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About the Synthesis of Fluorophores for Grafting via Two- or Multiphoton Absorption

MASTER THESIS

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STATUTORY DECLARATION

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, March 28th, 2012 Dominik Wohlmuth

Für meine Eltern

ABSTRACT

The scope of this master thesis was the development of functional molecules for selective immobilization using photoreactions triggered by two-photon absorption (TPA). For this purpose, the TPA chromophore BAC-M (2,6-bis(4-azidobenzylidene)-4-methyl-cyclohexanone) was selected and modified using so-called “click chemistry”. More precisely, thiol-ene chemistry and Cu-catalyzed [3+2] azide-alkyne cycloaddition were evaluated for this purpose.

Cysteamin and Propargylamin were treated with dansyl (5-(dimethylamino)naphthalene-1-sulfonyl) chloride to obtain the respective sulfonamides as substrates for thiol-ene and azide-alkyne click chemistry. A parallel approach was to use TCP-anhydride (1,6,7,12-Tetrachloroperylene-3,4,9,10-tetracarboxylic acid dianhydride) to obtain the corresponding asymmetrically substituted bisimides which were also evaluated within this master thesis.

A crucial part of this work was the identification of suitable reaction conditions for thiol-ene and azide-alkyne click chemistry. For this purpose, reactions were performed initially on smaller “model substrates”. Cinnamic acid ethyl ester was used as a model for thiol-ene chemistry whereas 2-azido-1-phenylethanone was reacted with different alkynes in copper catalyzed azide-alkyne “click” reactions.

Thus, it could be shown that due to the conjugated structure and the high degree of steric hindrance on the double bonds of BAC-M, a functionalization via thiol-ene chemistry was not possible. Because of the same reason, also azide-alkyne “click” reactions on this molecule were rather sluggish. The products from these model reactions were characterized using NMR, FT-IR, UV-Vis and fluorescence spectroscopy.

Finally, dansyl- and perylene- monofunctionalised BAC-M was obtained in a azide-alkyne „click“ reaction and it was attempted to graft these molecules to commercially available hydrogel pellets using a TPA process. While the TPA grafting process went smoothly for the dansyl derivative, an immobilization of the perylene-substituted molecule was not possible.

KURZFASSUNG

Diese Arbeit beschäftigt sich mit der Derivatisierung von Farbstoffmolekülen für selektive Immobilisierung mittels Zweiphotonenanregung. Dafür wurde der Zweiphotonenabsorptions-aktive Chromophor BAC-M (2,6-bis(4-Azidobenzyliden)-4-methyl-cyclohexanon) ausgewählt und mit geeignet derivatisierten Fluoreszenzfarbstoffen mittels sogenannter „Klickchemie“ umgesetzt. Im speziellen wurden dafür die Thiol-en Klick-Reaktion sowie die Kupfer-katalysierte [3+2] Azid-Alkin Cycloaddition evaluiert. Ausgehend von Dansylchlorid (5-(Dimethylamino)naphthalin-1-sulfonylchlorid) wurden mittels Umsetzung mit Cysteamin und Propargylamin die entsprechenden Sulfonamide als Substrate für die radikalische Thiol-En und Azid-Alkin Klickchemie erhalten. Parallel dazu wurden die oben genannten Amine mit TCP-anhydrid (1,6,7,12-Tetrachlorperylen-3,4,9,10-tetracarbonsäuredianhydrid) zu asymmetrisch substituierten Bisimiden umgesetzt die auch im Rahmen dieser Arbeit evaluiert wurden.

Ein wesentlicher Teil der Arbeit war die Identifikation von geeigneten Reaktionsbedingungen für die Thiol-En und Azid-Alkin-Klickchemie, wofür Reaktionen an Modellsubstanzen durchgeführt wurden. Für die Thiol-En Reaktion wurde Zimtsäureethylester als Modellsubstanz herangezogen, während die Umsetzung von verschiedenen Alkinen mit 2-Azido-1-phenylethanon in Gegenwart von CuI als Modellreaktion für die Azid-Alkin Klickchemie diente.

Dabei zeigte sich, dass eine Thiol-En Reaktion durch die konjugierte Struktur und die sterische Hinderung der Doppelbindungen von BAC-M nicht möglich war. Auch die Azid-Alkin-Klickreaktion verlief aus diesem Grund vergleichsweise langsam. Die so gewonnenen Moleküle sind mit Hilfe von NMR, FT-IR, UV-Vis und Fluoreszenz Spektroskopie charakterisiert worden.

Letztlich wurde versucht, über eine Azid-Alkin-Reaktion einseitig mit Dansyl beziehungsweise Perylen funktionalisiertes BAC-M mittels Zweiphotonenanregung auf kommerziell erhältliche Hydrogelpellets zu graften, was für die dansylierte Verbindung möglich war, während das Perylen-Derivat so nicht immobilisiert werden konnte.

ACKNOWLEDGEMENT

As usual on such occasions one is faced with the problem whom to mention and whom to leave out. Some people can be ignored on purpose because it makes a statement by omission, others might be forgotten and angered those who did not hit you. I apologize in advance.

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1 Introduction and Motivation

In future sensors will be utilized for many useful applications in the daily life for providing information about health conditions, environmental pollutants or hazardous substances for example.[1] Particularly, in agriculture and food industry it is necessary to check the condition and environments of plants, animals, and farm products in regular intervals. Other measurements may include the controlling of air and water pollutants, modern fire control systems, and numerous other applications. Unfortunately, there is a significant lack between the availability of sensors and their constantly rising demand.[2]

This increasing demand for miniaturized devices needs a selective immobilization of functional molecules at surfaces. One approach to solve this problem is photo grafting, where coupling reactions can be triggered by irradiation. TPA (Two Photon Absorption) has been identified as a suitable tool for selective grafting reactions. **Two-photon absorption (TPA)** is the simultaneous absorption of two photons of identical or different frequencies in order to excite a molecule.[3] The excited state of the molecule can then undergo a chemical reaction to form a covalent bond between the TPA active molecule and the polymer matrix. This technique allows inscribing of functional molecules (like sensor molecules) to complex three-dimensional structures. These structures could then be used in miniaturized devices like microsensor arrays.[4]

By the use of not innately functionalized TPA active molecules a technique was evaluated to enable these molecules via so-called “click chemistry”. Click Chemistry was evaluated to be the best choice so far. More precisely, thiol-ene click reaction and Cu-catalyzed [3+2] azide-alkyne cycloaddition reactions has been well studied. Inspired by nature, Sharpless et al. first developed the "click philosophy”, in 2001.[5] It was a totally new approach of synthetic strategy which has proven to be powerful, highly

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[1] *“Sensor Technology Handbook”;* Jon S. Wilson; *Newnes;* **2004**
[2] http://www.isotec-cluster.at/
[3] *Göppert-Mayer, M.; Analen der Physik;***1931**; 3; 273-294
[4] Aovsiani, A.; unpublished results
[5] Kolb, H. C.; Finn, M. G.; Sharpless, K. B.; *Angew. Chem. Int. Ed*.; **2001**; 40; 2004-2021

reliable and selective so far. Click chemistry is defined by a selection of reactions possessing the perfection to achieve selectivity and high yield products by meeting a few strict criteria. A click reaction must be carried out under simple and modular conditions including insensitivity to water and oxygen. It should have a wide application potential and give high yields. A high atom economy is sought, and any by-products should be non-toxic to humans and the environment. Possible side products should be removed with non chromatographic methods and the solvents should be easily removable after the reaction. Besides other necessary conditions, the easy availability of starting compounds and reagents is also important.[6]

[6] Kunz, D.; *Chem. Unserer Zeit*; **2009**; 43; 224-230

2 Scope of this Work

The objective of this work was to elaborate ways to synthesize functional molecules which can be photochemically immobilized at a surface using **two-photon absorption.** The functional molecule should be based on BAC-M and should contain a fluorescent dye. The conjugation of BAC-M and the dye should be done either via thiol-ene chemistry or via Cu-catalyzed [3+2] azide-alkyne cycloaddition reactions.

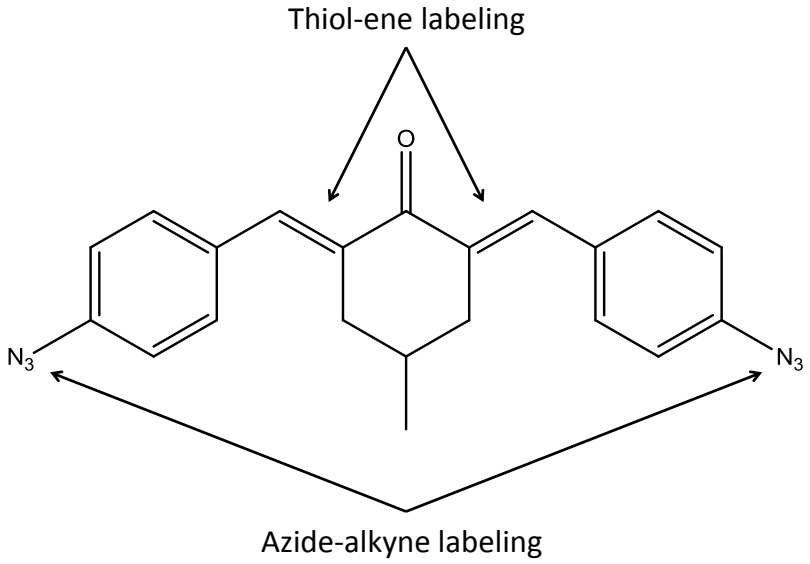


Figure 1: Active sites in TPA active molecule

In case of success, the new compounds should be used for inscription of a 3D pattern into poly(ethylene glycol) based hydrogels.

3 General Background

3.1 “Click Chemistry”

3.1.1 Concept of Click-Chemistry

In nature, countless substrates with large structural diversity and high degrees of reliability are synthesized in very short time, mostly in aqueous media. Water, being a universal solvent makes these reactions inevitable. The role of highly efficient combination of modular principles together with selective catalytic or enzyme systems is also important for the processes undergoing[6].

Inspired by the efficiency of such natural synthetic strategies, Sharpless et al. developed the "click philosophy” in synthetic chemistry at the beginning of the last decade.[5] The aim was, to develop a growing set of powerful, selective and “modular blocks” via a reliable approach in both small and large scale synthetic processes. This new approach was termed as “click chemistry” and there is a defined set of stringent criteria that needs to be fulfilled in this context.

Click reactions must be carried out under simple and modular conditions, have a wide application potential and give high yields. A high atom economy is sought, and any by-products which should be non-toxic to humans and the environment as well as easily removable without chromatographic methods are essential. Insensitivity to water and oxygen should be given. Another basic condition is the easy availability of starting compounds and reagents.[5]

An ideal click reaction is generally driven by a sufficiently high thermodynamic driving force. Usually, the energy gain of such reactions is about 20 kcal mol^{-1}. The reactions proceed very fast to complete conversion and also tend to be very selective for a single product.[6] It is commonly observed in carbon-carbon double bonds cleavage and carbon-heteroatom bond formation. The same is also observed for the breaking of strained ring systems. Thus, it is not surprising that nature prefers the formation of carbon-heteroatom bonds over carbon-carbon bonds. With respect to the conditions

of synthesis, which are offered by a chemical laboratory, the application of the concept of click chemistry has totally proven to be beneficial. In literature a lot of examples are available for such click reactions; some are given in Table 1.[7]

Table 1: Different types of click reactions (taken from reference 7)

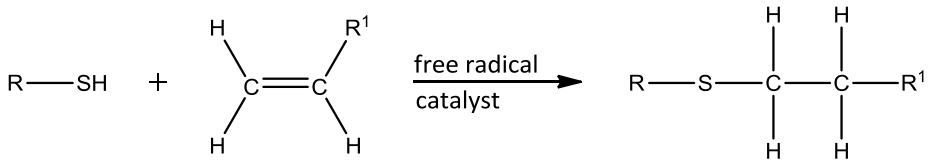
	Reagent A	Reagent B	Mechanism
1	**azide**	**alkyne**	**Cu-catalyzed [3+2] azide-alkyne cycloaddition (CuAAC)**
2	azide	cyclooctyne	strain-promoted [3+2] azide-alkyne cycloaddition (SPAAC)
3	azide	activated alkyne	[3+2] Huisgen cycloaddition
4	azide	electron-deficient alkyne	[3+2] cycloaddition
5	azide	aryne	[3+2] cycloaddition
6	tetrazine	alkene	Diels–Alder [4+2] cycloaddition with inverse electron demand
7	tetrazole	alkene	1,3-dipolar cycloaddition
8	dithioester	diene	hetero-Diels–Alder cycloaddition
9	anthracene	maleimide	[4+2] Diels–Alder reaction
11	**thiol**	**alkene**	**radical addition (thio click)**
12	thiol	enone	Michael addition
13	thiol	maleimide	Michael addition
14	thiol	*para*-fluoro	nucleophilic substitution
15	amine	*para*-fluoro	nucleophilic substitution

In this work, the two photon absorption active molecule BAC-M (2,6-bis(4-azidobenzylidene)-4-methyl-cyclohexanone, Figure 1) was chosen and the focus was emphasized on functionalization of this molecule via thiol alkene and Cu-catalyzed [3+2] azide alkyne Cycloaddition reactions.

[7] C. Remzi Becer, R. Hoogenboom, U.S. Schubert; *Angew. Chem. Int. Ed.*; **2009**, 48, 4900 – 4908

3.1.2 Thiol-ene Click-Chemistry

The highly efficient reaction concept of thiols with carbon-carbon double bonds (also abbreviated to “enes”) has already been well known since the beginning of the last century.[8] However, the ability of this chemistry to serve as a “click” reaction was only recognized recently.[9] Majorly, depending on the nature of the substrates, two types of reaction are possible; either the free-radical addition reaction of thiols to enes (both, electron-rich or electron-poor carbon–carbon double bonds), or the base-catalyzed, nucleophilic Michael addition reaction which is normally performed on electron-deficient olefins. (Scheme 1)[5] A large number of products has been reported by using both the reactions of thiols with olefins.



Scheme 1: Free radical thiol-ene reaction and catalyzed thiol Michael addition[5]

3.1.2.1 Free Radical Thiol-Ene Reaction

A series of thiol and ene functional group containing molecules have been evaluated by using conventional as well as high throughput methods and ranked according to their relative reactivity. Norbornenes and vinyl ethers are the most reactive for thiol-ene reactions followed by methacrylate, acrylonitrile, styrene, maleimides and conjugated dienes respectively.[10]

All the non-sterically hindered terminal olefins are able to participate in the thiol-ene reaction, however, the reactivity decreases with decreasing electron density at the carbon-carbon double bond. Hence, electron-donating olefins (e.g. vinyl ethers) or strained cyclic olefins (norbornene) react more easily than electron withdrawing olefins. In case of conjugated double bonds, carbon-centered radicals are very stable and produce radicals that have naturally low hydrogen-abstraction rate constants.

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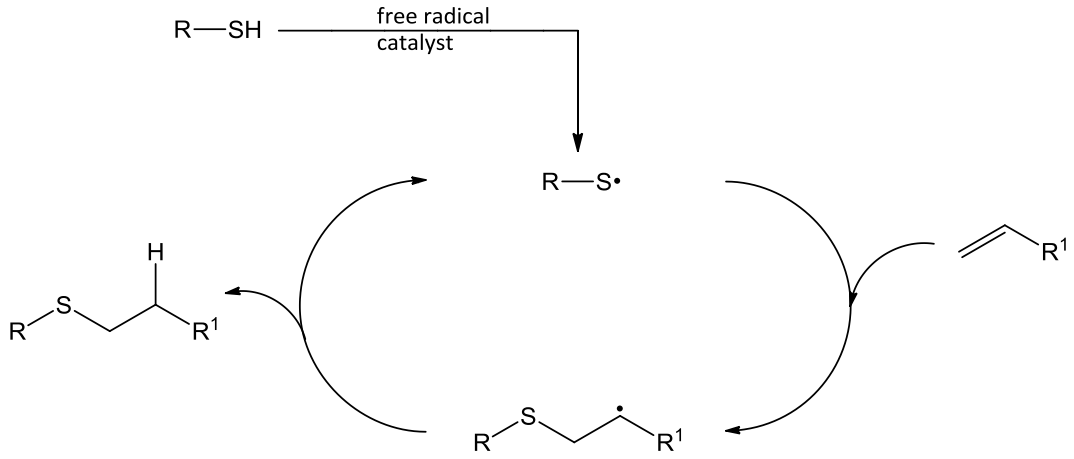
[8] Posner, T.; *Ber. Dtsch. Chem. Ges*; **1905;** 38; 646-657.
[9] Hoyle, C.E.; Bowman, C.N.; *Angew. Chem.*; **2010**, 122, 1584-1617

However, in case of strained or non-conjugated double bonds the addition of a thionyl radical is comparatively rapid.[10] The addition of the thiol to the alkene double bond is exothermic and reaction enthalpies ranges from -10.5 kcal mol^{-1} (for electron-rich double bonds e.g. vinyl ether) to -22.6 kcal mol^{-1} (for the electron-poor double bonds e.g. N-alkyl maleimide).[6]

The position of the double bond can also have a strong influence on the rate of conversion. Hoyle et. al.[9] showed that 1-hexene is much more reactive than *trans*-2-hexene, which is even more reactive than *trans*-3-hexen. The nature of the thiol is not as much decisive for the type of the reaction mechanism taking place i.e. either a free radical reaction or some other catalyst-initiated mechanism takes place. However, the structure of the participating olefin is more decisive for such reactions.



Scheme 2: Mechanism of thiol-ene reaction[7]

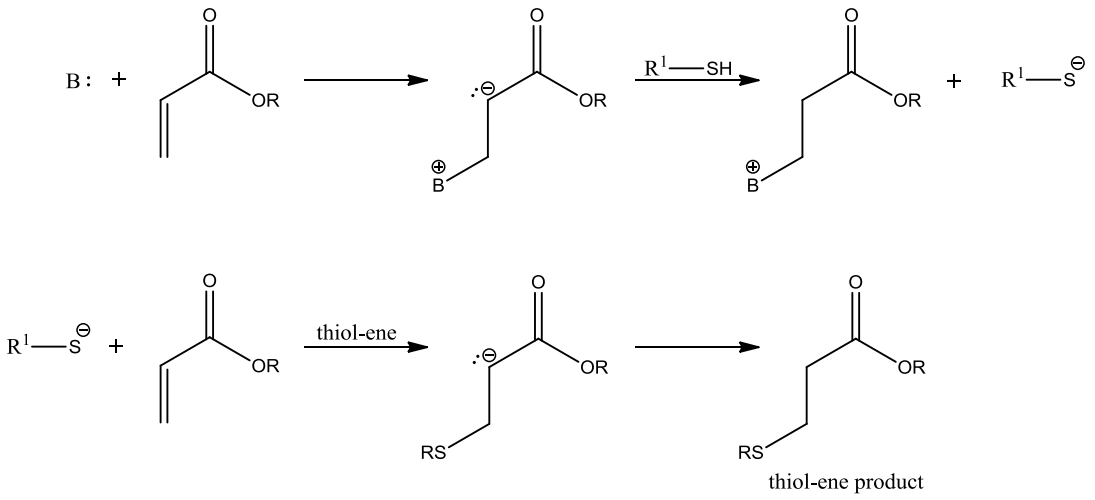
The reaction proceeding through radical mechanism can be either photochemical, thermal or can proceed by some other ways of free radical generation, but the first step always involves the homolytic cleavage of a thiol group to produce is a thionyl radical. This step is called initiation. In the next step this thionyl radical attacks the double bond of an olefin, producing another radical through a propagation step. This intermediate bears a carbon radical, which can abstract another hydrogen radical from further thiol functions and hence a chain reaction starts. This may lead to the formation of polymers or to the reaction with hydrogen radicals produced in the first

[10] Hoyle, C. E.; Lee, T. Y.; Roper, T.; *J. Polym. Sci.*; **2004**; 42; 5301-5338

step, to yield typical thiol-ene addition products. This step of absorption of a proton to the intermediate is called termination step.[11] The reaction mechanism to produce thiol-ene click products[9] or polymers or none of them is totally dependent upon the nature and concentration of the olefins and thiols and also upon the type of initiator used.

