*C-3, 11).

^{13}C NMR (126 MHz, CD_2Cl_2): δ (ppm) = 56.2 (C-1, 2), 100.5 (C-6, 14), 113.1 (C-2, 12), 116.1 (C-4, 8), 128.1 (C-3, 11), 158.3 (C-5, 9), 165.0 (C-1, 13), 175.3 (C-7).

MS (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + NH_4OAc (5 mM), γ ~ 0.1 mg cm^{-3}, m/z, %): [M+H$^+$] 257.1 (100), [M+2H$^+$] 258.1 (16.2).

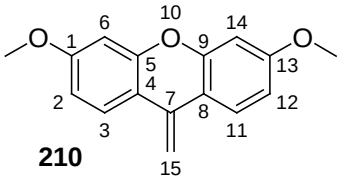
MS (EI; 70 eV, 150 °C, m/z): [M] 256.1.

FTIR (cm^{-1}): 1612, 1501, 1428, 1357, 1302, 1257, 1211, 1157, 1099, 1018, 979, 925, 825, 763, 663.

UV-Vis (C_2H_5OH, c = 1.27 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 209 (26700), 240 (43000), 266 (11000), 307 (22700).

Anal. calcd. for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; O, 24.97. Found: C, 70.18; H, 4.75; O, 25.07.

3,6-Dimethoxy-9-methylene-9H-xanthene **210**



210

Trimethylaluminium (1.1 mL, 2.2 mmol, 2 M solution in toluene, 1.13 eq.) was added dropwise to a stirred suspension of 3,6-dimethoxy-9*H*-xanthen-9-one **209** (500 mg, 1.95 mmol, 1 eq.) in a mixture of dry benzene (15 mL) and dry toluene (10 mL) under argon atmosphere at 25 °C to give a yellow mixture. It became homogeneous after warming to 50 °C. After cooling to 25 °C, trimethylaluminium (2.2 mL, 4.4 mmol, 2 M solution in toluene, 2.26 eq.) was slowly added (methane as a side-product is released). The reaction mixture was then heated at 65 °C for 90 min, cooled down to 0 °C (water–ice bath), and ice (20 g) and aq HCl (0.1 M, 3 mL) were cautiously added dropwise (methane is released). It was then extracted with

CH_2Cl_2 (3 × 15 mL), the combined organic layers were dried with Na_2SO_4, and the solvent was removed under reduced pressure to give 485 mg (98%) of the title product; yellow solid. **210**, both in the solid state or dissolved in polar solvents, is oxidized rapidly to a mixture of green products; therefore is should be stored in dark under N_2 atmosphere. If necessary, it can be easily purified by recrystallization from *n*-hexane.
This compound has also been characterized elsewhere.[107,175]

Mp: 144.5−145.8 °C (lit. 146–147 °C[107]).

^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) = 3.83 (s, 6H, H_3C-1, 2), 5.29 (s, 2H, H_2C-15), 6.62 (d, 2H, J = 2.6 Hz, *H*C-6, 14), 6.72 (dd, 2H, J_1 = 8.8 Hz, J_2 = 2.6 Hz, *H*C-2, 12), 7.66 (d, 2H, J = 8.8 Hz, *H*C-3, 11).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 3.80 (s, 6H, H_3C-1, 2), 5.39 (s, 2H, H_2C-15), 6.72 (d, 2H, J = 2.6 Hz, *H*C-6, 14), 6.78 (dd, 2H, J_1 = 8.8 Hz, J_2 = 2.6 Hz, *H*C-2, 12), 7.78 (d, 2H, J = 8.9 Hz, *H*C-3, 11).

^{13}C NMR (100.5 MHz, DMSO-d_6): δ (ppm) = 55.4 (C-1, 2), 97.2 (C-15), 100.8 (C-6, 14), 111.3 (C-2, 12), 113.3 (C-4, 8), 125.2 (C-3, 11), 130.5 (C-7), 150.7 (C-5, 9), 160.4 (C-1, 13).

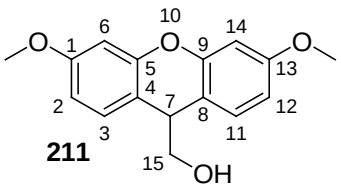
MS (EI; 70 eV, 150 °C, m/z)**:** [M] 254.1.

FTIR (cm^{-1}): 1625, 1619, 1599, 1567, 1467, 1462, 1440, 1425, 1386, 1330, 1265, 1247, 1207, 1171, 1160, 1109, 1098, 1078, 1028, 983, 946, 925, 838, 818, 783, 636.

UV-Vis (C_2H_5OH, c = 8.71 × 10^{-6} mol dm^{-3}): λ_{max}/nm (ε/m^{-1} cm^{-1}) = 220 (27700), 231 (32600), 273 (3200).

Anal. calcd. for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55; O, 18.88. Found: C, 75.55; H, 5.59; O, 18.86.

(3,6-Dimethoxy-9H-xanthen-9-yl)methanol **211**



211

BH_3•THF (1.0 M in THF, 3.5 mL, 3.5 mmol, 4.4 eq.) was added to a stirred solution of **210** (200 mg, 0.79 mmol, 1 eq.) in dry THF (25 mL) over a period of 20 minutes at 0 °C. The reaction mixture was stirred for 4 h at 20 °C, cooled to 0 °C (water–ice bath), and then water (2 mL, 10% solution in THF), aq NaOH (3.0 M, 2.5 mL, 7.50 mmol, 9.5 eq.), and aq hydrogen peroxide (30%, 2.7 mL, 26.4 mmol, 33 eq.) were cautiously added. The resulting mixture was stirred for 1.5 h at 20 °C, then

poured to water (50 mL), neutralized with aq. HCl (1 M, ~8 mL) to pH = 7, and the organic material was extracted with diethyl ether (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried with Na_2SO_4, filtered, and the solvent was removed under reduced pressure to give 193 mg (90 %) of the title product. No further purification was necessary; pale yellow solid.

Mp: 83.9−85.0 °C.

^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 3.43 (t, 2H, J = 5.7 Hz, H_2C-15), 3.86 (s, 6H, H_3C-1, 2), 3.86 (t, 1H, J = 6.1 Hz, HC-7), 4.81 (t, 1H, J = 5.3 Hz, –OH), 6.63 (d, 2H, J = 2.5 Hz, HC-6, 14), 6.68 (dd, 2H, J_1 = 8.4 Hz, J_2 = 2.6 Hz, HC-2, 12), 7.23 (d, 2H, J = 8.5 Hz, HC-3, 11).

^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) = 39.7 (C-7), 55.2 (C-1, 2), 68.2 (C-15), 100.8 (C-6, 14), 109.4 (C-2, 12), 114.9 (C-4, 8), 130.2 (C-1, 8), 152.0 (C-5, 9), 158.8 (C-1, 13).

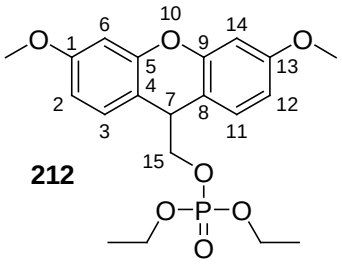
MS: (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ~ 0.1 mg cm^{-3}, m/z, %): [M-OH$^-$] 255.1 (32.4), [M] 272.1 (3.1), [M+H$^+$] 273.0 (100), [M+NH_4^+] 289.7 (3.9), [M_2+NH_4^+] 561.5 (33.4).

FTIR (cm^{-1}): 3299, 3053, 2941, 2921, 2875, 2831, 1633, 1614, 1574, 1500, 1462, 1435, 1425, 1325, 1276, 1259, 1205, 1184, 1161, 1149, 1097, 1053, 1034, 1022, 983, 976, 925, 824, 815, 638, 617.

UV-Vis (C_2H_5OH, c = 1.19 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/m^{-1} cm^{-1}) = 212 (46400), 278 (300).

Anal. calcd. for $C_{16}H_{16}O_4$: C, 70.58; H, 5.92; O, 23.50. Found: C, 70.63; H, 6.06; O, 23.31.

(3,6-Dimethoxy-9H-xanthen-9-yl)methyl Diethyl Phosphate **212**



212

Diethyl chlorophosphate (0.11 mL, 0.75 mmol, 1.2 eq.) in dry dichloromethane (20 mL) was added to a solution containing **211** (170 mg, 0.625 mmol, 1 eq.) and 4-dimethylaminopyridine (91 mg, 0.75 mmol, 1.2 eq.). The resulting solution was stirred at 20 °C under argon atmosphere for 24 hours, and water (20 mL) was added. The reaction mixture was extracted with ethyl acetate (3 × 20 mL), and the combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The

remaining yellow oil was purified by column chromatography (*n*-hexane/ethyl acetate, 60:40, v/v) to give 199 mg (78 %) of the pure title product; slightly yellow oil.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.07 (dt, 6H, J_1 = 7.1 Hz, J_2 = 0.8 Hz, H_3C–CH$_2$–O–), 3.72 (m, 4H, H$_3$C–CH_2–O–), 3.76 (s, 6H, H_3C–O–), 4.02 (t, 2H, J = 4.9 Hz, >CH-CH_2–O–), 4.24 (dt, 1H, J_1 = 4.5 Hz, J_2 = 1.5 Hz, *H*C-7), 6.67 (d, 2H, J = 2.5 Hz, *H*C-6, 14), 6.73 (dd, 2H, J_1 = 8.5 Hz, J_2 = 2.6 Hz, *H*C-2, 12), 7.30 (d, 2H, J = 8.6 Hz, *H*C-3, 11).

^{1}H NMR (600 MHz, CD_2Cl_2): δ (ppm) = 1.19 (dt, 6H, J_1 = 7.1 Hz, J_2 = 0.9 Hz, H_3C–CH$_2$–O–), 3.80 (s, 6H, H_3C–O–), 3.87 (m, 4H, H$_3$C–CH_2–O–), 4.01 (t, 2H, J = 5.9 Hz, >CH-CH_2–O–), 4.18 (t, 1H, J = 5.9 Hz, *H*C-7), 6.64 (d, 2H, J = 2.5 Hz, *H*C-6, 14), 6.68 (dd, 2H, J_1 = 8.4 Hz, J_2 = 2.6 Hz, *H*C-2, 12), 7.21 (d, 2H, J = 8.4 Hz, *H*C-3, 11).

^{13}C NMR (126 MHz, CD_2Cl_2): δ (ppm) = 16.2 (H$_3$*C*–CH$_2$–O–), 38.7 (>*C*H–CH$_2$–O–), 55.8 (–O*C*H$_3$), 64.0 (H$_3$C–*C*H$_2$–O–), 72.6 (>CH–*C*H$_2$–O–), 101.7 (C-6, 14), 110.3 (C-2, 12), 113.6 (C-4, 8), 130.5 (C-3, 11), 153.5 (C-5, 9), 160.5 (C-1, 13).