3.1.2.2 Thiol-Michael Addition

Besides the thiol–ene free-radical reaction, there is another reaction of a thiol and an electron-deficient ene that also occurs readily (Scheme 1). It is named Thiol-Michael Addition, a reaction which constitutes a key reaction in biosynthesis.[12] Most of these reactions are base catalyzed however, also attempts with metals, Lewis acids[13], primary and secondary amines[9] or even catalyst free reactions[12] were reported. The reactions proceed with very high conversions via a very efficient anionic chain process which is the same like the mechanism of free radical reaction (Scheme 2). The only difference is that instead of radicals, anions are used. The mechanism starts with the addition of the nucleophilic catalyst to the electron poor alkene followed by a proton abstraction of the thiol (Scheme 3). These almost quantitative thiol–Michael addition reactions are finished in very short times with durations of a few minutes or even seconds in some cases at room temperature.

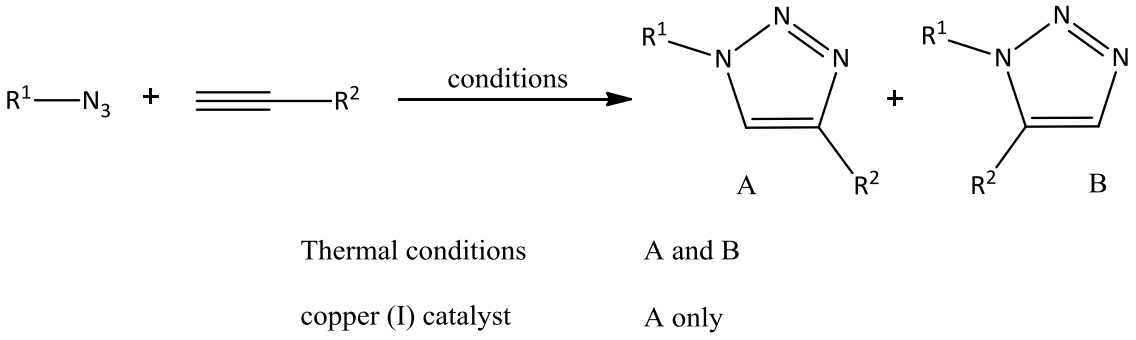


Scheme 3: Mechanism of Thiol-Michael addition (taken from reference nr. 9)

[11] Dondoni, A.; *Angew. Chem., Int. Ed;.* **2008**; *47*; 8995-8997
[12] Movassagh, B.; Shaygana, P.; *Arkivoc*; **2006**; 12; 130-137
[13] Mather, B. D.; Viswanathan, K.; Miller, K. V.; Long, T. E.; *Prog. Polym. Sci.*; **2006**; 31; 487-531

3.1.3 Cu-Catalyzed [3+2] Azide-Alkyne Cycloaddition

Among all reactions associated with the classification of click chemistry, 1,3-dipolar cycloaddition of azides and alkynes is one of the best, fulfilling the conditions of ideal click reactions.[9] This reaction was introduced even before the beginning of last century[6] but Huisgen et al. started intensively studying 1,3-dipolar cycloaddition reactions in late sixties.[6] Since then a lot of reactions have been reported with various functional groups on the side chain for particularly yielding the above mentioned substrates. However, a modern approach of click reactions was presented in 2001 by Sharpless who entitled these reactions as „Crème de la crème of click reactions".[6] The real acceleration of the conversion and the regioselective progress was made a year later as reactivity of these reaction was boosted by using Cu(I) catalysts. 1,2,3-triazoles synthesis is one of the most important reaction of such types where an azide is reacted with terminal alkynes which is also called Huisgen's method (Scheme 4).

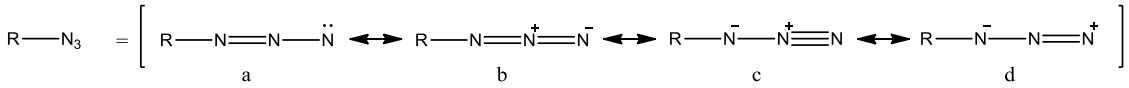


Scheme 4: 1,2,3-Triazol synthesis

The majority of this studied reactions, follow the principle proposed by Huisgen. The reaction proceeds through an aromatic transition state to yield a stereo specific syn addition product by the involvement of two π-electrons of acetylene and four π-electrons of the azide forming a cyclic product. For this reason the formation of the triazoles is also called [π4s-π2s] cycloaddition.

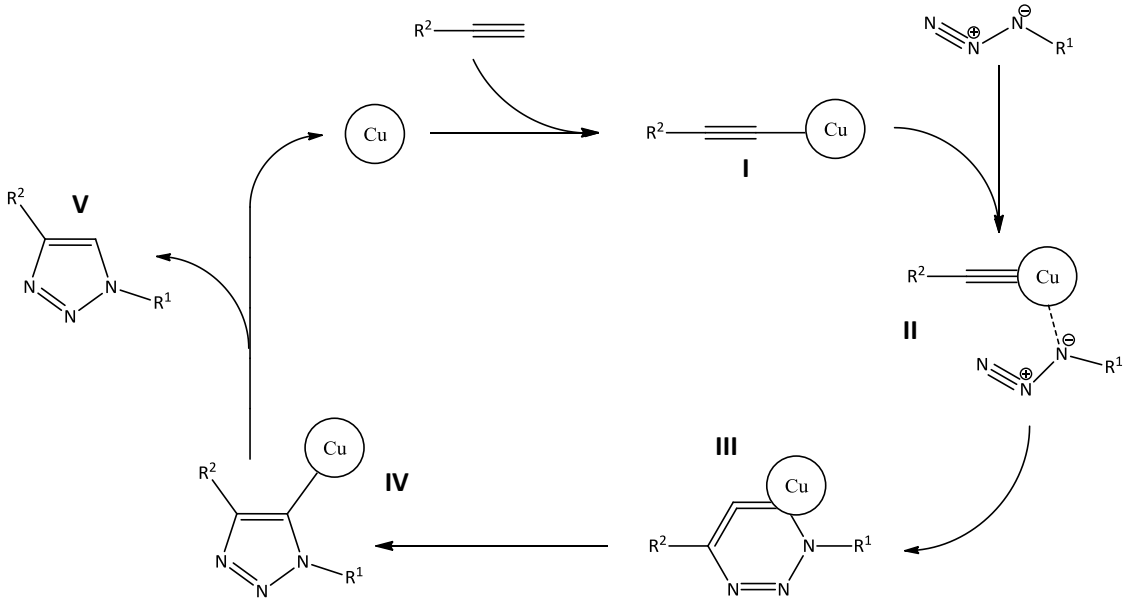
The role of Azide functional group is also important in such type of reactions. Out of four resonance structures in azide, three represent 1,3-dipole propargyl anion type structures, which make it more stable against hydrolysis and intensively inert to

oxygen in air (Scheme 5). Beside these advantages, easy insertion into molecules of variant types makes it highly suitable for click chemistry.[14]



Scheme 5: Resonance structures of the azide functionality, the 1, 3 - dipolar structure (d) explains the reactivity as 1, 3-dipole[14]

The role of Cu (I) salt as a catalytically active component (CuAAC) is also important for azide based click chemistry reactions as it provides significant difference in the reactions. In general, the reaction is accelerated by Cu (I) with a factor of 10^7 whereas the selectivity of formation of 1,4 triazole products (Scheme 4, A) is favored and yields of more than 95 % are reached. Without using Cu (I) as catalyst, the reaction leads to a product mixture of 1,4 - and 1,5 - triazole (Scheme 4, A and B).



Scheme 6: Early proposed mechanism for the CuAAC.

Presumably, the reaction mechanism proceeds via formation of copper acetylide in the first step (**I**). In case of internal acetylene this complex is not formed easily due to the lack of reactivity of internal acetylenes towards Cu(I) salts. In the next step, the

[14] Bräse, S.; Gil, C.; Knepper, K.; Zimmermann ,V.; *Angew. Chem. Int. Ed.*; **2005;** 33; 5188-5246

activation of azide takes place by the coordination with copper to form the complex (**II**). This aspect can also be explained by the regional specificity of Cu(I)-catalyzed complexes. Both reaction components are bound to the copper ion before the bond-formation step takes place the endothermic formation of a six-membered metallacycle (**III**). In the next step, triazole-copper derivative (**IV**) is formed and finally triazole (**V**) is released (Scheme 6).

3.2 TPA – Two Photon Absorption

3.2.1 Historical Background of TPA

In 1931, the German physicist Maria Göppert-Meyer started the prediction of the phenomenon of two photon absorption[3] by forecasting the ability of simultaneous absorption of two photons in some molecules to reach higher excited levels. However, since it required a very high photon density flux no experimental results to prove this could be obtained. In 1961 Kaiser et. al.[15] performed a series of appropriate experiments using laser equipment. Subsequently, the first organic fluorescent dyes and the absorption cross sections of these dyes e.g. of Rhodamine were investigated.[16] With the development of sub-picosecond pulsed lasers in the 1990s more intensified studies of two photon absorption commenced. The development of two-photon fluorescence microscopy by Webb et al. and the rapid implementation of this technology boosted the interest in multi-photon processes till then.[17] Meanwhile, a variety of applications based on the principle of two-photon absorption are evolved. In the late nineties, the first chromospheres were optimized according to TPA ability. The driving force was mainly due to the application of these substances in military technology such as optical power limiting and high density optical data storage. A very modern application of TPA is 3D micro fabrication with selective photocatalyzed polymerization e.g. micro-structures can be built.[18] (Figure 2)

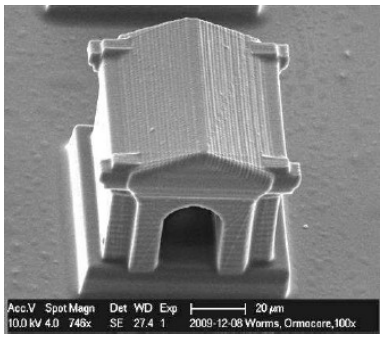


Figure 2: 3D microfabrication via selective photocatalyzed polymerization

[15] Kaiser, W.; Garrett, C. G. B.; *Phys. Rev. Lett.* **1961**, 7, 229 - 231
[16] Topp, M. R.; Rentzepis, R. M.; *Phys. Rev.;* **1971**; 3; 358-364.
[17] Marder, S. R.; *Chem. Commun*.; **2006**; 131–134
[18] Torgersen, J.;*Masterthesis*; **2010**

3.2.2 Basics of Molecule Spectroscopy

Today a large variety of optical spectroscopy techniques are available, which allows obtaining information about the energetic position of the electronic states of organic molecules. A generalized overview of the molecular energy levels and the possible transitions in a molecule is illustrated by the Jablonski diagram which is presented in Figure 3.

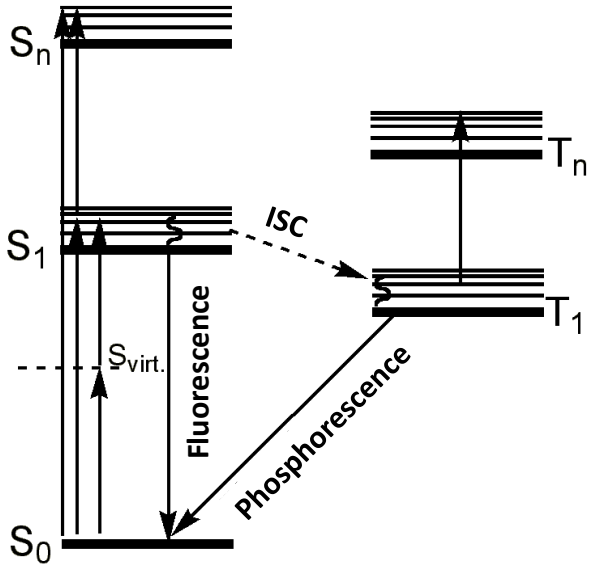


Figure 3: Simplified Jablonski diagram

The principle of electronic states of a molecule and the transitions between them is as followed. By irradiating the molecule with light it is raised from the ground state S_0 to an electronically higher energy level S_1 or S_n representing a singlet state. The intensity (I) of the light beam along its direction of propagation (z) is reduced by the medium due to absorption processes. This can be calculated by the differential equation (1).

$\frac{dI}{dz} = -\alpha I$, with the solution $I(z) = I(0) - e^{\alpha z}$ **(1)**

After absorption, when the molecule is raised from the ground state (S_0) to the excited state (e.g. S_1), the oscillation energy is transferred without radiation (internal conversion) until the molecule reaches the lowest vibrational level of the S_1 state again. Afterwards different transitions of the excited system may occur. Either it will

return with the release of energy in the form of fluorescence radiation back into the ground state, or it can undergo intersystem crossing (ISC) in which the molecule passes into the triplet state (T_1). During this transition the multiplicity is changed by spin conversion. By absorption of additional photons, the molecule can shift to other higher levels (Sn or Tn). This process takes place consecutively and can eventually lead to the destruction of the dye (photobleaching).

3.2.3 State Model of TPA

By low intensity light just the absorption of a single photon is possible. In contrast, light of higher intensity allows under certain circumstances, the simultaneous, non-resonant absorption of several photons. The transmitted intensity (I) along the propagation direction (z) of light turns out to: α, β, and γ are the absorption coefficient for the case of one-, two-and three-photon absorption. In case of the simultaneous absorption of two photons equation (2) it can be canceled after second term. The intensity of the signal is thus proportional to the square of light intensity. It is therefore a nonlinear optical process.

$$\frac{dI}{dz} = -\alpha I - \beta I^2 - \gamma I^3 - \ldots \qquad \textbf{(2)}$$

The TPA is a non-linear optical process, in which two photons are absorbed simultaneously to raise a molecule in an excited state. In other words with TPA an excited state can be reached by using photons with half of the energy (or twice the wavelength) that is also required for the corresponding one photon transition.

If TPA is described with a state model, it must be distinguished between centrosymmetric chromophores and non.-centrosymmetric dipolar chromophores. In this work we used only centrosymmetric chromophores so this process is reported in detail.

All static dipole moments are zero in centrosymmetric molecules. Therefore, the three states of this model have alternating symmetries: The wave functions for the ground

state (g) and the final state (f) are even (symmetric relating to an inversion center), whereas the wave function for the intermediate state (i) is odd (anti-symmetric).

One-photon transitions via electric-dipole are allowed for both (g)↔(i) and (i)↔(f). In the case of TPA, the optical frequency (v) is out of resonance with both of these transitions, but it creates a nonstationary state that is a superposition (or mixture) of (g) and (i), which only exists while the chromophore experiences in the field of the first photon (about 5 fs). In this virtual state the induced polarization is decoupled from the intermediate state by a frequency difference that corresponds to the energy $\Delta = E_{gi} - hv$. Due to the transient occurrence of (i) with uneven parity in this overlay, a second photon of frequency (v) can induce the transition of electric dipole to the final state (f) with even parity. Therefore the transition (g)↔(f) is allowed for TPA, while OPA would be forbidden.

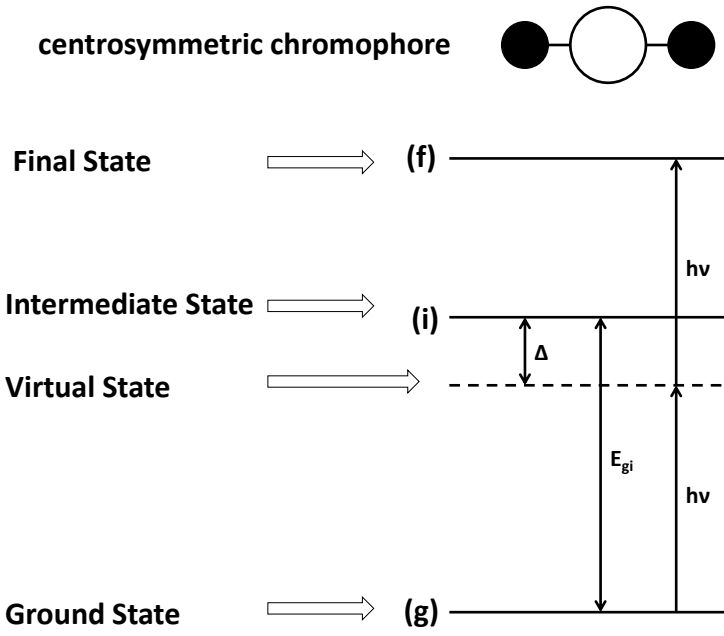


Figure 4: Energy level diagrams for the essential states in centrosymmetric chromophores.[19]

[19] Pawlicki, M; Collins, H. A.; Denning, R. G.; Anderson, H. L.; *Angew. Chem.*; **2009**; 121; 3292-3316

3.2.4 Structure of TPA Chromophores

Since the first experiments to confirm the phenomenon of TPA experimentally, only a few research groups have tried to find out more about the structural features of TPA molecules. In the late 90s of last century, a few research groups began with the targeted synthesis of TPA dyes. Initially, the emphasis has been placed on thiophene, stilbene and bisstyrylbenzole.[20]

It turned out that the chromophores need to have a high symmetry in the ground as well as in the excited state. Furthermore, the dyes should have an extensive π-system to allow a sufficiently large polarization of the molecule. Electron-withdrawing (A) and electron donating (D) groups should be bound to the π-system in a way that a highly symmetric but well-polarizable chromophore is formed. Two well suited structural motifs for TPA chromophores are the [D-A-D] and [A-D-A].[21] (Figure 5)

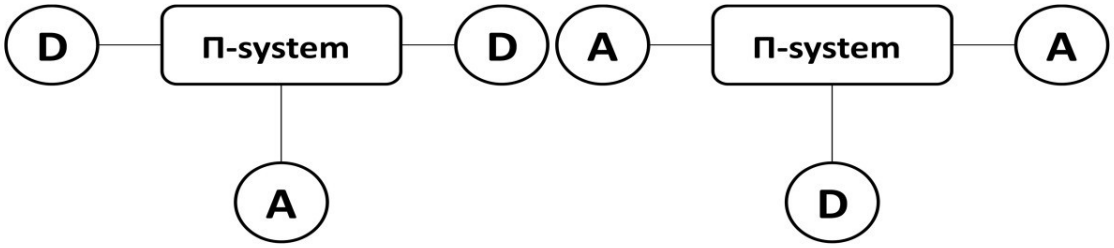


Figure 5: Design concepts of TPA chromophores according to Marder et. al

3.2.5 TPA Cross-Section, Measurement and Unit

The Beer-Lambert law for one-photon absorption (OPA) changes from

to ——— for TPA with residual light intensity as a function of path length or cross section x as a function of concentration c and the initial light intensity I_0. The absorption coefficient α now becomes the **TPA cross section** β.[22]

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[20] Kröner, M.; *Dissertation*; **2004**

[21] Albota, M.; Beljonne, D.; Bredas, J.-L.; Ehrlich, J. E.; Fu, J.-Y.; Heikal, A. A.; Hess, S. E.; Kogej, T.; Levin, M.; Marder, S. R.; McCord-Maughon, D.; Perry, J. W.; Rockel, H.; Rumi, M.; Subramaniam, G.; Webb, W. W.; Wu, X. L.; Xu, C.; *Science;* **1998**; *281*; 1653–1656

[22] http://en.wikipedia.org/wiki/Two-photon_absorption

The molecular two-photon cross-section is usually quoted in the units of Goeppert-Mayer (**GM**) where 1 GM is 10^{-50} cm^4 s $photon^{-1}$.[23] Considering these units, one can see that it results from the product of two areas (one for each photon, each in cm^2) and a time (within which the two photons must arrive to be able to act together). The large scaling factor is introduced so that two-photon absorption cross-sections of common dyes will have convenient values.

Figure 6 shows an overview of some TPA chromophores and the measurement conditions.