^{31}P NMR (162 MHz): δ (ppm) = –1.47.

MS (FAB, m/z, %): [M^{2-}] 406.1 (0.74), [M^-] 407.1 (4.66), [M] 408.1 (1.34), [M+H^+] 409.1 (3.85), [M+2H^+] 410.1 (1.0).

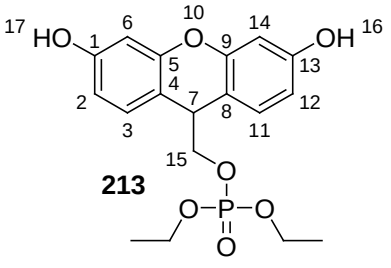
MALDI-MS (positive mode, m/z): 406.978, 407.977, 408.979.

FTIR (cm^{-1}): 2980, 2940, 2906, 2836, 2360, 2332, 1733, 1634, 1614, 1599, 1574, 1500, 1464, 1437, 1427, 1394, 1369, 1326, 1258 (P=O), 1202, 1196, 1162 (P–O–C), 1121, 1101, 1014 (P–O–C), 971 (P–O–C), 891, 830, 800, 733.

UV-Vis ($CHCl_3$): λ_{max}/nm (relative intensities) = 241 (100), 278 (62).

Anal. calcd. for $C_{20}H_{25}O_7P$: C, 58.82; H, 6.17; O, 35.01. Found: C, 57.90; H, 6.25; O, 35.85.

(3,6-Dihydroxy-9H-xanthen-9-yl)methyl Diethyl Phosphate **213**



213

Boron tribromide (1 M in dichloromethane, 9.56 mL, 9.56 mmol, 13 eq.) was added dropwise to a solution of (3,6-dimethoxy-9*H*-xanthen-9-yl)methyl diethyl phosphate (**212**, 300 mg, 0.735 mmol, 1 eq.) in dry CH_2Cl_2 (30 mL) under

nitrogen atmosphere at –78 °C. The reaction mixture was stirred and left to warm to –10 °C in 24 h. Water (30 mL) was then added, and the mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (50 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The resulting solid was dried under reduced pressure to give 276 mg (99 %) of the pure title product; beige powder.

Mp: 135 °C (decomp.).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 1.09 (t, 6H, J = 7.1 Hz, H_3C–CH_2–O–), 3.74 (m, 4H, H_3C–CH_2–O–), 3.94 (t, 2H, J = 5.2 Hz, >CH–CH_2–O–), 4.12 (t, 1H, J = 4.3 Hz, *H*C-7) 6.45 (d, 2H, J = 2.2 Hz, *H*C-6, 14), 6.53 (dd, 2H, J_1 = 8.3 Hz, J_2 = 2.2 Hz, *H*C-2, 12), 7.15 (d, 2H, J = 8.4 Hz, *H*C-3, 11), 9.56 (bs, 2H, –OH).

^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) = 15.70 (H_3C–CH_2–O–), 15.76 (H_3C–CH_2–O–), 37.1 (>*C*H–CH_2–O–), 62.87 (H_3C–*C*H_2–O–), 62.93 (H_3C–*C*H_2–O–), 72.2 (>CH–*C*H_2–O–), 102.3 (C-6, 14), 110.8 (C-2, 12), 111.3 (C-4, 8), 129.8 (C-3, 11), 152.3 (C-5, 9), 157.3 (C-1, 13).

^{31}P NMR (162 MHz): δ (ppm) = –4.77.

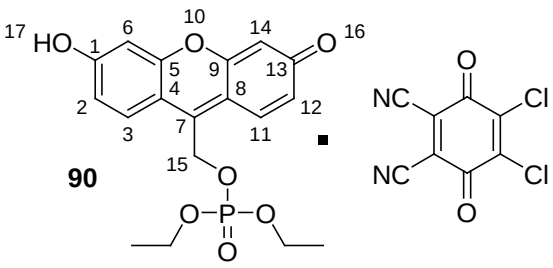
MS (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ~ 0.1 mg cm^{-3}, m/z): [M^+] 380.7, [M+H^+] 381.7.

FTIR (cm^{-1}): 3296, 2960, 2925, 2873, 2853, 2357, 2332, 1736, 1612, 1589, 1502, 1487, 1458, 1365, 1309, 1285, 1262, 1227 (P=O), 1208, 1198, 1174, 1147, 1098, 1069, 1032 (P–O–C), 984 (P–O–C), 859, 851, 820, 801, 778, 668, 651.

UV-Vis (CH_3OH, c = 1.20 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/m^{-1} cm^{-1}) = 211 (34700), 279 (4200).

Anal. calcd. for $C_{18}H_{21}O_7P$: C, 56.84; H, 5.57. Found: C, 56.90; H, 5.77.