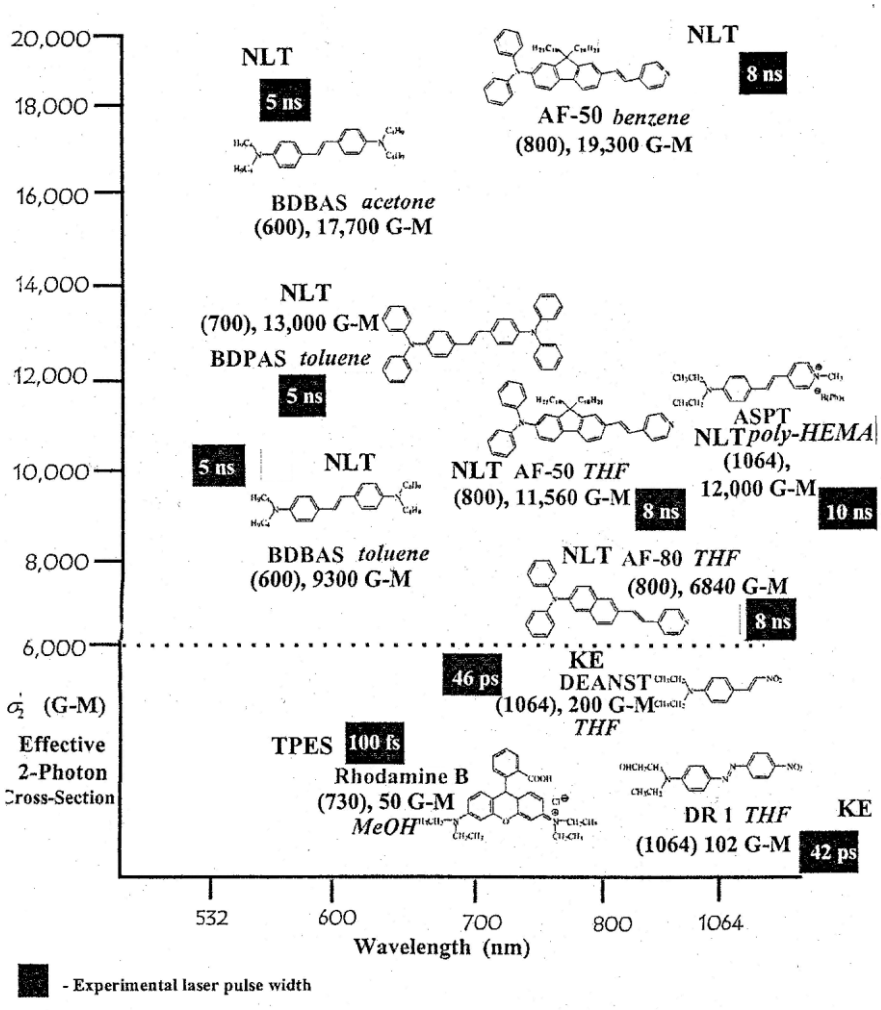

Figure 6: Application of the TPA cross-section for the excitation wavelength for some chromophores, indicating the structure and measurement conditions[17]

[23] PowerPoint presentation @ chem.ucsb.edu www.chem.ucsb.edu/~ocf/lecture_ford.ppt Link

4 Results and Discussion

The aim of this work was the functionalization of the polymer cross linking agent BAC-M (2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone) with the two fluorescence dyes 5-(dimethylamino)naphthalene-1-sulfonyl chloride (dansyl) and 1,6,7,12-tetrachloroperylene-3,4,9,10-tetracarboxylic acid dianhydride (perylene). Firstly, these two dyes were derivatized with a functional group such as a thiol[24] or an azide[25]. Then, some attempts were made to use these functional groups for the synthesis of molecules via thiol-ene reaction[9], Michael addition[12] and azide-alkyne[26] reaction.

In order to identify appropriate reaction conditions, different model reactions were performed on BAC-M and resembling molecules. In this regard, the azide-alkyne labeling reaction was identified as the most suitable for such reactions.

The second part of this work deals with the selective grafting of the obtained dye onto hydrogels. The free azide groups of these molecules decompose upon excitation with ultraviolet light to yield nitrenes. Besides proton abstraction, these highly reactive intermediates were found to form a covalent bond between the functional molecule and a polymer matrix via insertion mechanism.[27]

Herein the synthesis of the products and some unexpected issues faced during the synthesis are discussed. For the characterization of the molecules mainly NMR spectroscopy was used. The characterization of the free azides was evaluated using FT-IR spectroscopy and for control of grafting, laser scanning microscope (LSM) technique was used in collaboration with Vienna University of Technology.

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[24] Robinson, C.; Hartman, R. F.; Rose, S. D.; *Bioorganic Chem.*; **2008**, 36, 265-270

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

[26] Li, C.; Henry, E.; Kumar Mani, N.; Tang, J.; Brochon, J.C.; Deprez, E.; Xie, J.; *J. Org. Chem.*; **2010**, 2395-2405

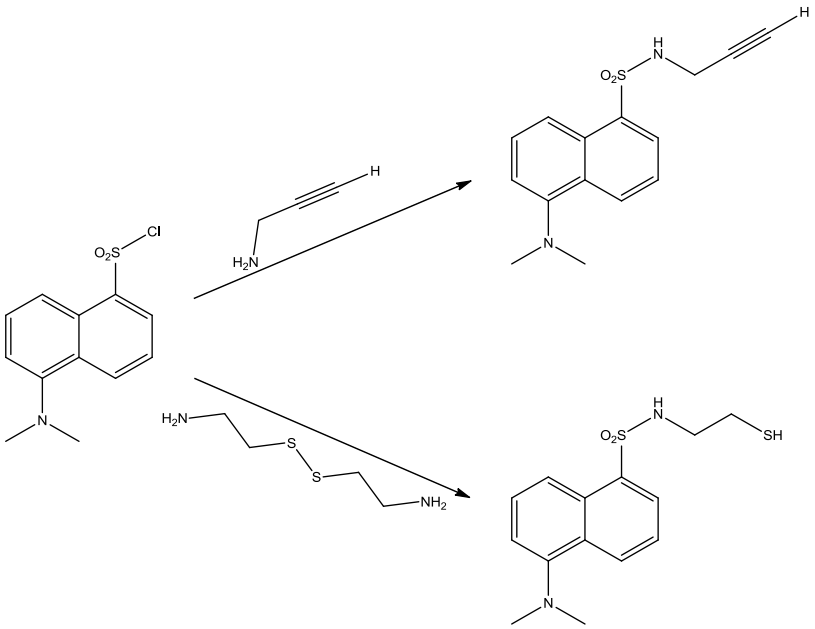
[27] Rupp, B. ; Ebner , C.; Rossegger, E.; Slugovc , C.; Stelzer, F.; Wiesbrock, F,; *Green Chem.;* **2010,** 12, 1796-1802

4.1 Functionalization of pH Sensitive Dyes

4.1.1 Functionalization of Dansyl Dye

In selective reaction of cystamine with dansyl dye, 30:1 mixture of water and acetone was used. The reaction was performed in a Schlenk tube at room temperature. For a better solubility of the cystamine, a little amount of sodium hydrogencarbonate was added. The pH was set to a value between seven and eight and adjusted using HCl (2 M) or $NaHCO_3$ (saturated solution) if required.

The disulfide bond was cleaved using zinc and acetic acid in ethanol. The stirring of the reaction mixture during this reaction is very important because of possible formation of zinc precipitate in the tube. This reaction performed well, an overall yield of 86 % was obtained but the product was not stable due to reformation of disulfide bond from free thiols.



Scheme 7: Functionalization of dansyl chloride with propargylamine and cysteamine

In the next step, the reaction of propargylamine with dansyl dye was performed in a Schlenk tube at room temperature. The reaction was performed under nitrogen atmosphere with dry solvents to avoid the formation of hydrolyzed product i.e. 5-(dimethylamino)-1-naphthalenesulfonic acid.

For the initial step, the dansyl chloride was dissolved together with triethylamine in dry dichloromethane and after adding the propargylamine the solution immediately turned from dark yellow to yellow fluorescent.[25] The reaction worked very well with 98% overall yield. No further issues regarding stability of the products were observed in this case.

The product was characterized by using ^{1}H NMR spectroscopy.

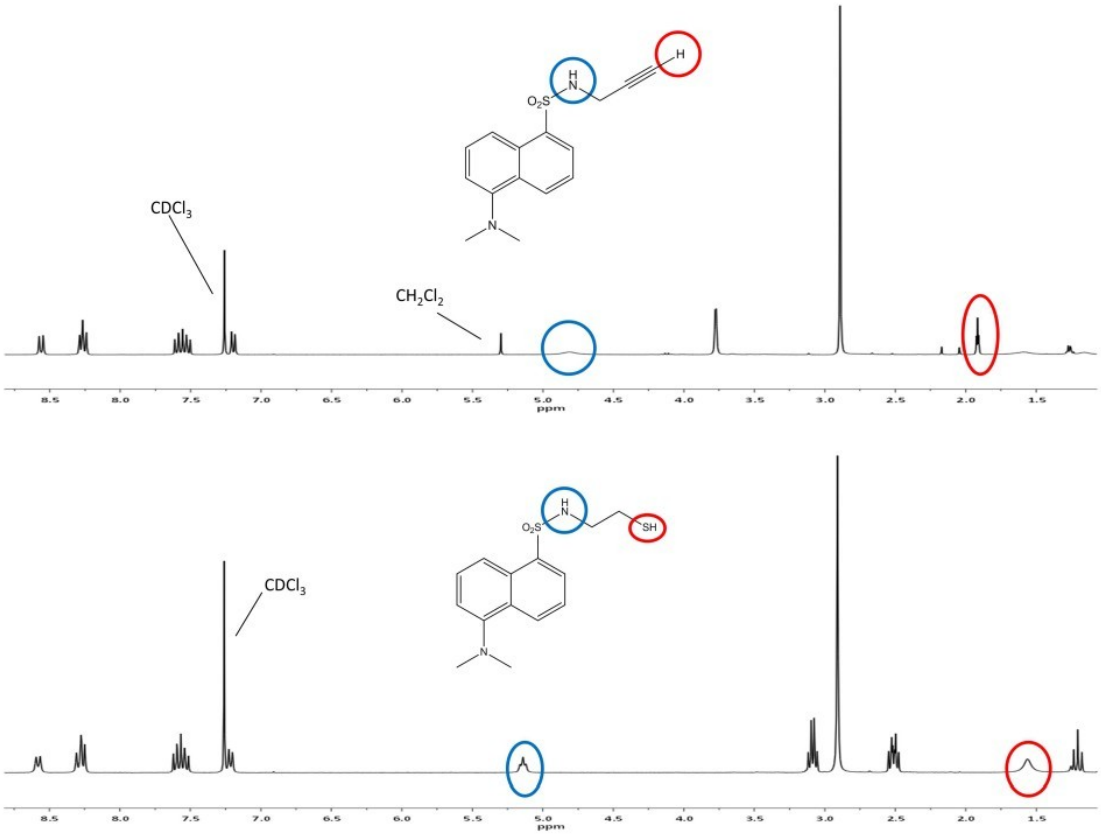


Figure 7: ^{1}H-NMR of dansyl propargylamine and dansyl cysteamine

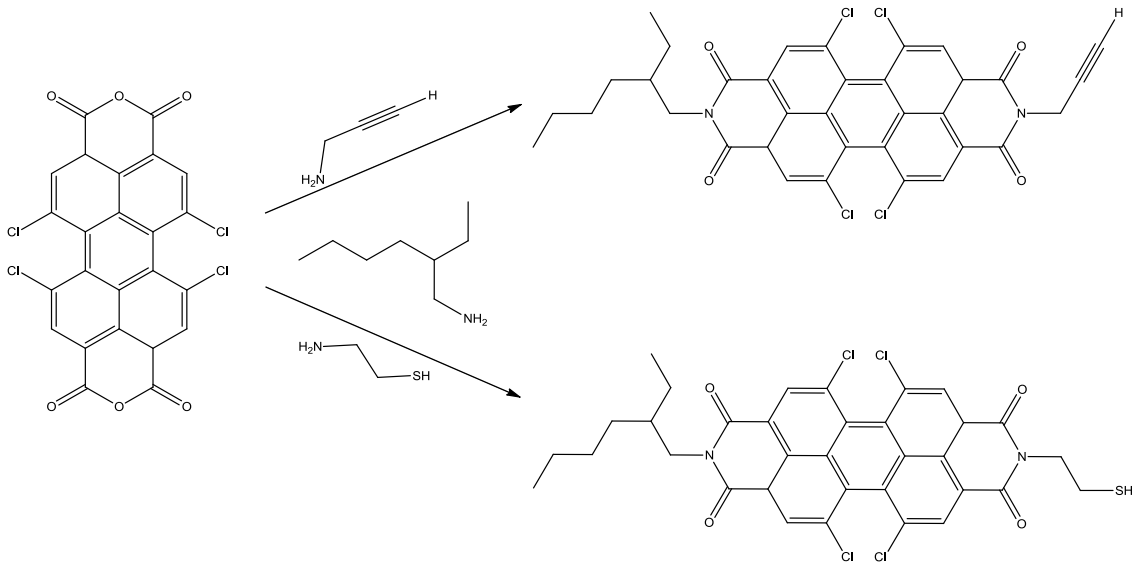
The spectra shown above (Figure 7) illustrate the results of the functionalization reaction of dansyl chloride with propargylamine and cystamine executed during this work. Both spectra were recorded in $CDCl_3$ at 25 °C.

The NMR-characterization showed the exact peaks in good ratio and connection to each other and leads to the conclusion the products were obtained without impurities.

4.1.2 Functionalization of Perylene Dye[28]

For the functionalization of perylene dye two reactions were performed. Both reactions were performed under the same conditions. Perylene and toluene were put in Schlenk tubes. Subsequently, in one tube 2-aminoethanethiol and in the other tube propargylamine together with the 2-ethyl-hexylamine were added simultaneously. The color of the reaction mixture turned dark red.

The mixture was heated to 110 °C to dissolve all the educts. After one day CH_2Cl_2 was added and transferred into another flask to remove the solvent under vacuum. For further purification a column chromatography with silica gel was done. It was started with pure CH_2Cl_2 after color change acetic acid was added.



Scheme 8: Functionalization of Perylene with Propargylamine and Cysteamine

Previously, various methods have been described to obtain such functionalized dyes. For example via desymmetrization with KOH and NH_3 in DMF[29] and another path yielded the products via saponification with KOH and CH_3COOH/HCl.[30] In both cases the yield was not satisfactory. Hence, one pot reaction was performed to overcome this issue.

[28] Gallas, K.; unpublished results
[29] Tröster, H.; *Dyes & Pigments*; **1983**; *4*; 171-177
[30] Langhals, H.; *Helvetica Chimica Acta*; **2005**; *88*; 1309 - 1343

In this regard, the addition of 2-ethyl-hexylamine was found necessary to increase the solubility of perylene because solubility of perylene is only possible at higher temperatures without using amine in solvents like imidazole, toluene or NMP. At first, a few reactions were performed using all of these solvents but toluene figured out to be the best due to its working up procedure after the reaction as compared to NMP or imidazole.

30% obtained final yield of this reaction was impressive considering the possibility of formation of three major products. At one side either the ethylhexylamine or the aminoethanethiol can react with perylene respectively with the propargylamine. The other possibility which was also most desirable, is the connectivity of the linker at one side and ethylhexylamine on the other side of perylene.

For the characterization of products ^{1}H-NMR was recorded.

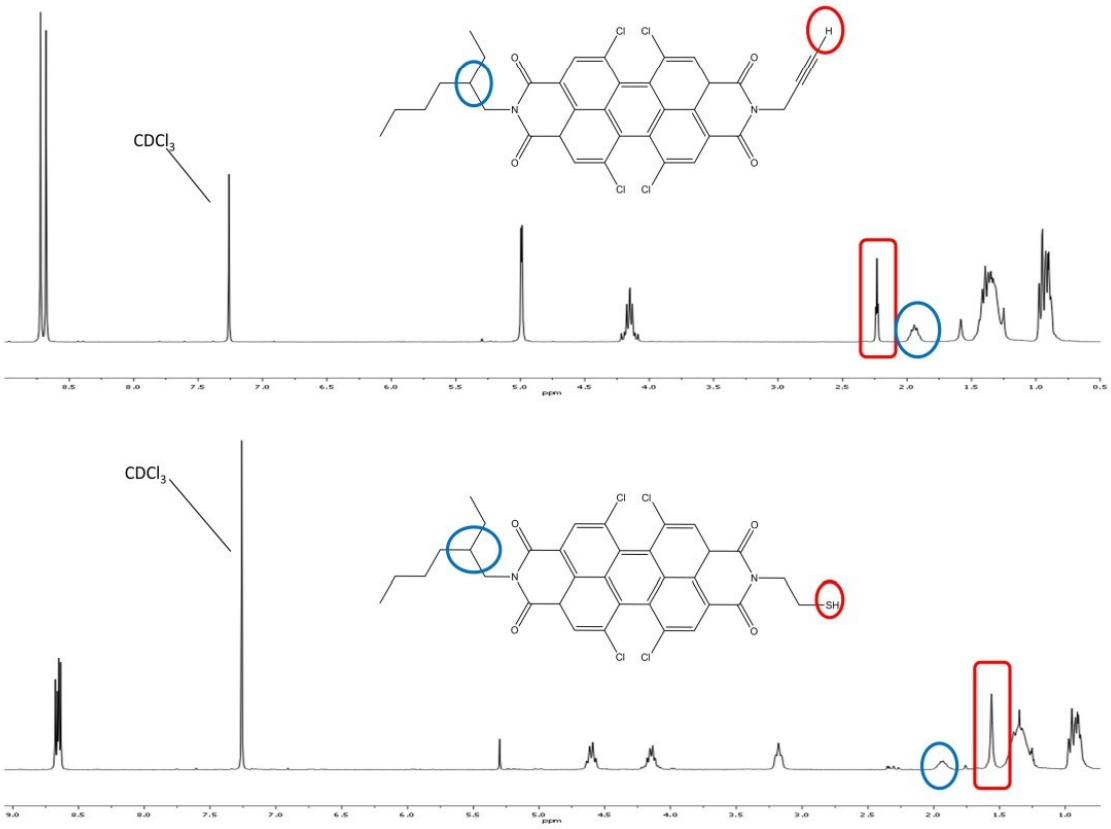


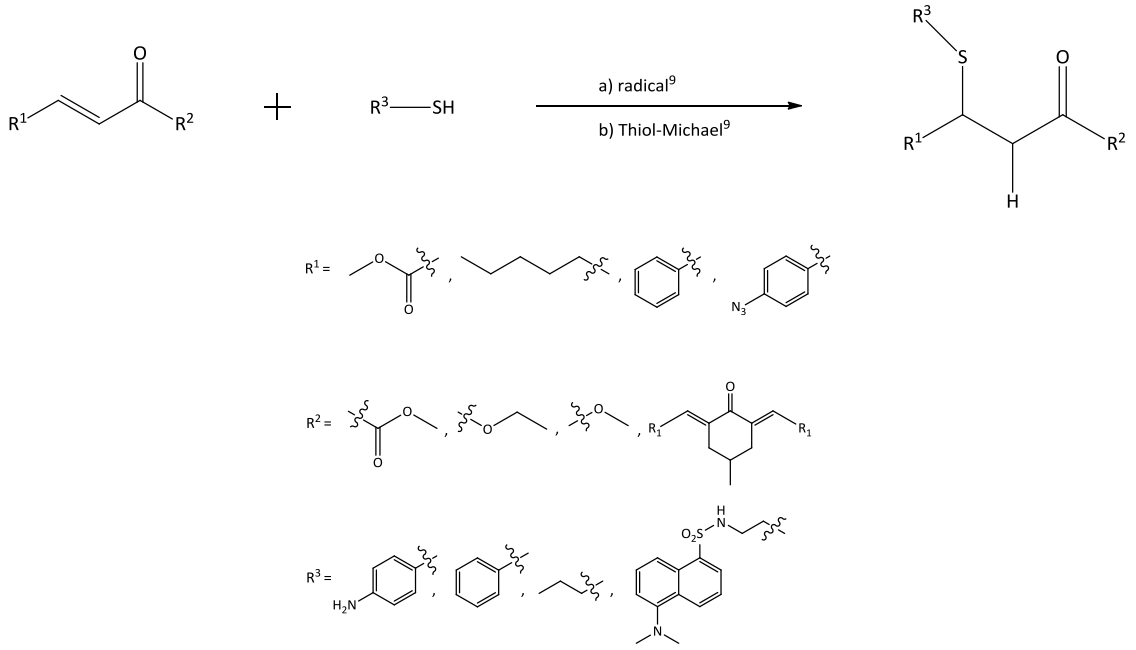
Figure 8: ^{1}H-NMR of perylene propargylamine and perylene cystamine

The spectra (Figure 8) illustrate the results of the functionalization reaction of perylene with propargylamine and cysteamine. Both spectra were recorded in $CDCl_3$.

In both cases the spectrum appears as expected, specially the spectra of propargylamine functionalized perylene. All peaks are in correct ratio with others. Also the Cysteamine functionalized perylene bears no unusual features in the NMR spectrum; however, the isolated product was not completely purified.

4.2 Thiol-Ene Labeling of Compounds

In the first approach we focused on addition reaction of thiols to various olefins. This reaction is also favorable due to weak sulfur–hydrogen bonds in thiols. In past, quantitative yields of the products using this technique with a large variety of materials under various reaction conditions are reported.[9]



Scheme 9: Synthesis for preparation of products via thiol-ene coupling. a) Free radical and b) Michael addition.

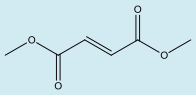
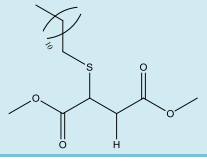
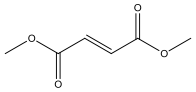
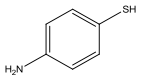
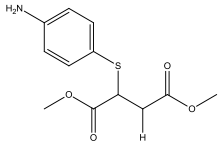
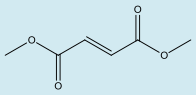
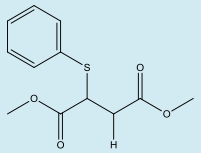
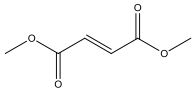
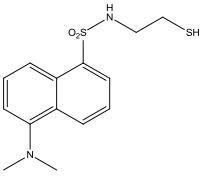
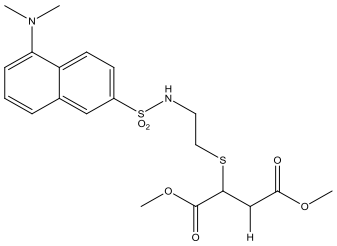
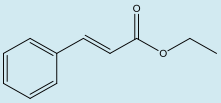
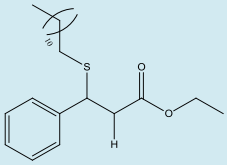
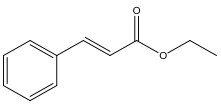
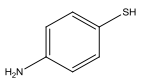
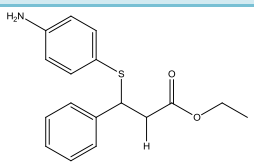
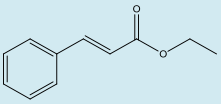
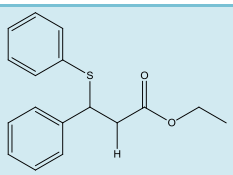
Original photo induced thiol–ene reactions utilized abstraction-type photo initiators, such as benzophenone or benzoyl peroxide. These precursors used the excited triplet state of the benzophenone to abstract hydrogen from the thiol and thus initiated the reaction.[9]

In this specific case of a photo initiator it was not possible to get required product because of the quenching effect of the used educts. Hence, two other approaches have been pursued. On one hand a nucleophilic addition of the thiol to an unsaturated carbonyl compound was performed which is also called Michael addition.[12] On the

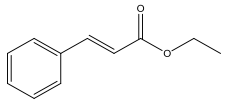
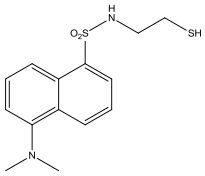
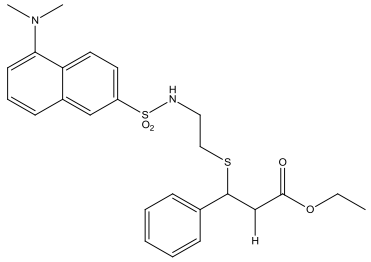
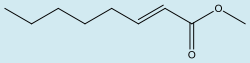
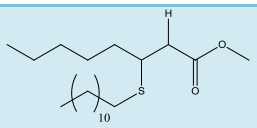
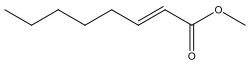
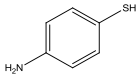
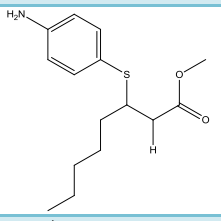
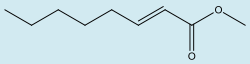
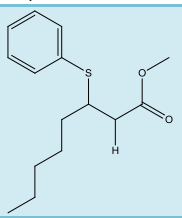
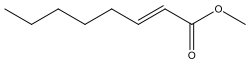
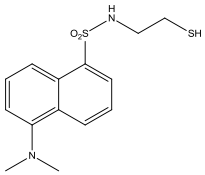
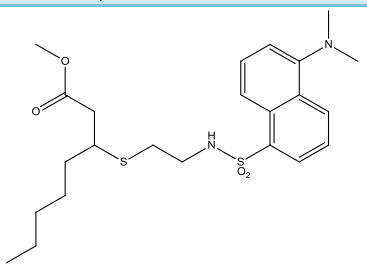
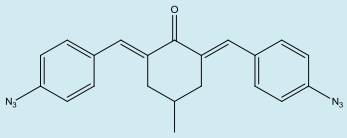
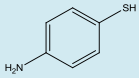
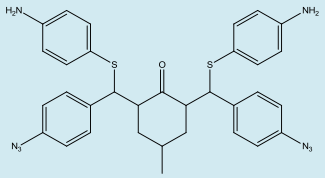
other hand a thermally initiated free radical addition to the carbon-carbon double bonds was performed.[31]

In Table 2 thiol-ene reactions performed in our lab are summarized.

Table 2: Performed reactions with olefins and thiols and expected products.

Entry	Olefin	Thiol	Product
1			
2			
3			
4			
5			
6			
7			

[31] Lowe, A., B.; *Polym. Chem.*; **2010**; 1; 17-36

Entry	Olefin	Thiol	Product
8			
9			
10			
11			
12			
13			

4.2.1 Thiol-Ene Labeling via Free Radical Reaction

Initially, different free radical reactions with two different radical initiators were tested. In the first step, few reactions were performed with BPO (benzoyl peroxide, Figure 9, **I**). BPO is an organic compound of the peroxide family. The bond between the two oxygen atoms is labile and breaks under energy consumption, for example by

elevated the temperatures or light exposure whereas the thermal decomposition starts at 80 °C.[32]

Various olefins and thiols presented in Table 2 were reacted together to get thiol-ene addition products. The reaction procedure is as followed: A Schlenk tube was charged with olefin and thiol dissolved in toluene. Subsequently BPO was added and the reaction mixture was heated to 110 °C. The reaction progress was monitored via TLC but in none of the reactions an addition product was observed even after 72 hours.

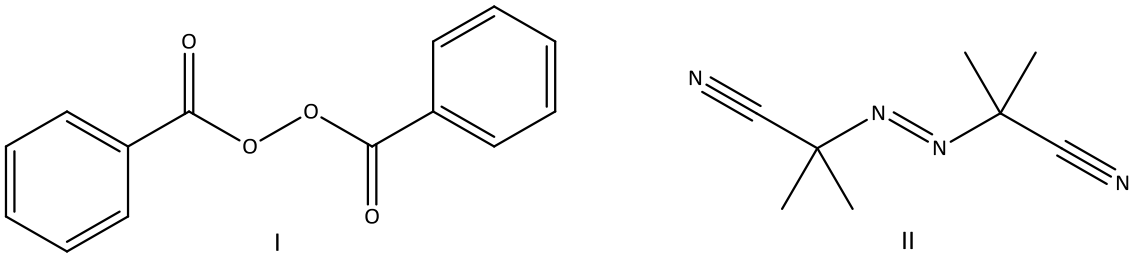


Figure 9: The two radical initiators BPO (benzoyl peroxide, I) and AIBN (azo-bis-(isobutyronitril), II)

In the next approach AIBN (azo-bis-(isobutyronitril), **II**) was used instead of BPO as radical initiator. AIBN (Figure 9, **II**) is an organic compound from the family of diazo compounds. It decomposes normally at a temperature above 25 °C and releases molecular nitrogen and two isobutyronitrile radicals. The formation of a stable nitrogen species is the driving force for the decomposition reaction which liberates free radicals.[33]

Using AIBN, the reactions were performed in chloroform as a solvent. The respective olefin and thiol were dissolved in the solvent. Here, it was necessary to use as little solvent as possible for the stabilization and enhanced activity of free radical initiator. The substrates were added in following ratio: 1 eq olefin, 1.5 eq thiol and 0.5 eq radical initiator. After adding the substrates, the reaction temperature was raised to 60 °C and it was stirred for 60 to 72 hours depending on the different educts. The reaction

[32] Entry to CAS-Nr. 94-36-0 at GESTIS database of the IFA
[33] Entry to CAS-Nr. 78-67-1 at GESTIS database of the IFA

progress was monitored via TLC. In all cases where AIBN was used as initiator the desired products were not obtained (Table 2, entries 1-13)

The reactivity of Thiol-ene chemistry to form various products also depends upon the chemical structure of alkenes due to sterical reasons. The nature of olefins can significantly affect reactivity in thiol–ene reactions because of differences in the steric strain and susceptibility of olefin to thiyl radical attack for hydrogen abstraction subsequently. It is proven that electron-rich (vinyl ether) or strained alkenes (e.g. norbornene) react more rapidly than electron-deficient olefins.

4.2.2 Thiol-Ene Labeling via Michael Addition

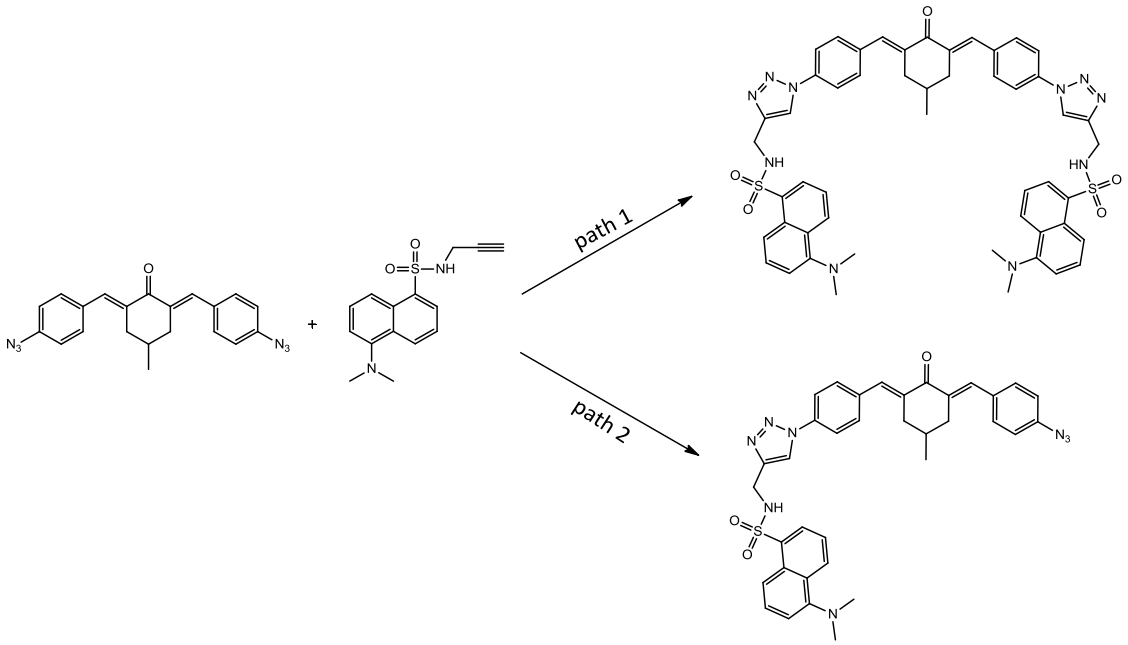
Another possibility for thiol–vinyl reactions between a thiol and an electron-deficient olefin is called Thiol-Michael-Addition.[9] Prerequisite condition is that olefin should be electron-deficient, such as (meth)acrylates, maleimides, α,ß-unsaturated ketones, fumarate esters, acrylonitrile, cinnamates, and crotonates.

In this case no catalyst was used and the reaction was performed in methanol.[12] The educts were reacted with various thiols in a Schlenk tube und stirred for 96 hours. No significant product was detected via TLC at this temperature and later the temperature was raised to 40 °C and then tested the products again via TLC. However, again no significant conversions were detected.

4.3 Azide-Alkyne Labeling

In the second part of this work, copper-catalyzed 1,3-dipolar cycloaddition of the cross linking agent BAC-M and the two fluorescent dyes dansyl alkyne and perylene alkyne was evaluated. The reaction conditions were optimized using the dansyl derivative because of its better solubility in solution. Later the optimized conditions were also applied to the second fluorescent dye, perylene.

Two approaches were pursued to functionalize the BAC-M. On the one hand the dye should be labeled before grafting the molecule on the polymer matrix. (Scheme 10, path **2**) On the other hand it was assumed that the BAC-M is already grafted and there are residual azide groups. For this case a model reaction (Scheme 10, path **1**) was performed.



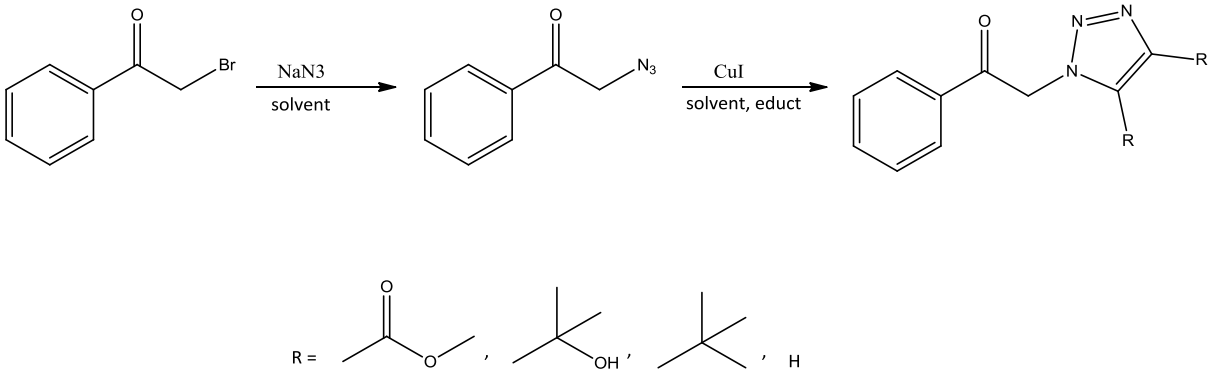
Scheme 10: Formation of di- and mono-labeled BAC-M via 1,3-dipolar cycloaddition.

However, according to the results of Aovsiani et. al.[4] there are no more alkyne groups left after grafting the BAC-M on the polymer matrix. Hence, the assumption that the residual groups of such already grafted bisazides can be used for the formation of the 1,2,3-triazole ring was confuted.

Thus, the reaction shown in path **1**, was used for model substance for the mono-functionalized BAC-M which is labeled on one side with azide group, grafting reaction was performed on the other side of the molecule. (Scheme 10, path **2**).[34]

4.3.1 Model Reactions for Azide-Alkyne Labeling

The molecule selected for the model reaction of azide alkyne labeling has similar structural elements like the cross linking agent BAC-M. Hence it was used to identify the favored reaction conditions for introducing a copper-catalyzed 1,3-dipolar cycloaddition.



Scheme 11: General reaction scheme of the 2 step model reaction, started with 2-bromo-1-phenylethanone and various alkynes.

In the reaction scheme above the general conditions for the cycloaddition are shown. Reactants, solvents and methods were varied in order to get products with high yields. According to literature[35] it is possible to perform a one step reaction of α-bromo ketone with sodium azide.

In the first attempt 2-bromo-1-phenylethanone was dissolved in a *tert*-BuOH/H_2O 1:1 mixture. Subsequently sodium azide, dimethyl acetylenedicarboxylate and copper (I) iodide to catalyze the reaction, were added. The mixture was stirred at room temperature for 24 hours and the reaction progress was monitored via TLC. However no products in the reaction mixture were detected.

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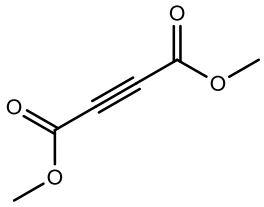
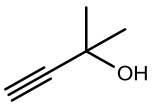
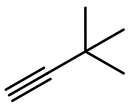
[34] Rodionov, V. O.; Fokin, V. V.; Finn, M. G.; *Angew. Chem. Int. Ed.*; **2005**; 44; 2210 –2215
[35] Kumar, D.; Patel, G.; Reddy, V.B.; *Synlett*; **2009**; 3; 399-402

Therefore, some other solvents were also tried such as THF or CH_2Cl_2, but even by changing the solvents no considerable change in reaction products were detected. Presumably the solubility of educts was the major issue in performing the reaction.

In the next step, it was decided to try the reaction in a stepwise synthesis. In the first step the 2-bromo-1-phenylethanone and the sodium azide were dissolved in a *tert*-BuOH/H_2O 1:1 mixture. The reaction batch was stirred again at room temperature and the progress was monitored via TLC. After eight hours the α-bromo ketone was consumed.

In the next step to the yellow solution dimethyl acetylenedicarboxylate and the copper (I) Iodide as a salt were added. Now the temperature was raised and after one day there was no more azide detected.

Table 3: Overview of various reaction conditions

Reactants	Solvents	Methods
 dimethyl acetylenedicarboxylate	CH_2Cl_2, THF, *tert*-BuOH/H_2O (1:1)	single step reaction stepwise reaction
 2-methylbut-3-yn-2-ol	*tert*-BuOH/H_2O (1:1)	stepwise reaction
 3,3-dimethylbut-1-yne	*tert*-BuOH/H_2O (1:1)	stepwise reaction

These findings from initial tests showed that the variation of solvents and also the different parameters have influence on the conversion. It turned out that a stepwise

reaction with the primary conversion of the bromo ketone to an azido ketone and the subsequent formation of the 1,2,3-triazole ring is the method of choice. The *tert*-BuOH/H_2O = 1:1 mixture used as a solvent was the best choice of solvents because both the bromo ketone and sodium azide dissolve in this mixture of solvents easily.

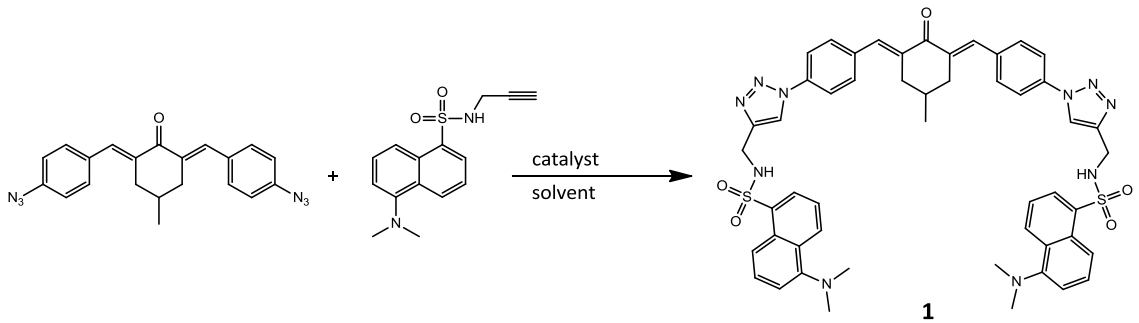
Based on these optimized conditions further reactions with different alkynes were performed which are shown in Table 3. 2-bromo-1-phenylethanone and the different acetylenes reacted smoothly to afford diverse 1,4 disubstituted 1,2,3-triazoles in good yields. After the respective completion of the reaction, the products were isolated by evaporation of the solvent, taking up the residue in CH_2Cl_2 and extraction with saturated $NaHCO_3$.