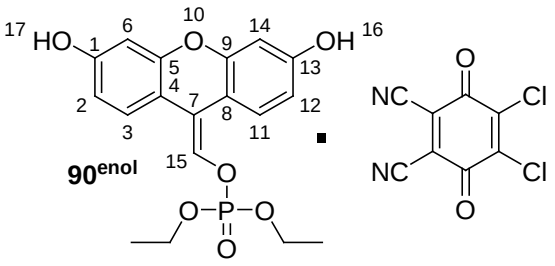
Diethyl (6-Hydroxy-3-oxo-3H-xanthen-9-yl)methyl Phosphate•DDQ Complex (**90**•DDQ)



2,3-Dichloro-5,6-dicyano-1,4-benzochinone (DDQ, 23 mg, 0.1 mmol, 1.25 eq.) was added to a solution of (3,6-dihydroxy-9*H*-xanthen-9-yl)methyl diethyl phosphate (**213**, 31 mg, 0.08 mmol, 1 eq.) in dry acetonitrile (2 mL) at 20 °C,

and the mixture was stirred at this temperature for 20 min. The resulting red precipitate was filtered off, washed with acetonitrile (20 mL), and dried under reduced pressure to give 25 mg (50%) of the pure title complex; orange-red powder.

Mp: 170 °C (decomp.).



90$^{\mathbf{enol}}$

^{1}H NMR (600 MHz, DMSO-d_6): *enol form* **90**$^{\mathbf{enol}}$ δ (ppm) = 1.04 (t, 3H, J = 7.0 Hz, H_3C-CH_2-O-), 1.17 (t, 3H, J = 7.0 Hz, H_3C–CH_2–O–), 3.74 (m, 2H, H_3C–CH_2–O–), 3.94 (m, 2H, H_3C–CH_2–O–), 5.89 (d, 1H, $^{3}J_{P\text{-}H}$ = 7.1 Hz, *H*C-15), 6.47 (dd, 2H, J_1 = 3.8 Hz, J_2 = 2.4 Hz, *H*C-6, 14), 6.63 (dt, 2H, J_1 = 8.7 Hz, J_2 = 2.2 Hz, *H*C-2, 12), 7.53 (d, 1H, J = 8.6 Hz, *H*C-3), 7.57 (d, 1H, J = 8.6 Hz, *H*C-11), 9.66 (s, 2H, –OH).

Signals of ethyl groups of the diethylphosphate mioiety are split because of the existence of two rotamers similar to some malonic acids[176].

The peak at 5.89 (d, 1H, J = 7.1 Hz) is split to a doublet by the ^{31}P atom, which was proved by a phosphorus decoupled ^{1}H{^{31}P} NMR.

^{1}H NMR (300 MHz, phosphate buffer in D_2O, pH = 7.4, I = 0.1 M): fast equilibration, δ (ppm) = 0.99 (t, 3H, J = 6.9 Hz, H_3C–CH_2–O–), 1.07 (t, 3H, J = 6.9 Hz, H_3C–CH_2–O–), 3.79–3.94 (m, 4H, H_3C–CH_2–O–), 6.55 (s, 2H, *H*C-15), 6.90 (d, 2H, J = 9.2 Hz, *H*C-6, 14), 7.14 (d, 2H, J = 5.4 Hz, *H*C-2, 12), 7.84–8.85 (2 × bs, 2H, *H*C-3, 11).

^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) = 15.4, 15.5 (H_3C–CH_2–O–), 15.6, 15.7 (H_3C–CH_2–O–), 63.6 (C-15), 68.1 (H_3C–CH_2–O–), 68.2 (H_3C–CH_2–O–), 101.3, 101.5 (C-6), 103.1, 103.2 (C-14), 110.3 (C-4), 110.6 (C-2), 111.9 (C-11), 112.7 (C-3), 114.2 (C-12), 128.7 (C-8), 129.4 (C-7), 134.0 (C-9), 151.6 (C-5), 151.8 (C-1), 158.1 (C-13)

The weak C_q signals from DDQ were not observed; the compound probably decomposes in DMSO.

^{13}C NMR (126 MHz, phosphate buffer in D_2O, pH = 7.4, I = 0.1 M): δ (ppm) = 15.1 (H_3C–CH_2–O–), 15.2 (H_3C–CH_2–O–), 66.4 (H_3C–CH_2–O–), 96.9 (C-6), 98.6 (C-14), 114.9 (C-4, 2), 117.2 (C-11, 3), 132.3 (C-12), 133.6 (C-8), 139.5 (C-7), 143.1 (C-9), 158.7 (C-1, 5), 166.2 (C-13)

^{31}P NMR (162 MHz, DMSO-d_6): δ (ppm) = –2.92 (q, J = 6.4 Hz).

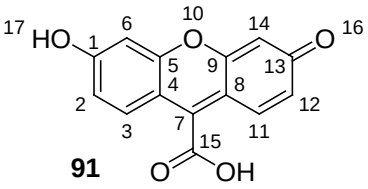
FTIR (cm^{-1}): 3151, 2712, 2586, 2367, 2336, 1722 (C=O), 1599, 1561, 1552, 1480, 1463, 1424, 1413, 1384, 1361, 1323, 1266, 1240 (P=O), 1152, 1125, 1090, 1039 (P–O–C), 992, 954 (P–O–C), 925, 863, 793, 641.

UV-Vis (aq phosphate buffer, pH = 7.0, I = 0.1 M): λ_{max}/nm (ε/M^{-1} cm^{-1}) = 214 (37300), 245 (46600), 332 (6500), 528 (39100).

HRMS (TOF ES^+): calcd. for $C_{26}H_{20}Cl_2N_2O_9P$ [M+H^+]: 605.0283 ($C_{26}H_{19}{}^{35}Cl^{35}ClN_2O_9P + H^+$), 607.0254 ($C_{26}H_{19}{}^{35}Cl^{37}ClN_2O_9P + H^+$). Found: 605.0283, 607.0272.

Anal. calcd. for $C_{26}H_{19}Cl_2N_2O_9P$: C, 51.59; H, 3.16; N, 4.63. Found: C, 51.16; H, 3.48; N, 4.52.