All the products were characterized via ^{1}H-NMR.

4.3.2 Synthesis of 5-(dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide (Di-Functionalized BAC-M, 1)

Consequently, it was decided to perform the reaction under the same reaction conditions as previously used with the cross linking agent BAC-M and the fluorescent dye dansyl alkyne. Therefore 1 eq. of BAC-M and 1 eq. of dansyl propargylamine were again dissolved in *tert*-BuOH/H_2O 1:1. Subsequently copper (I) iodide was added to the solution and stirred at room temperature.

However, after two days there was no detectable conversion, the only new product was 5-(dimethylamino)-1-naphthalenesulfonic acid. Again the problem was the solubility of the educts. Both, the BAC-M and the dansyl derivative did not dissolve in the *tert*-BuOH/H_2O mixture.



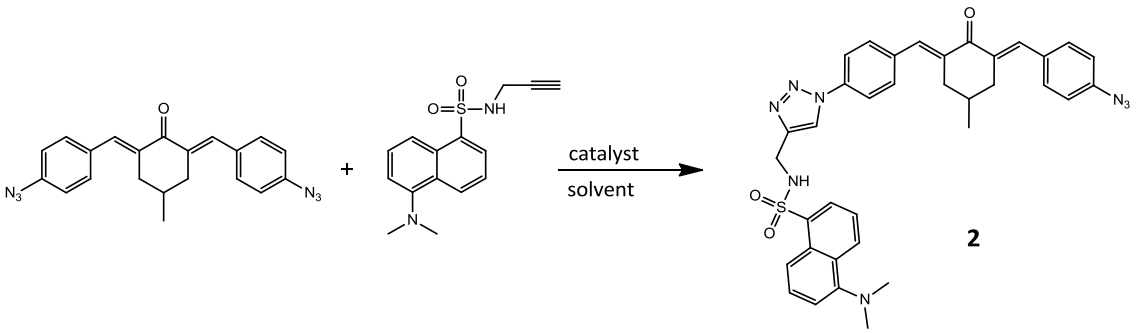
Scheme 12: Synthesis of di-functionalized BAC-M

Hence, in the next step, degassed CH_2Cl_2 was used in place of *tert*-BuOH/H_2O solvent mixture. It is known from earlier that both BAC-M and dansyl propargylamine are well soluble in this solvent. In a Schlenk tube the BAC-M and the dansyl propargylamine were combined in a 1:2 ratio. Then the reaction mixture was heated to accelerate the reaction progress. Subsequently, the copper (I) iodide was added to the yellow solution. The reaction progress was monitored via TLC.

After one day full conversion was detected, therefore, it was quenched with water and extracted with CH_2Cl_2. The product was isolated as dark orange powder. Under these conditions and formation of above mentioned side-reaction, formation of dansylic acid, was avoided and yield of >85% was obtained. ^{1}H-NMR was used for characterization. The spectrum is shown in chapter 4.4.

4.3.3 Synthesis of N-((1-4-((E)-3-(4-azidobenylidene)-5-methyl-2-oxocyclohexylidene)-methyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(dimethylamino)naphthalene-1-sulfonamide (Mono-Functionalized BAC-M, 2)

In the second attempt, mono functionalized BAC-M was prepared (Scheme 13). Therefore it was important to "click" the dye just on one side of the cross linking agent. The other side should retain the azide group to allow TPA grafting as described in chapter 4.7. The emerging problem was the symmetry of the molecule. According to literature even a large excess of alkyne leads to provided ditriazole.[35]

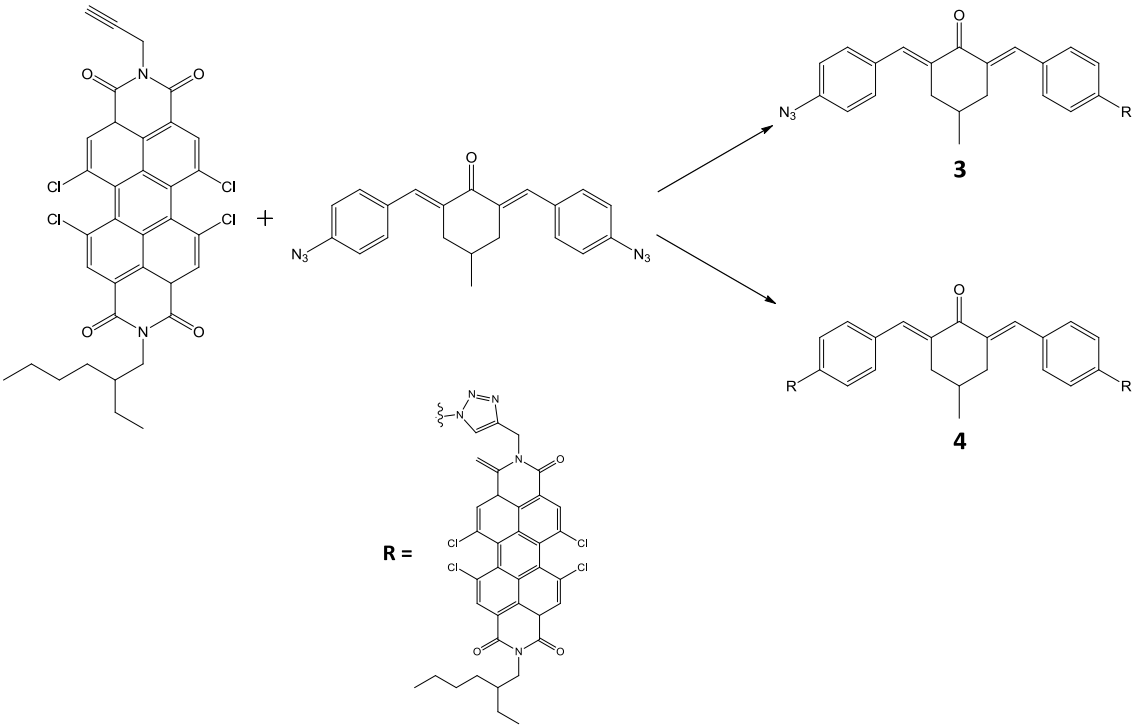


Scheme 13: Synthesis of mono-functionalized BAC-M

However, it was decided to perform the reaction under the same reaction conditions like in the preparation of di-functionalized BAC-M (**1**). Hence, the diazide was dissolved in dry and degassed CH_2Cl_2, and the diazide was added in an excess of ten equivalents over alkyne. Finally the Cu (I) salt was added and the mixture was stirred at 30 °C.
The reaction progress was monitored via TLC. Although the diazide is still visible after one day, which is of no surprise due to excess of azide, there is a new spot at R_f = 0.49. After stirring another day the product was purified via column chromatography and obtained with a yield of >85 %. For characterisation of reaction, ^{1}H-NMR was recorded. The spectrum is shown in chapter 4.4.

4.3.4 Synthesis of Mono- and Di-Functionalized BAC-M with Perylene-Propargylamine, 3 and 4[28]

Based on the results of the reactions with dansyl dye the next step was the implementation of the copper-catalyzed 1,3-dipolar cycloaddition of BAC-M with perylene. Because of the already known fact, that there are no more free azides at the cross linking agent after grafting on polymermatrix[4] the focus was set on the preparation of the mono-functionalized BAC-M. For characterization and comparison with the dansyl labeled molecule the di-functionalized BAC-M was also prepared.



Scheme 14: Synthesis of mono- and di-functionalized BAC-M with perylene-propargylamine

The corresponding reactions were performed in a Schlenk tube under the same conditions like the reactions before. The only difference kept was the ratio of educts. For the synthesis of **3**, 1.3 equivalents of diazide were used with respect to alkyne while in previously 10 equivalents of diazides were used and for the synthesis of **4** the ratio was one equivalent of diazide with respect to two equivalents of alkyne. For both cases distinctly longer reaction time of five days was needed and after purification with column chromatography the obtained yields of **3** and **4** were 24% and 12 %

respectively. For characterization of products ^{1}H-NMR spectra were recorded which are shown in chapter 4.4.

4.4 ^{1}H-NMR Measurements

All ^{1}H-NMR spectra where made in $CDCl_3$. In the Figure 10 the spectra of dansyl propargylamine and the cross linking agent BAC-M are shown to get an overview of the molecule. The characteristic peaks are marked in the spectra and the including H-atoms are accentuated in the molecules.

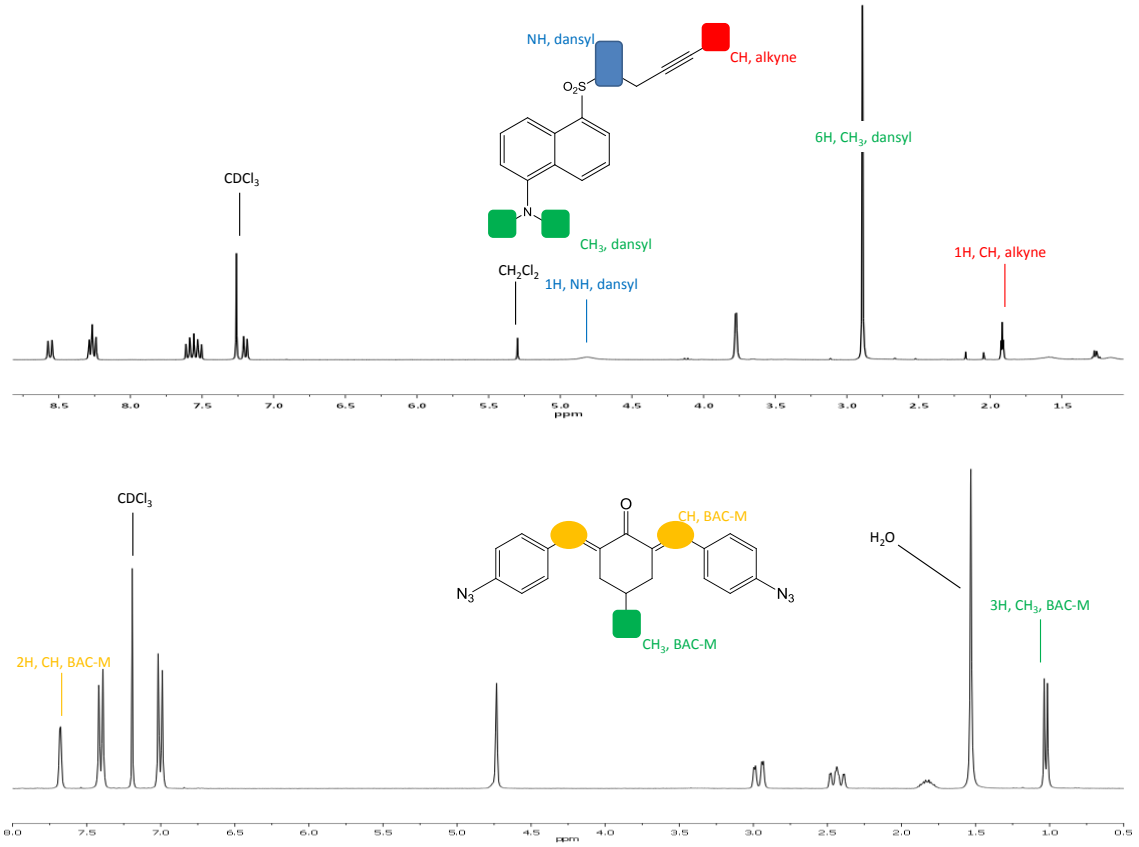


Figure 10: ^{1}H-NMR of dansyl propargylamine and BAC-M

In both cases the spectra show respective peaks as expected in the product. The green marks are helpful in detecting the presence of mono- or the di-functionalized BAC-M derivatized products. The H_2O peak in the lower spectra appears due to 35-45% of water present as stabilizer in BAC-M.

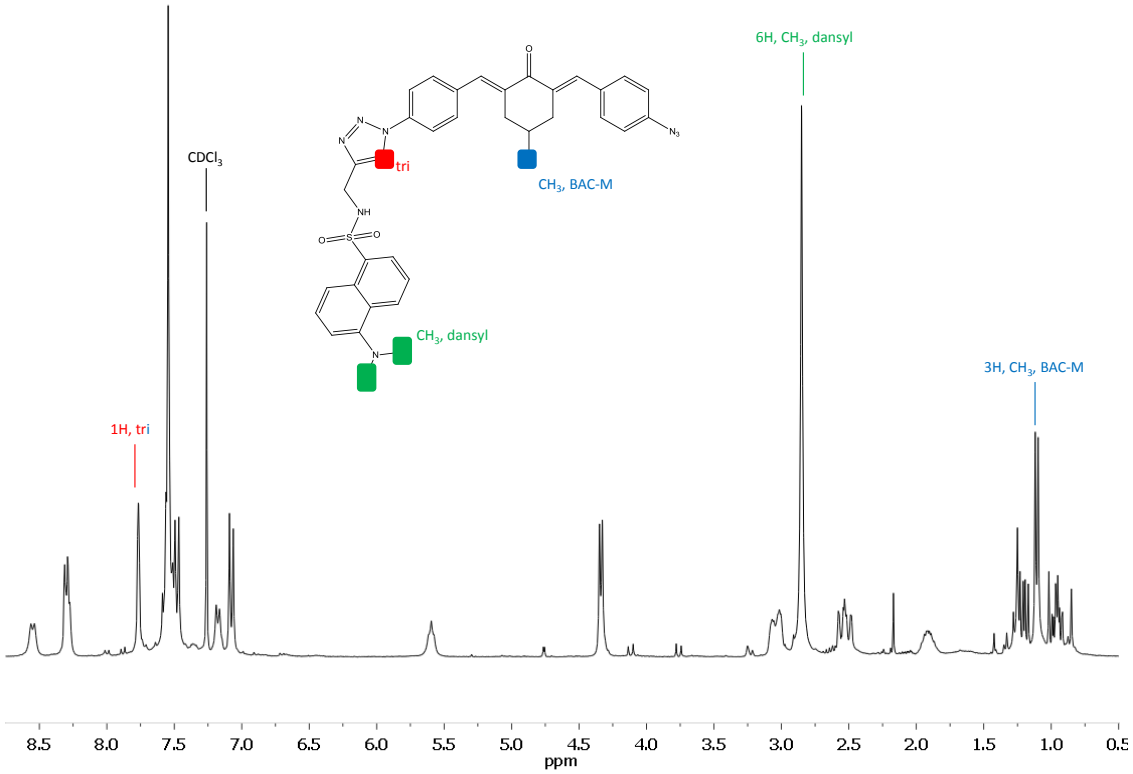


Figure 11: ^{1}H-NMR of mono-functionalized BAC-M with dansyl propargylamine, 2

The spectrum shown above (Figure 11) illustrates the result of one of the functionalization reactions of BAC-M with dansyl propargylamine executed during this work.

Due to the fact, that the blue marked CH_3 groups of the BAC-M and the green marked CH_3 groups of the dansyl propargylamine appear in a ratio one on two, the presented product is the mono-functionalized cross linked.

All the other peaks also arose as expected and are in a good ratio to each other, which also shows that the product obtained through this reaction is relatively pure. The undefined peaks in the region about 0.5 to 1.5 are stabilized products of the used solvents during the reaction.

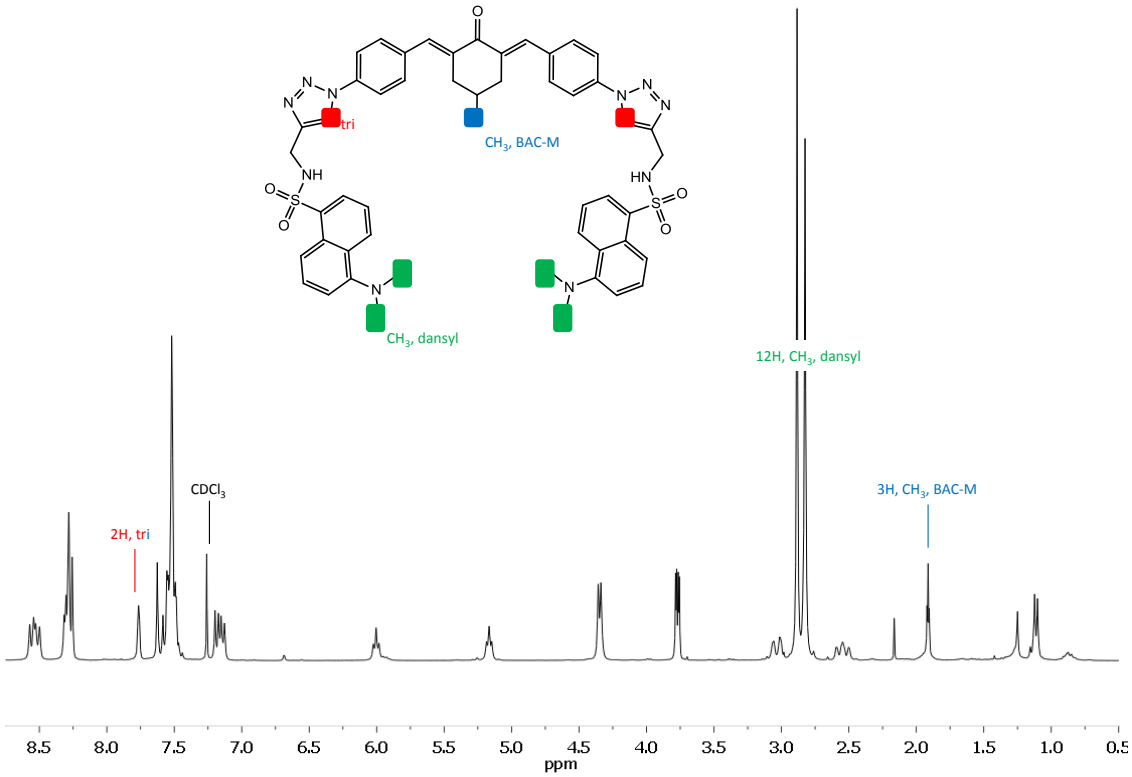


Figure 12: ^{1}H-NMR of di-functionalized BAC-M with dansyl propargylamine, 1

In Figure 12 the spectrum of the di-functionalized BAC-M with dansyl propargylamine is shown. The difference of mono-functionalized product with di-functionalized product is the ratio of CH_3 groups of the dansyl propargylamine and the cross linking agent. The red marked H atoms of the triazole rings clearly indicate the presence of the desired product. Because of the problems with stabilizer in the solvents in the last reaction only pure solvents were used for this reaction and there were no undefined peaks between 0.5 to 1.5 ppm.

Once again all the other peaks appeared as expected and are in a quite good ratio to each other.

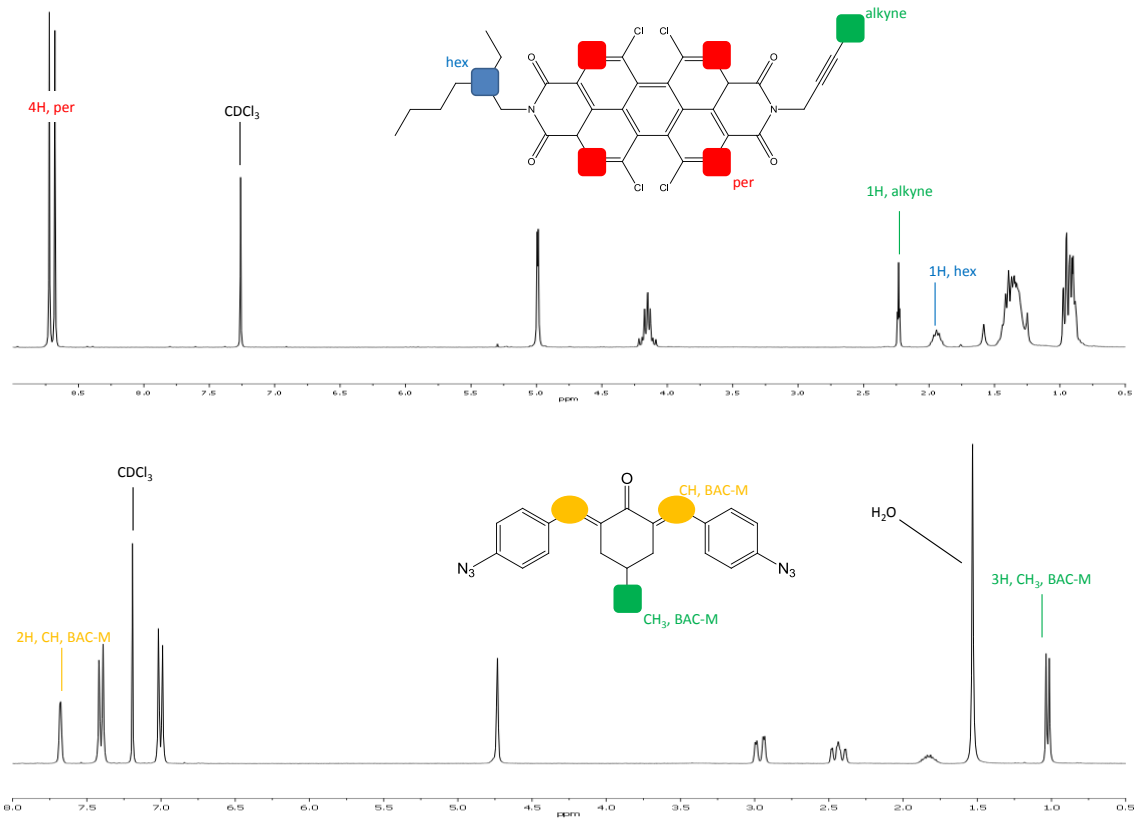


Figure 13: ^{1}H-NMR of functionalized perylene and BAC-M

In the spectra above (Figure 13) the perylene derivative and once again the BAC-M are shown. The red marks in the upper spectra are the characteristic H atoms of the perylene whereof it can be seen either the mono- or the di-functionalized cross linking agent. The peaks around 0.5 to 2.0 are no more stabilization peaks, this are the CH_2 and CH_3 peaks of the ethyl-hexyl chain.

All in all the peaks appeared as expected and they are in a good shape and ratio to each other. Although the reaction time with about seven days is quite very long the product is yielded after purified via column chromatography quite pure.

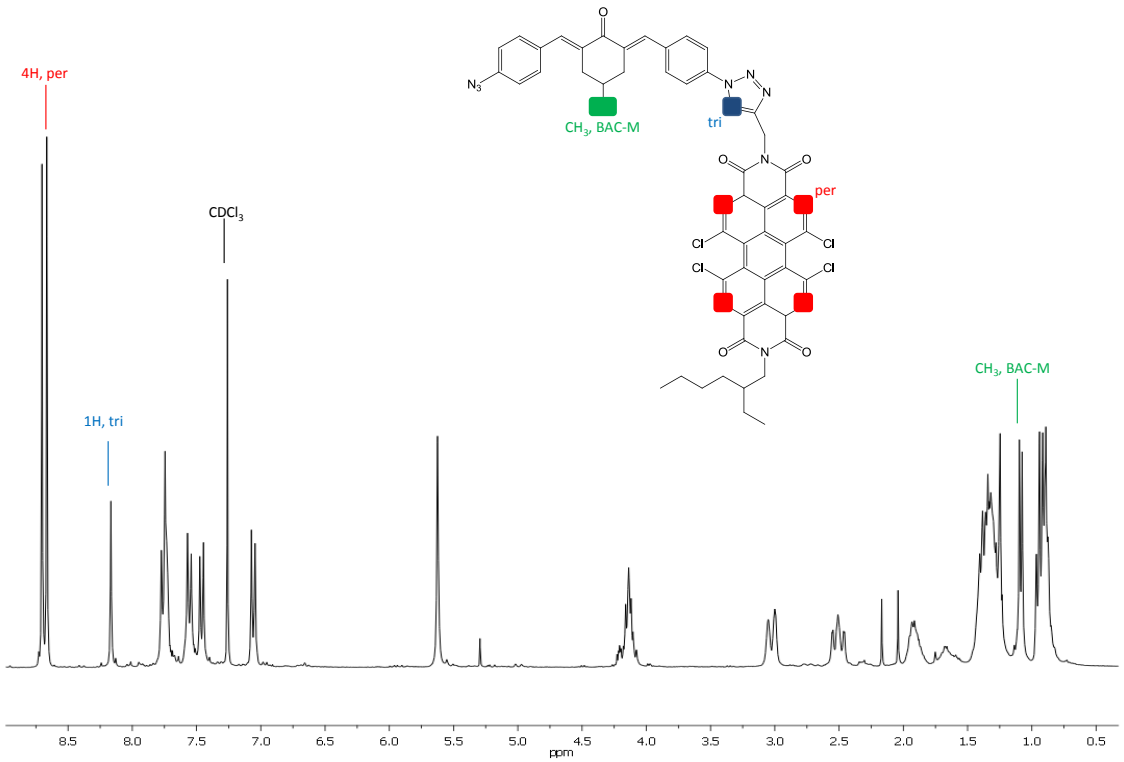


Figure 14: ^{1}H-NMR of mono-functionalized BAC-M with perylene, 3

The last ^{1}H-NMR which is presented here is the spectrum of the mono-functionalized BAC-M with the perylene. In comparing the spectra of Figure 13 with the spectrum above there appears a new peak at 8.17 ppm (blue marked) which is the H atom of the triazole ring. Due to the fact that there are four H atoms from the perylene, one of the triazole ring and three of the BAC-M it is proved that the obtained product is the expected one.

4.5 FT-IR Measurements

It was predicted by evaluating the ^{1}H-NMR spectra of the received products **1**, **2** and **3** that the obtained products are the expected ones but it was not sure if there were still any residual azide groups left because azide groups cannot be detected through ^{1}H-NMR. So a complimentary method was required for characterization.

For this purpose FT-IR measurements were chosen. The spectra were recorded under different conditions like solid products, in solution in CH_2Cl_2 or in DMF. However the best method was to combine the solid product with Nujol in a mortar and to make a mull which was directly placed in the spectrometer. Nujol is a heavy paraffin oil so it is chemically inert and shows a relatively simple IR spectrum.

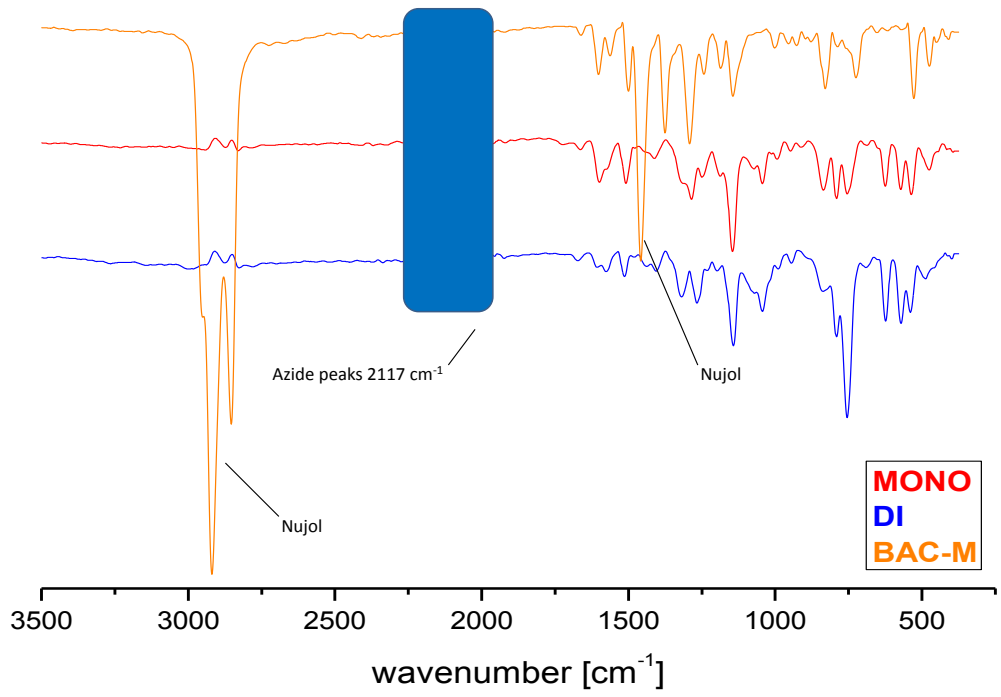


Figure 15: FT-IR spectra of BAC-M as well was mono- and di-functionalized BAC-M the with dansyl derivative (1 and 2)

The spectra shown above (Figure 15) illustrate the results of the functionalization reactions of BAC-M with dansyl propargylamine. The characteristic azide peaks arise at 2117 cm^{-1}.

It was good to observe the peak of BAC-M, which has two azide groups, is relatively bigger compared to the mono-functionalized BAC-M. In the di-functionalized product there are no azide peaks observed. Hence also the FT-IR spectra of the dansylic derivative figure gave the expected results.

When preparing the BAC-M sample it was saturated with Nujol. Hence the Nujol peaks are dominating the spectrum, see peaks between 2950-2800, 1465-1450, and 1380–1370 cm^{-1}.

In the spectra down (Figure 16) the mono- and the di-functionalized BAC-M with the perylene derivative are shown.

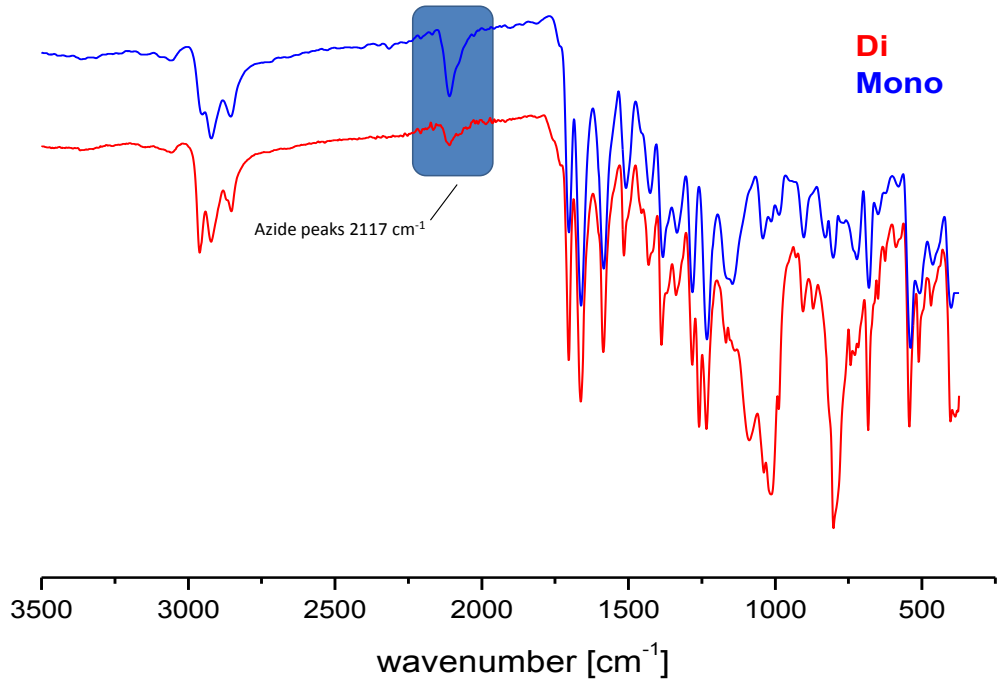


Figure 16: FT-IR spectra of mono- and di-functionalized BAC-M with the perylene derivative (3 and 4)

Also in these spectra it can be seen clearly that the azide peak had decreased.

4.6 Optical Properties of the Compounds

All presented UV/VIS spectra were measured in dichloromethane with a concentration of $3 \cdot 10^{-3}$ g/ml. They were recorded in a quartz cuvette of 1 cm thickness. The excited range reached from 200 nm to 600 nm. However, the predicted range was from 240nm to 600 nm because of the self-absorption of the solvent.

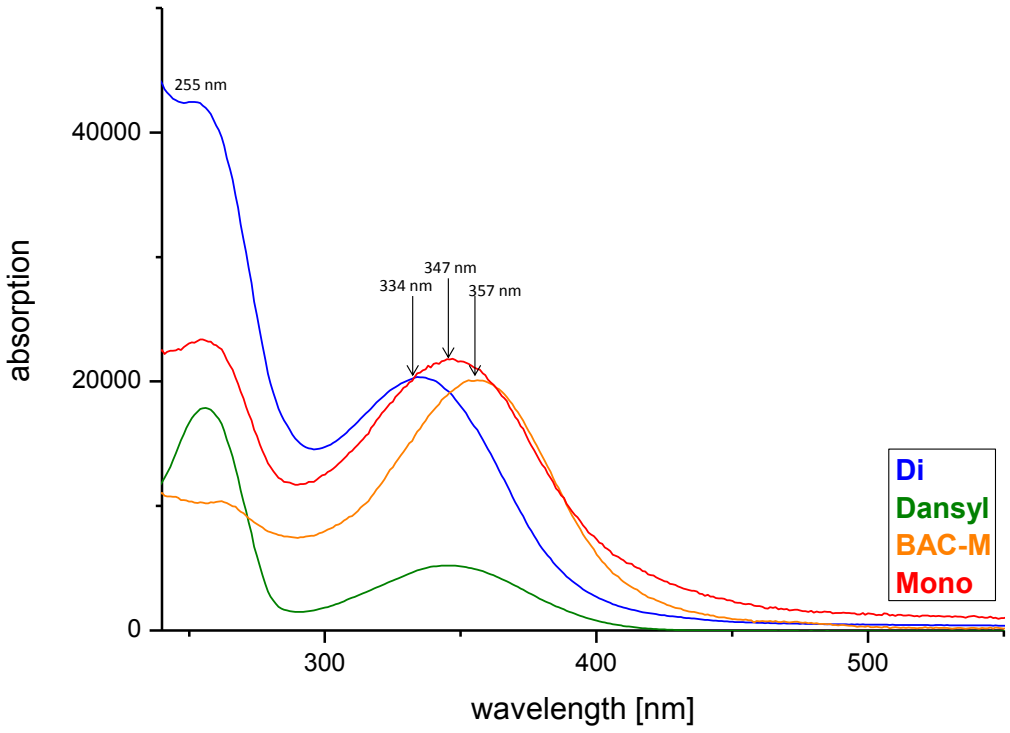


Figure 17: UV/VIS spectra of BAC-M, dansyl propargylamine as well as mono- and di-functionalized BAC-M the with dansyl derivative (1 and 2)

In the spectra shown above (Figure 17) it is clearly visible that there are two peak maxima. The one is around 350 nm and the other one is at 255 nm. If the yellow and the green line are compared it can be seen that the lower maxima belongs to the dansyl and the higher one to the BAC-M. Because of the electronic modification of the cross linking agent there occurs a hypsochromic shift. Without any dansyl derivative the maximum is at 357, the maximum of the mono-functionalized is at 347 nm and the one of di-functionalized is at 334 nm.

Another phenomenon for the successful function of the reaction is the ratio of the BAC-M and the dansyl maximum. In case of *Mono* it is almost 1 : 1, for the *Di* it is 1 : 2. This means, that there is the double amount of dansyl on BAC-M at *Di*.

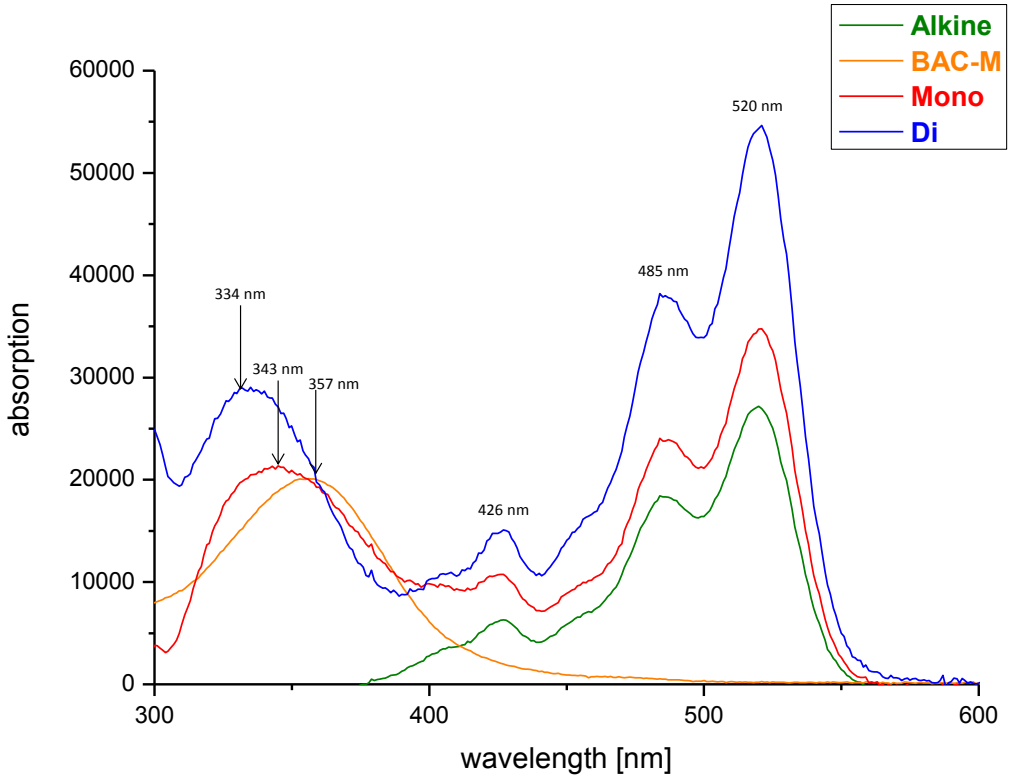


Figure 18: UV/VIS spectra of BAC-M, perylene derivative as well as mono- and di-functionalized derivative (3 and 4)

In case of the perylene derivatives **3** and **4** similar results were obtained. The BAC-M peak also befalls a hypsochromic shift if the cross linking agent is functionalized. Hence, the peak maxima changes in the same way like in the UV/VIS spectra before (Figure 17).

However, a difference can be seen clearly when comparing the dansyl spectra with the perylene where two peaks maxima are observed, one at 520 nm and another one at 485 nm, and they are at higher wavelengths. Also the ratio of the perylene peak to the BAC-M peak changes a little. For the *Di*-functionalized it is still 2 : 1, but for the *Mono* it is 1.5 : 1 now.

The predicted fluorescence spectra were measured in dichloromethane with a concentration of $3 \cdot 10^{-4}$ g/ml. They were recorded in a quartz cuvette with 1 cm thickness. Excitation was at a wavelength of 345 nm for dansyl, BAC-M and **2** and 335 nm for **1**. Emission was recorded at a wavelength of 507 nm expect BAC-M which was recorded at 400 nm.