6-Hydroxy-3-oxo-3H-xanthene-9-carboxylic Acid **91**



91

Obtained upon irradiation of **90**•DDQ in aqueous phosphate buffer. A ~10 mmol dm^{-3} solution of **90**•DDQ in phosphate buffer (D_2O, pH = 7.4, I = 0.1 M) was irradiated in an NMR tube. A part (~40%) of the starting material precipitated and was filtered off. The filtrate was acidified with aq CF_3COOH to form a red precipitate. It was filtered, washed with aq CF_3COOH (3 × 1 mL), and dried. Yield: 50% (calculated on the basis of the starting material consumed); dark red powder.
The literature claims that this compound has also been prepared before,[177,178] but we were unable to reproduce successfully these procedures.

^{1}H NMR (300 MHz, phosphate buffer in D_2O, pH = 7.4, I = 0.1 M): fast equilibration, δ (ppm) = 6.67 (d, 2H, J = 2.0 Hz, *H*C-6, 14), 6.84 (dd, 2H, J_1 = 9.0 Hz, J_2 = 2.0 Hz, *H*C-2, 12), 7.63 (d, 2H, J = 9.0 Hz, *H*C-3, 11).

^{1}H NMR (300 MHz, CD_3OD/CD_3CN, 1:1, (v/v)): fast equilibration, δ (ppm) = 6.56 (d, 2H, J = 2.6 Hz, *H*C-6, 14), 6.64 (dd, 2H, J_1 = 8.6 Hz, J_2 = 2.6 Hz, *H*C-2, 12), 7.23 (d, 2H, J = 8.6 Hz, *H*C-3, 11).

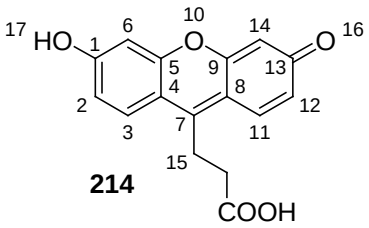
^{13}C NMR (126 MHz, 0.1 M phosphate buffer in D_2O, pH = 7.4): δ (ppm) = 104.2 (C-7), 108.5 (C-6, 14), 123.6 (C-4, 8), 131.1 (C-2, 12), 154.0 (C-3, 11), 159.7 (C-5, 9), 171.9 (C-1, 13), 180.5 (C-15).

HRMS (TOF MS ES^+): calcd. for $C_{14}H_9O_5$ [M+H^+] 257.0444, found 257.0452

UV-Vis (CH_3OH): λ_{max}/nm (relative intensity) = 491 (100), 321 (16), 242 (101)

UV-Vis (0.1 M aq phosphate buffer, pH = 7.4): λ_{max}/nm (relative intensity) = 488 (100), 318 (11), 241 (81)

6-Hydroxy-3-oxo-3H-xanthene-9-propanoic acid (succinylfluorescein) **214**



214

Procedure A:

A stirred mixture of succinic acid anhydride (2.50 g, 25 mmol, 1 eq.) and resorcinol (2.75 g, 25 mmol, 1 eq.) in aq sulfuric acid (30 mL, 73% (v/v)) was heated to 140 °C for 6 hours. The reaction mixture was cooled to 20 °C and poured into water (500 mL). The stirred solution was alkalinized with aq NaOH (50%) to pH = 13, while the temperature was kept at ~20 °C. Acetic acid was added to the solution until pH = 4 was obtained and the brown precipitate was filtered. The filtrate was washed with water (3 × 25 mL), dried under reduced pressure, washed with hot 1,4-dioxane (15 mL) and hot methanol (15 mL), and dried under reduced pressure to give 5.7 g (80 %) pure title compound; dark brown solid.

This compound has also been characterized elsewhere.[110]

Procedure B[90]*:*

Resorcinol (10 g, 91 mmol, 2.1 eq.) and succinic acid (5 g, 42 mmol, 1 eq.) were crunched and powdered in a mortar to create an intimate powder mixture. The mixture has been heated on a silicon oil bath to 170 °C. At 110 °C resorcinol melted down and the succinic acid dissolved in it. After 30 min at 170 °C anhydrous zinc (II) chloride (0.5 g, 3.7 mmol, 0.09 eq.) was added. HCl (g) was evolved and the colour changed from pale yellow to reddish brown. After 210 min of heating when the temperature did not exceed 180 °C the heating was stopped and the mixture solidified. Acetone (25 mL) has been added and the mixture was sonicated to dissolve rests of resorcinol. The insoluble part has been filtered off and washed with 20 mL of acetone. The amorphous brown solid was refluxed in 5 % aq HCl for 1 hour and after cooling down to 0 °C sparkling small crystals of product were created. The product was filtered off and dried in vacuum to give 5.5 g (46 %); dark brown solid.

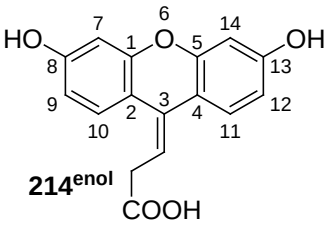
The structure of the product was confirmed by comparison with a standard prepared by Procedure A.