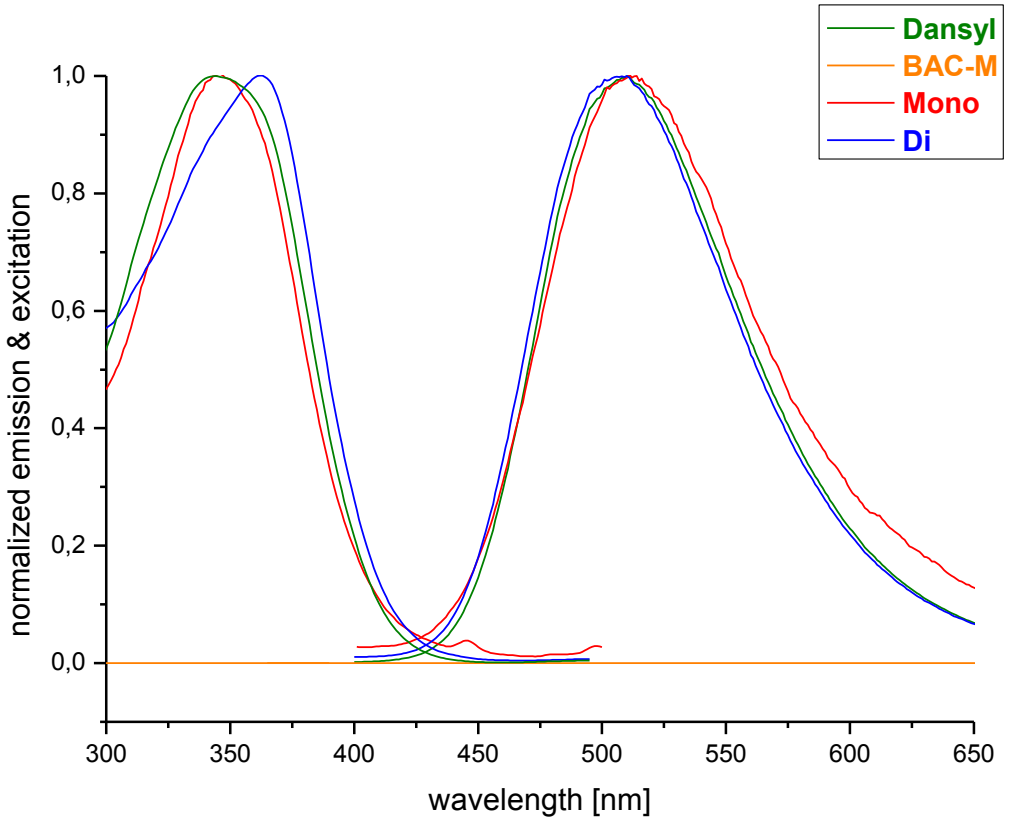


Figure 19: Fluorescence spectra of BAC-M, dansyl propargylamine as well as di- and mono-functionalized BAC-M with the dansyl derivative (1 and 2)

In the spectra above (Figure 19) the normalized excitation and emission peaks of BAC-M, the dansyl derivative, the mono- (**2**) as well as the di-functionalized (**1**) BAC-M are presented. It is clearly observable that the cross linking agent (yellow line) does not show any fluorescence at all. All the other curves also do not differ significantly; especially the emission is similar for all. In excitation of **1** a hyperchromic shift visible. This might be due to different excitation wavelength.

The spectra of the perylene und its derivatives were again measured in dichloromethane with a concentration of $2 \cdot 10^{-4}$ g/ml. Now the excitation wavelength was set to 519 nm and the emission wavelength to 550 nm.

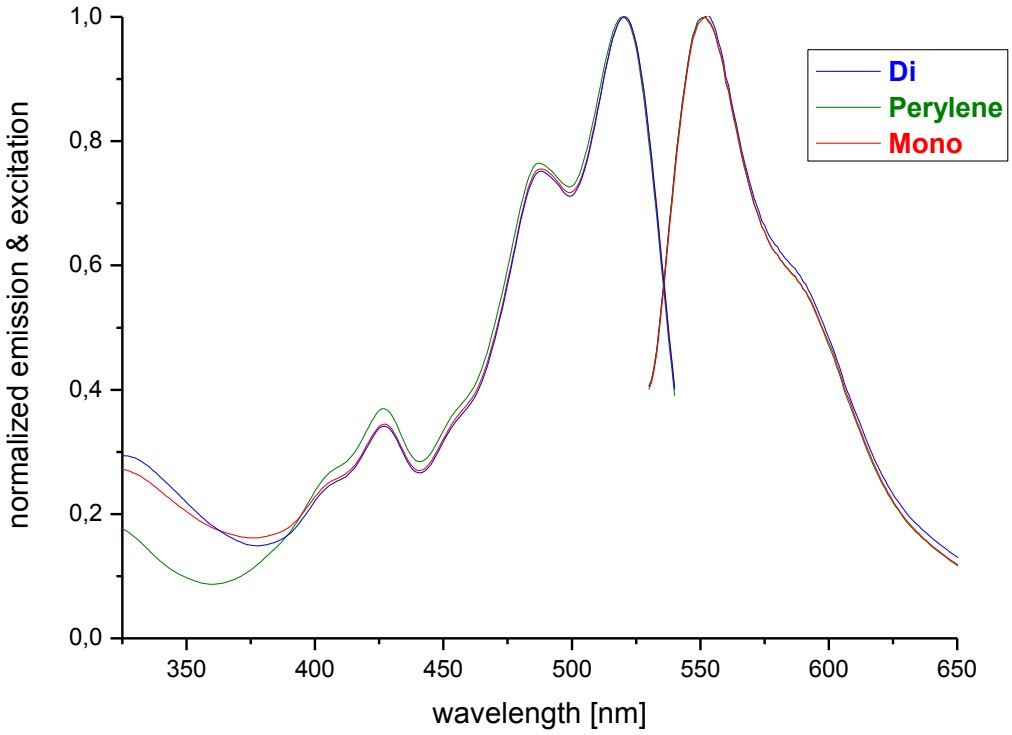


Figure 20: Fluorescence spectra of perylene propargylamine as well as mono- and di-functionalized BAC-M with the perylene derivative (3 and 4)

In the predicted excitation and emission of Figure 20 there is absolutely no difference. The BAC-M curve was omitted because there was no florescence observed.

4.7 Results of Grafting Experiments at Vienna University of Technology

The grafting experiments where performed in collaboration with Vienna University of Technology. Therefore, 2% by weight solution in DMF was made for both the mono-functionalized BAC-M with dansylic derivative as well as the perylene derivative. In this solution a commercially available hydrogel pellet was added and expanded for about one hour. Afterwards the pellet was placed at the sample holder in the TPA-system according to adjustments described in the experimental part, the dyes where grafted on the polymer matrix. As in Figure 21 it can be seen clearly that the inscribed structures are made of single lines which were created crosswise with a line distance of 0.5 µm. The resulting structures had dimensions of 50 x 50 x 40 µm.

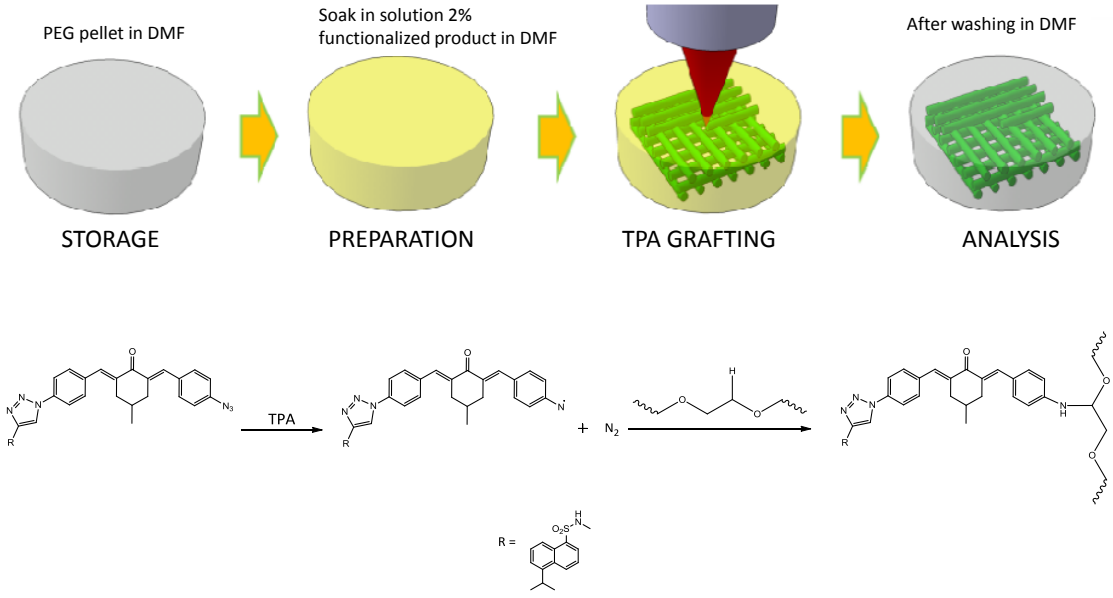


Figure 21: Method of grafting to dyes on the polymer matrix

The main idea behind this reaction is that the azide groups of BAC-M decompose upon excitation with ultraviolet light and create nitrenes. Besides proton abstraction, these highly reactive intermediates were found to form a covalent bond between the functional molecule and a polymer matrix via an insertion mechanism.[27]

In the first attempt when the dansylic derivative was used no particular matrix was formed. Probably due to formation of solution was made 24 hours ago and the azides are not stable in solution for such a long time. Another approach for reaction not working might be the concentration. Prior tests were made with BAC-M, therefore a solution of 2% by weight was used. The failure of reaction might also be due to heavy nature of functionalized BAC-M. Hence, in the next attempt 4% by weight solution which was prepared just before starting the experiment.

In the grafting experiment a fixed array was created whereas only the power of the laser (40 – 290 mW) and the inscribing speed (1 – 5 mm/s) was varied. Visualization was performed with a LSM (laser-scanning-microscopy) with an integrated laser which excited at 488 nm. In this wavelength range only the excitation of BAC-M was observed and not of the dansyl derivative.

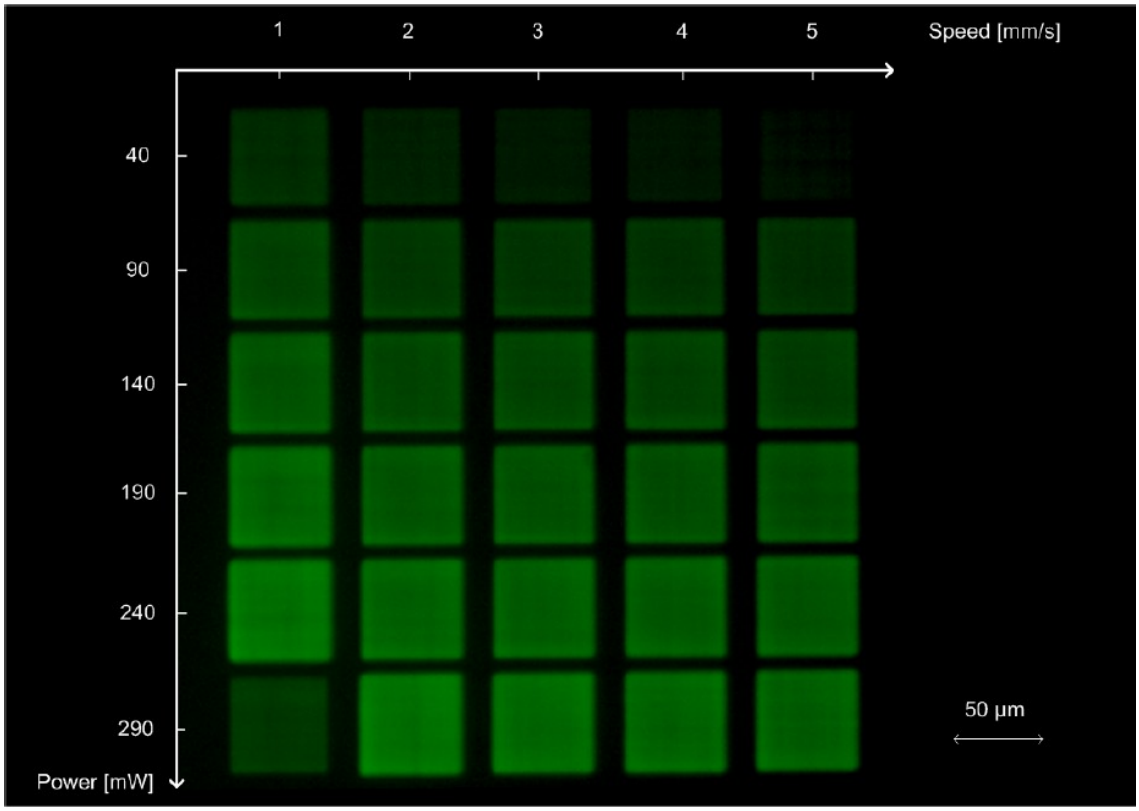


Figure 22: Result of the grafting experiment with the dansylic derivative, 2

In the figure above (Figure 22) the results of the grafting experiment with the dansyl functionalized BAC-M (**2**) are shown. This picture was taken with the LSM. Along the x-axis the changes of the time is plotted against the change in intensity of the laser along the y-axis. Hence, the brightest picture should lie in the lower left corner, but because

of problems in initiating of the femto second laser this spot is unclear so neglected. However, it is clear to see that at faster inscribing speed and at lower laser power the amount of dye grafted is minimum on the polymer matrix. The green color of the spots is not the real color of the fluorescence of BAC-M, it is actually a changed color which comes at a filter in the LSM.

In the picture (Figure 23) below there is another grafting experiment shown with the non-functionalized BAC-M. As the laser of the LSM excites with a wavelength of 488 nm and in this range only BAC-M is excited there must be a difference in brightness which is comparable with the images given in Figure 22. The wavelength of the laser could not be varied in this experiment; hence it was not possible to measure the excitation of dansyl dye.

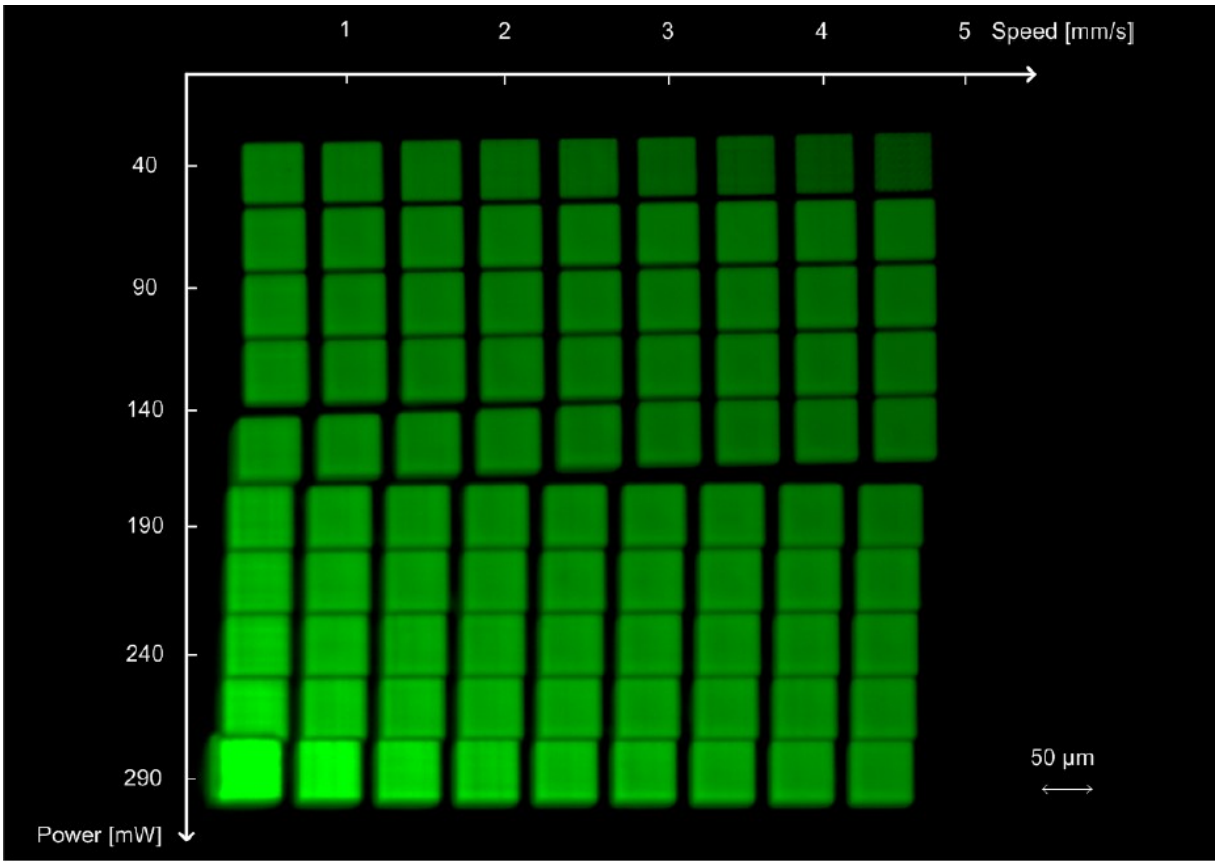


Figure 23: Result of the grafting experiment of BAC-M

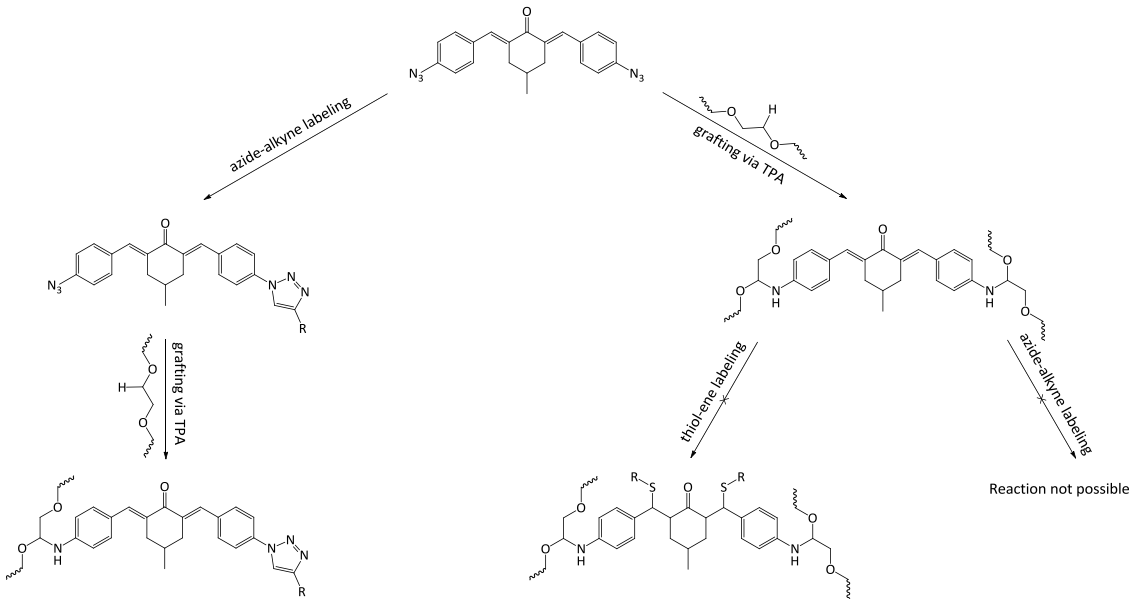
Summarizing the results it can be stated that the grafting experiments with the mono-functionalized BAC-M was working well as compared to dansyl, which could not be excited in the wavelength range of the BAC-M dye.

The second series of experiments performed in Vienna was the grafting experiment of the perylene derivative. It was performed in the similar way like the dansyl and BAC-M derivatives. However, this reaction with perylene did not work at all. During grafting, the combustion of polymer matrix was observed at the spot when it was irradiated with the laser beam. As this observation was pursued via CCD camera (Figure 24) the combustion was observed clearly.

5 Conclusion

In order to develop functional molecules for selective immobilization at surfaces, the two-photon absorption (TPA) active molecule BAC-M was modified with two different fluorescence dyes via thiol-ene click reaction and Cu-catalyzed [3+2] azide-alkyne cycloaddition reactions.