Procedure C:

Resorcinol (10 g, 91 mmol, 2.1 eq.), succinic acid (5 g, 42 mmol, 1 eq.), and anhydrous zinc (II) chloride (0.5 g, 3.7 mmol, 0.09 mmol) were crunched and powdered in a mortar to create an intimate powder mixture. The mixture was put into a glass tube of a microwave reactor and a stirrer with a temperature detector has been installed. The emissivity factor has been set to 0.185 and the optimized temperature program (temperature set: 110 °C; 2 min of max 100 % power, 2 min of max 40 % power and 54 min of max 30 % power) was applied.

The mixture melted at 110 °C and the true temperature of the reaction mixture after equilibration was 130 °C. After the 58 minutes sequence had ended the mixture solidified and was worked up analogically to the procedure B to give 2.4 g (20 %) of the title product.

Mp. 300 °C (decomp.) (lit. 155–160 °C[110] (decomp.)).



214$^{\mathbf{enol}}$

^{1}H NMR (300 MHz, DMSO-d_6): *enol form* **214**$^{\mathbf{enol}}$ δ (ppm) = 3.40 (d, 2H, J = 7.2 Hz, =CH-CH_2-COOH), 5.80 (t, 1H, J = 7.3 Hz, =C*H*-CH_2-COOH), 6.52 (d, 1H, J = 2.3 Hz, *H*C-7), 6.56 (d, 1H, J = 2.3 Hz, *H*C-14), 6.62 (dd, 2H, J_1 = 8.6 Hz, J_2 = 2.3 Hz, *H*C-9, 12), 7.43 (dd, 2H, J_1 = 8.6 Hz, J_2 = 2.0 Hz, *H*C-10, 11), 9.89 (s, 1H, –O*H*), 9.74 (s, 1H, –O*H*), 12.36 (bs, 1H, –COO*H*).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ (ppm) = 34.6 (-CH_2-*C*H_2-COOH), 102.2 (-*C*H_2-CH_2-COOH), 102.6 (C-6), 110.8 (C-14), 111.8 (C-2), 111.9 (C-4), 112.7 (C-11), 115.8 (C-12), 124.3 (C-3), 126.5 (C-8), 128.3 (C-7), 151.2 (C-9), 152.9 (C-5), 157.9 (C-1), 158.1 (-COOH), 172.9 (C-13)

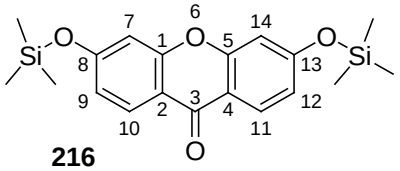
MS (EI$^+$, 70 eV, m/z, %): 284 (35), 255 (1), 239 (100), 229 (50), 223 (7), 213 (10), 200 (3), 181 (5), 165 (6), 152 (11), 137 (7), 115 (9).

FTIR (KBr, cm^{-1}) = 3450 (br), 3053, 2968, 1745, 1633, 1599, 1463, 1391, 1329, 1250, 1202, 1159, 1116, 1037, 846.

UV-Vis (aq phosphate buffer, pH = 7.0, I = 0.1 M), (c = 1.00 × 10^{-5} mol dm^{-3}): λ_{max}/nm (ε/m^{-1} cm^{-1}) = 238 (53800), 486 (95000).

HRMS (ESI$^+$): calcd. for $C_{16}H_{13}O_5$ [M+H$^+$] 285.0757. Found: 285.0758.

3,6-Bis(trimethylsilyloxy)-9H-xanthen-9-one **216**



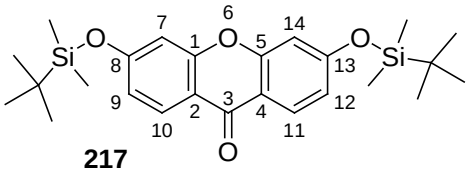
216

To the solution of 3,6-dihydroxy-9*H*-xanthen-9-one **205** (500 mg, 2.2 mmol, 1 eq.) in dry DMF (40 mL) a solution of imidazole (150 mg, 22 mmol, 10 eq.) in dry DMF (5 mL) was added under nitrogen atmosphere. Freshly distilled trimethylsilyl chloride (1.68 mL, 13.2 mmol, 6 eq.) was added dropwise at 20 °C over 20 min. The reaction mixture was stirred for 30 minutes and then it has poured on ice (50 g) and extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to give 743 mg (91 %) of the title product; white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.15 (d, J = 9.1 Hz, 2H, *H*C-10, 11), 6.76 (dd, J_1 = 9.1 Hz, J_2 = 2.1 Hz, 2H, *H*C-9, 12), 6.64 (d, 2H, J = 2.1 Hz, *H*C-7, 14), 0.31 (s, 18H, –Si(CH_3)$_3$)

MS (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ~ 0.1 mg cm^{-3}, m/z): [M$^+$] 372.1

3,6-Bis(tert-butyldimethylsilyloxy)-9H-xanthen-9-one **217**



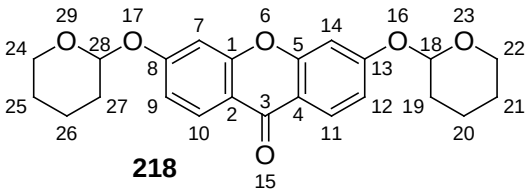
217

To the solution of 3,6-dihydroxy-*9H*-xanthen-9-one **205** (250 mg, 1.1 mmol, 1 eq.) in dry DMF (20 mL) a solution of imidazole (750 mg, 11 mmol, 10 eq.) in dry DMF (5 mL) was added under nitrogen atmosphere. A solution of *tert*-butyldimethylsilyl chloride (995 mg, 6.6 mmol, 6 eq.) in dry THF (5 mL) was added dropwise at 20 °C. The reaction mixture was stirred for 30 minutes and then it was poured on ice (20 g) and extracted with DCM (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and evaporated to give 502 mg (98 %) of the title product; white feathery solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.20 (d, J = 9.3 Hz, 2H, *H*C-10, 11), 6.86 (d, J = 9.3 Hz, 2H, *H*C-9, 12), 6.83 (s, 2H, *H*C-7, 14), 1.02 (s, 18H, –Si(CH_3)$_2$*t-Bu*), 0.29 (s, 12H, –Si(CH_3)$_2$*t*-Bu)

MS (ESI$^+$; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ~ 0.1 mg cm^{-3}, m/z): [M$^+$] 456.2, [M+H$^+$] 457.2

3,6-Bis(tetrahydro-2H-pyran-2-yloxy)-9H-xanthen-9-one **218**



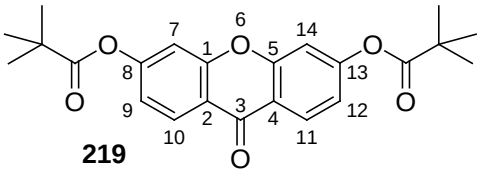
218

3,4-Dihydro-*2H*-pyran (17.0 mL, 187 mmol, 8.5 eq.) was added dropwise to the mixture of 3,6-dihydroxy-*9H*-xanthen-9-one **205** (5.0 g, 22 mmol, 1 eq.) and pyridinium *p*-toluenesulfonate (274 mg, 1.1 mmol, 0.05 eq.) in dry DCM (80 mL) over 2 hours under nitrogen atmosphere. After 1 hour of stirring, another portion of 3,4-dihydro-*2H*-pyran (3.0 mL, 33 mmol, 1.5 eq.) was added. After stirring the reaction at 20 °C overnight, aqueous K_2CO_3 (100 mL, 10 %) was added and the mixture was extracted with dichloromethane (3 × 50 mL). The combined organic extracts were washed with brine (80 mL), dried over $MgSO_4$ and solvents were evaporated under reduced pressure. The crude product was purified by column chromatography (petrolether : ethylacetate – 5 : 1 (v/v)) to give 7.1 g (82 %) of the pure title product as a mixture of diastereomers; white crystalline solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.25 (d, *J* = 8.8 Hz, 2H, *H*C-10, 11), 7.11 (d, *J* = 2.2 Hz, 2H, *H*C-7, 14), 7.04 (dd, *J* = 8.8, 2.2 Hz, 2H, *H*C-9, 12), 5.58 (m, 2H, *H*C-18, 28), 3.95 – 3.82 (m, *J* = 23.6 Hz, 2H, H_2C-22, 24), 3.73 – 3.62 (d, *J* = 11.3 Hz, 2H, H_2C-22, 24), 2.16 – 1.98 (m, 2H, H_2C-19, 27), 1.98 – 1.88 (m, 2H, H_2C-19, 27), 1.80 – 1.48 (m, 8H, H_2C-20, 21, 25, 26).

MS (EI^+, 70 eV, m/z, %): 396 (40), 311 (30), 226 (100), 85 (18)

9-Oxo-9H-xanthene-3,6-diyl bis(2,2-dimethylpropanoate) **219**



219

Triethylamine (0.27 mL, 1.93 mmol, 2.2 eq.) was added to a suspension of 3,6-dihydroxy-9*H*-xanthen-9-one **205** (200 mg, 0.876 mmol, 1 eq.) in dry DCM (30 mL) under nitrogen atmosphere dropwise for 15 min at 20 °C and the suspension became homogenous. Pivaloyl chloride (0.24 mL, 1.93 mmol, 2.2 eq.) was added dropwise and the solution turned yellowish. After 30 min of stirring at 20 °C, another portion of pivaloyl chloride (0.12 mL, 0.97 mmol, 1.1 eq.) was added dropwise to complete the conversion and the mixture was stirred for more 45 min. The reaction was quenched by addition of HCl (5 % aq, 10 mL), organic layer was separated, washed with brine, and dried over $MgSO_4$. Evaporation of the solvent gave 337 mg (97 %) of title product; white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) = 8.37 (d, 2H, J = 8.2 Hz, *H*C-10, 11), 7.28 (d, 2H, J = 2.1 Hz, *H*C-7, 14), 7.13 (dd, 2H, J_1 = 8.2 Hz, J_2 = 2.1 Hz, *H*C-9, 12), 1.42 (s, 18H, *t*Bu)

MS (ESI^+; CH_3OH/H_2O, 1:1 (v/v) + 5 mM NH_4OAc, γ ~ 0.1 mg cm^{-3}, m/z): $[M^+]$ 396.4, $[M+H^+]$ 397.4, $[M+2H^+]$ 398.5

Conclusion

The general aim of this thesis was the synthesis and investigation of photoactivatable comounds. The work consists of three different projects, all based on the synthesis of a molecule capable of defined transformation upon irradiation.

The aim of the first project, the Photoremovable Chiral Auxiliary (PCA) project was to prove the hypothesis of possible chiral induction of benzoin PPG for Diels-Alder reaction of benzoin acrylate **93** with cyclopentadiene **92** and to optimize the reaction conditions (solvent, temperature, catalyst) and the structure of the chiral auxiliary to give the highest induced enantioselectivity. The project was partially solved in my bachelor thesis[139]), namely the usage of *(R)*-benzoin and *(S)*-2'chlorobenzoin as PCA. This work deals with the synthesis of acrylates ***(S)*-93a** and ***(S)*-93b**, which was accomplished by a two-step procedure with excellent yield (85 – 90 %). The enantioselectivity of the Diels-Alder reaction was optimized up to 96 % for reaction catalyzed by 2 eq. of $SnCl_4$ at –78 °C using *(S)*-2-methoxybenzoin as PCA. The project has been published.[80]

The second project, Phenanthroline-flavin for photocatalysis, was concerned with the synthesis of the phenanthrolin extended derivative of flavin **89**. Despite of many attempts of synthetizing of the target molecule, the flavin **89** has not been prepared and sufficiently characterized. Three different multistep synthetic approaches were investigated, and in all cases the final condensation reaction did not result in the desired product. Several new uracil and 1,10-phenanthroline derivatives have been synthetized and fully characterized. The syntheses of the phenanthroline and uracil derivatives are going to be published soon as a part of the project of synthesis of extended flavins.

The aim of the last project, (6-Hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl: A Photoremovable Protecting Group, was to synthetize diethyl (6-hydroxy-3-oxo-3*H*-xanthen-9-yl)methyl phosphate **90** and its photoproduct, 6-hydroxy-3-oxo-3*H*-xanthene-9-carboxylic acid **91**. Instead of the pure compound **90**, its complex with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (**90**•DDQ) was synthetized by a seven-step procedure with excellent overall yield 32 %. The acid **91** was synthetized photochemically by irradiation of **90**•DDQ by green light (λ = 546 nm) in aqueous buffer (pH = 7) with 50 % yield.

Abbreviation index

2-NB	2-Nitrobenzyl
ATP	Adenosine triphosphate
A. U.	Arbitrary units
BHc	6-Bromo-7-hydroxy-4-hydroxymethyl coumarin
Bnz	Benzoin
BuLi	*n*-Butyl lithium
cAMP	Cyclic adenosine monophosphate
Cbz	Carboxybenzyl
CF	Carboxyfluorescein
CFDA-CE	Carboxyfluorescein diacetate succinimidyl ester
CMNB	5-Carboxymethoxy-2-nitrobenzyl
Cys	Cysteine
DCM	Cichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DDQ-H2	2,3-Dichloro-5,6-dicyanohydroquinone
DIC	*N,N'*-Diisopropylcarbodiimide
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	2,5-Dimehtylphenacyl
DMS	Dimethyl sulfate
DMSO	Dimethyl sulfoxide
EA	Electron affinity
EDTA	Ethylenediaminetetraacetic acid
EI	Electron ionization
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
FAB	Fast atom bombardment
FAD	Flavin adenine dinucleotide
FITC	Fluorescein isothiocyanate
FMN	Flavin mononucleotide
Fmoc	Fluorenylmethyloxycarbonyl
FTIR	Fourier transform infrared spectroscopy
GABA	γ-Aminobutyric acid
GC	Gas chromatography
GM	Göppert-Mayer
HOBT	Hydroxybenzotriazole
HOMO	Highest Occupied Molecular Orbital
HPLC	High performance liquid chromatography
HPLC-HRMS	High performance liquid chromatography coupled with high resolution

	mass spectrometry
HRMS	High resolution mass spectrometry
IAF	Iodoacetamido-fluorescein
IP	Ionization potential
IUPAC	International Union of Pure and Applied Chemistry
LED	Light-emitting diode
LG	Leaving group
LUMO	Lowest Unoccupied Molecular Orbital
MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
MCM	(7-Methoxycoumarin-4-yl)methyl
mCPBA	Meta-Chloroperoxybenzoic acid
MeLi	Methyl lithium
mRNA	Messenger RNA
MW	Microwave
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	*N*-Bromosuccinimide
NHS	Carboxyfluorescein succinimidyl ester
NMO	*N*-Methylmorpholine N-oxide
NMR	Nuclear magnetic resonance spectroscopy
NPE-HCCC1/Me	(2-Nitrophenyl)ethyl-caged 6-chloro-7-hydroxycoumarin-3-carboxamide of dimethyl-D-glutamate
NPE-HPTS	(2-Nitrophenyl)ethyl-caged 8-hydroxypyrene-1,3,6-tris-sulfonic acid
oNB	*ortho*-Nitrobenzyl
PCA	Photoremovable chiral auxiliary
PET	Photoinduced electron transfer
PG	Protecting group
*p*HP	*para*-Hydroxyphenacyl
Piv	Pivaloyl
PPG	Photoremovable protecting group
PPTS	Pyridinium p-toluenesulfonate
PTFE	Polytetrafluoroethylene
RFT	Riboflavin tetraacetate
RuBi-GABA	(bis(2,2'-Bipyridine-N,N')trimethylphosphine)-4-aminobutyric acid ruthenium hexafluorophosphate complex
SCE	Saturated calomel electrode
Sn1	Nucleophilic substitution of 1st order
SNAFL	Carboxyseminaphthofluorescein
SOMO	Single occupied molecular orbital
SPS	Solid-phase synthesis
TBAF	Tetra-n-butylammonium fluoride
TBDMS	*tert*-Butyldimethylsilyl
THF	Tetrahydrofuran

THP	Tetrahydropyranyl
TLC	Thin layer chromatography
TMEDA	Tetramethylethylenediamine
TMS	Trimethylsilyl
TsOH	*p*-Toluenesulfonic acid
Tyr	Tyrosine
Z	Carboxybenzyl

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