In general, two approaches were pursued. One path to achieve this challenge was selective grafting of BAC-M on polymer matrices and further modification of reactive sites in the molecule via “click chemistry”. The second approach was to functionalize these molecules with dyes in first step and later graft them on the polymer matrix. (Scheme 15)



Scheme 15: General scheme of pursued approaches

For thiol-ene click chemistry, a series of α-,β-unsaturated esters was tried with thiols commonly available in laboratory via both free radical and Michael addition schemes. The reason for choosing this reaction was mainly the weak sulfur–hydrogen bonds and its easy addition upon the olefins. The reaction with electron rich olefins successfully yielded addition products under various reaction conditions, but not much products were reported with the electron withdrawing olefins.[9] To achieve this approach, two

different radical initiators AIBN and BPO were selected and reactions at various temperatures and time durations were performed. But none of the thiol-ene reactions between α-,β-unsaturated esters and thiols was working. The main reason of the products not forming might be due to sterically hindered terminal alkenes or the conjugation of olefinic double bonds with carbonyl group of ester giving least possibility for thiol-ene addition reaction. Hence, these alkenes are incapable of participating in the radical-mediated thiol–ene process. Another reason might be the reduced accessibility of thiol group to the secondary carbon radical of the *trans* isomer of the double bond. Finally, this may also be due to the size of the substituent attached to the carbon radical site.[34]

In the second approach a copper-catalyzed 1,3-dipolar cycloaddition of the cross linking agent BAC-M and the two fluorescent dyes dansyl alkyne and perylene alkyne was evaluated. The reaction conditions were optimized using 2-azido-1-phenylethanone and different alkynes containing either an aliphatic or an aromatic side chain, in presence of CuI as an initiator. Later the optimized conditions were also applied to the fluorescent dyes. Herein, two approaches were pursued to functionalize the BAC-M. Firstly, the dye should be labeled before grafting the molecule on the polymer matrix. Secondly, it was assumed that the BAC-M is already grafted and there are residual azide groups still available. However, according to the results of Aovsiani et. al.[4] there are no more azide groups left after grafting the BAC-M on the polymer matrix. Hence, the assumption that the residual groups of such already grafted bisazides can be used for the formation of the 1,2,3-triazole ring was confuted. Both, the mono and the di-functionalized BAC-M were characterized after synthesis and isolation using major spectroscopic techniques including NMR, FT-IR, UV/VIS and fluorescence spectroscopy.

In the final part of this work, the grafting of this mono-functionalized cross linking agent on a polymer matrix was applied. The azide groups of BAC-M generally decompose upon excitation with ultraviolet light and create nitrenes. These highly reactive intermediates form a covalent bond between the functional molecule and a polymer matrix via an insertion mechanism.[27] In case of the dansyl- derivative the grafting experiment was achieved successfully. A fixed array was made, whereas power of the laser and the inscribing speed was varied. Visualization was performed

with a LSM (Laser-Scanning-Microscopy) and it was observed that at faster inscribing speed and at lower laser power the amount of dye grafted is minimum on the polymer matrix. The second series of experiments were performed with the grafting experiment of the perylene derivative. The reaction procedure was similar to the dansyl derivative. However, the reaction with perylene could not be established to yield any grafted polymer as it was successfully done with dansyl. During grafting, the combustion of polymer matrix was observed at the spot where it was irradiated with the laser beam. As this observation was pursued via CCD camera so the combustion was observed clearly.

6 Experimental

6.1 Materials

All chemicals used for this work were purchased from commercial sources (Fluka, Sigma Aldrich, Lancaster, Merck or ABCR) and unless mentioned otherwise, applied without further purification. BAC-M (2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone) was obtained from Sigma-Aldrich.

Thin layer chromatography was performed with aluminium sheets with silica gel 60 F_{254} from Merck. Via UV light irradiation at 365 nm and a 0.5 % dip solution of $KMnO_4$ the spots were visualized.

Nitrogen was used for reactions conducted under inert gas atmosphere. Silica gel 60 (220-440 mesh ASTM) was used for column chromatography.

NMR spectroscopy (^{1}H, ^{13}C) was done on a Bruker Avance III 300 MHz spectrometer. Deuterated solvents such as $CDCl_3$, MeOD and DMSO-d_6 purchased from Cambridge Isotope Laboratories, Inc., were used according to different solubilities of the probes. The following abbreviations were taken to indicate different peak shapes: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), b (broad), bs (broad singlet), dd (doubletic-doublet).

For IR spectroscopy a Bruker ALPHA FT-IR Spectrometer was used. Measurements were performed in ATR mode. For liquid measurement solvents such as CH_2Cl_2, Acetone and Nujol were purchased from Aldrich.

UV/VIS absorption spectra were taken with a Cary 50 UV/VIS spectrophotmeter from Varian. The measurements were done with a silica glass bulb applicative for a spectral range from 300 to 800 nm from Hellma. In all spectra the absorption was measured.

For fluorescence analyses a Hitachi F-7000 was taken. Measurements were performed in a silica glass bulb and dichloromethane was used as solvent. In all spectra excitation and emission was recorded.

The basic setup of two photon absorption (TPA) system applied is given in Figure 24. The principle follows as: a femto-second laser beam is passed through the shutter of

acousto-opticmodulator first. This shutter diffracts the laser beam so that only the first order waves can be used for grafting reactions. The intensity is adjusted by a λ/2 waveplate and with a polarizing dependant beam-splitter. Before the beam exposes into the resin it is focused to be observed by a microscope. The motion along x- and y-axes of the laser beam is observed by high precision air bearing axes. The motion along z-axes which carries the resin container is also observed by similar process. The mirror system ensures that the movements along x- and y-axes are parallel to the direction of the laser beam, so that the line of beam remains stable throughout the whole structuring process. For this inline process observation, a camera views the laser beam and particularly focuses on the grafting spot. The axes are mounted on a hard stone frame, which is designed to damp vibrations. The base of whole setup is an optical table with an air friction damping which again suppresses vibrations. For the control of the machine, the axes and the laser intensity power meter is plugged to an electronic device, which processes the commands given by the control computer. The sample is illuminated using a red LED lamp to prevent premature polymerisation.[18]

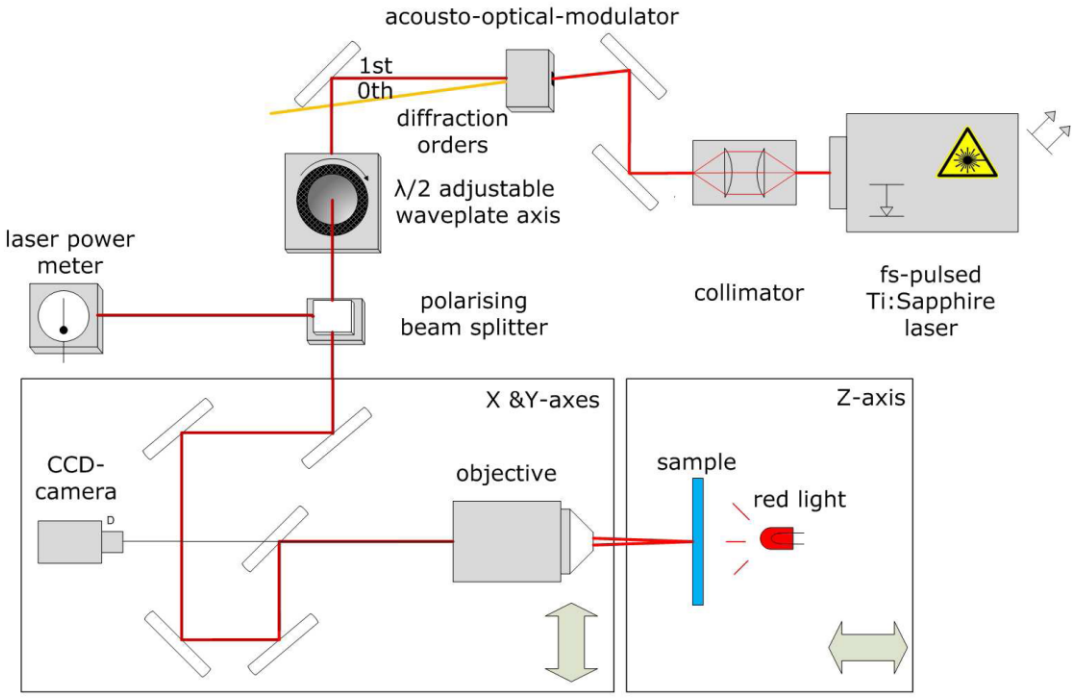
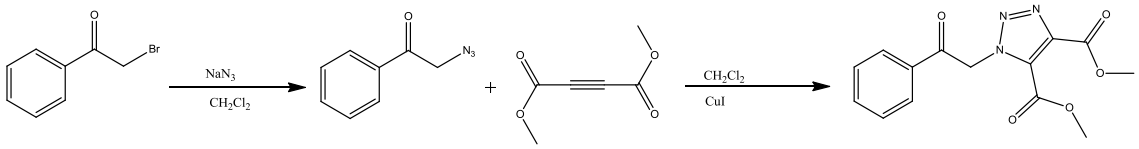


Figure 24: Schematic picture of the TPA experimental setup

6.2 Alkyne-Azide Labeling

6.2.1 Synthesis of Dimethyl-1-(2-oxo-2-phenylethyl)-1H-1,2,3-triazole-4,5-dicarboxylate



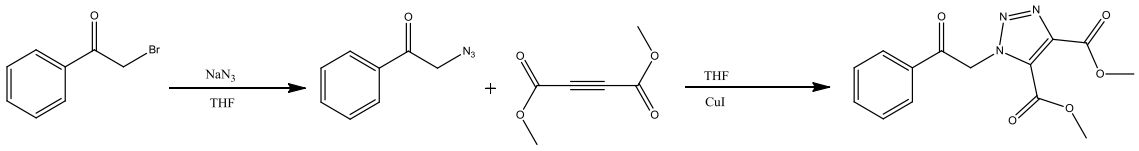
100 mg of 2-bromo-1-phenylethanone (0.50 mmol, 1 eq) and 36 mg of sodium azide (0.55 mmol, 1.1 eq) were dissolved in 2 mL CH_2Cl_2 abs. and stirred over night at room temperature. The reaction progress was monitored via TLC in CH:EE = 1:1.

After 24h the reaction was finished and to the yellow solution was then added 71 mg of dimethyl acetylenedicarboxylate (0.50 mmol, 1 eq). The temperature was increased to 60°C. After all reagents have been dissolved 5 mg of copper (I) iodide (5 mol%) were added and the reaction was stirred over night again at 60°C. Afterwards solids were removed by filtration and the solvent was evaporated. The residue was taken up in about 100 mL CH_2Cl_2 and extracted with saturated $NaHCO_3$ solution four times, brine solution two times followed by H_2O dist. three times. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

yield: 0.0 mg (0% of theoretical yield)

TLC: R_f = 0.79 (CH/EE, 1:1, (v:v))

6.2.2 Synthesis of Dimethyl-1-(2-oxo-2-phenylethyl)-1H-1,2,3-triazole-4,5-dicarboxylate



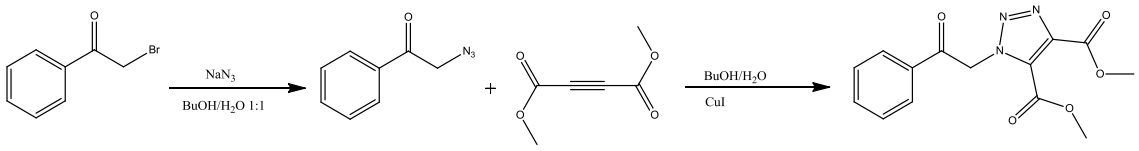
100 mg of 2-bromo-1-phenylethanone (0.50 mmol, 1 eq) and 36 mg of sodium azide (0.55 mmol, 1.1 eq) were dissolved in 2 mL THF 1:1 and stirred over night at room temperature. The reaction progress was monitored via TLC in CH:EE = 1:1.

After 24h the reaction was finished and to the yellow solution was then added 71 mg of dimethyl acetylenedicarboxylate (0.50 mmol, 1 eq). The temperature was increased to 60°C. After all reagents have been dissolved 5 mg of copper (I) iodide (5 mol%) were added and the reaction was stirred over night again at 60°C. Afterwards solids were removed by filtration and the solvent evaporated. The residue was taken up in about 100 mL CH_2Cl_2 and extracted with saturated $NaHCO_3$ solution 4 times, brine solution 2 times followed by H_2O dist. three times. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

yield: 45.2 mg (24%)

TLC: R_f = 0.67 (CH/EE, 1:1, (v:v))

6.2.3 Synthesis of Dimethyl-1-(2-oxo-2-phenylethyl)-1H-1,2,3-triazole-4,5-dicarboxylate



100 mg of 2-bromo-1-phenylethanone (0.50 mmol, 1 eq) and 36 mg of sodium azide (0.55 mmol, 1.1 eq) were dissolved in 2 mL *tert*-BuOH/H_2O 1:1 and stirred over night at room temperature. The reaction progress was monitored via TLC in CH:EE = 1:1.

After 24h the reaction was finished and to the yellow solution was then added 71 mg of dimethyl acetylenedicarboxylate (0.50 mmol, 1 eq). The temperature was increased to 60°C. After all reagents have been dissolved 5 mg of copper (I) iodide (5 mol%) were added and the reaction was stirred over night again at 60°C. Afterwards solids were removed by filtration and the solvent was evaporated. The residue was taken up in about 100 mL CH_2Cl_2 and extracted with saturated $NaHCO_3$ solution 4 times, brine solution 2 times followed by H_2O dist. three times. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

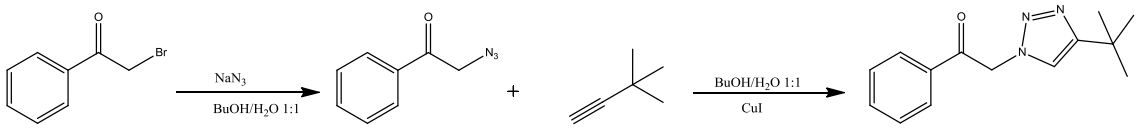
yield: 184.4 mg (98%)

TLC: R_f = 0.66 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 7.98 (d, 2H, $^3J_{HH}$ = 7.22 Hz, ph^2, ph^6), 7.69 (t, 1H, ph^4), 7.56 (m, 2H, ph^3, ph^5), 6.16 (s, 2H, $(C_6H_5)COCH_2N$), 3.91 (s, 6H, $O(CH_3)_2$)

^{13}C-NMR: (δ, 20°C, $CDCl_3$, 75.53 MHz): 190.17 (1C, CO), 161.11 (1C, COO), 160.42 (1C, COO), 141.21 (1C, 1,2,3-triazole), 135.41 (1C, ph^1), 134.21 (2C, ph^2, ph^6), 133.91 (1C, ph^4), 130.11 (1C, 1,2,3-triazole), 129.71 (2C, ph^3, ph^5), 54.12 (1C, $(C_6H_5)COCH_2N$), 53.22 (2C, $O(CH_3)_2$)

6.2.4 Synthesis of 2-(4-(tert-butyl)-1H-1,2,3-triazol-1-yl)-1-phenylethanone



100 mg of 2-bromo-1-phenylethanone (0.50 mmol, 1 eq) and 36 mg of sodium azide (0.55 mmol, 1.1 eq) were dissolved in 2 mL *tert*-BuOH/H_2O 1:1 and stirred over night at room temperature. The reaction progress was monitored via TLC in CH:EE = 1:1.

The next morning the reaction was finished and to the yellow solution was then added 41 mg of 3,3-Dimethyl-1-butyne (0.50 mmol, 1 eq). The temperature was increased to 60°C. After all reagent have been dissolved 5mg of copper (I) iodide (5 mol%) were added and the reaction was stirred over night again at 60°C. Afterwards solids were removed by filtration and the solvent was evaporated. The residue was taken up in about 100 mL CH_2Cl_2 and extracted with saturated $NaHCO_3$ solution 4 times, brine solution 2 times followed by H_2O dist. three times. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

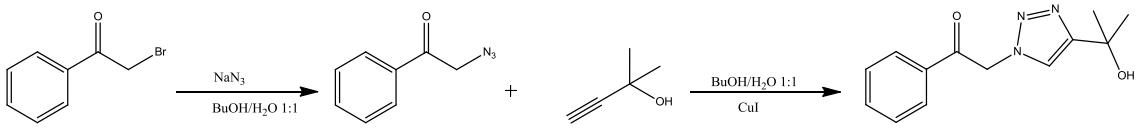
yield: 134.4 mg (89%)

TLC: R_f = 0.47 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 7.99 (d, 2H, $^3J_{HH}$ = 7.24 Hz, ph^2, ph^6), 7.68 (t, 1H, ph^4), 7.55 (m, 2H, ph^3, ph^5), 5.86 (s, 2H, $(C_6H_5)COCH_2N$), 1.38 (s, 9H, $C(CH_3)_3$)

^{13}C-NMR: (δ, 20°C, $CDCl_3$, 75.53 MHz): 190.19 (1C, CO), 134.67 (1C, ph^1), 134.06 (2C, ph^2, ph^6), 133.81 (1C, ph^4), 130.56 (1C, 1,2,3-triazole), 128.91 (2C, ph^3, ph^5), 123.09 (1C, 1,2,3-triazole), 56.52 (1C, $(C_6H_5)COCH_2N$), 34.78 (1C, $C(CH_3)_3$), 29.87 (3C, $C(CH_3)_3$)

6.2.5 Synthesis of 2-(4-(2-hydroxypropan-2-yl)-1H-1,2,3-triazol-1-yl)-1-phenyl-ethanone



100 mg of 2-bromo-1-phenylethanone (0.50 mmol, 1 eq) and 36 mg of sodium azide (0.55 mmol, 1.1 eq) were dissolved in 2 mL *tert*-BuOH/H_2O 1:1 and stirred over night at room temperature. The reaction progress was monitored via TLC in CH:EE = 1:1.

The next morning the reaction was finished and to the yellow solution was then added 42 mg of 2-Methyl-3-butyn-2-ol (0.50 mmol, 1 eq). The temperature was increased to 60°C. After all reagents have been dissolved 5 mg of copper (I) iodide (5 mol%) were added and the reaction was stirred over night again at 60°C. Afterwards solids were removed by filtration and the solvent was evaporated. The residue was taken up in about 100 mL CH_2Cl_2 and extracted with saturated $NaHCO_3$ solution 4 times, brine solution 2 times followed by H_2O dist. three times. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

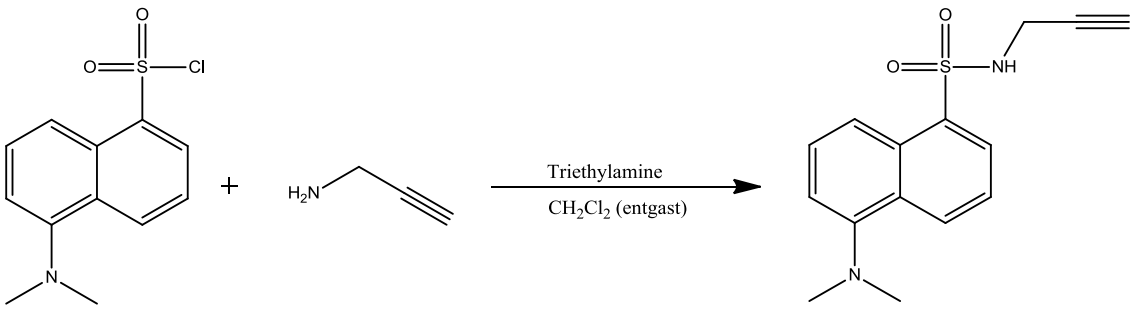
yield: 143.5 mg (95%)

TLC: R_f = 0.51 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.00 (d, 2H, $^3J_{HH}$ = 7.23 Hz, ph^2, ph^6), 7.68 (t, 1H, ph^4), 7.55 (m, 2H, ph^3, ph^5), 5.86 (s, 2H, $(C_6H_5)COCH_2N$), 2.49 (bs, 1H, OH), 1.38 (s, 6H, $COH(CH_3)_2$)

^{13}C-NMR: (δ, 20°C, $CDCl_3$, 75.53 MHz): 190.17 (1C, CO), 134.59 (1C, ph^1), 134.03 (2C, ph^2, ph^6), 133.77 (1C, ph^4), 130.54 (1C, 1,2,3-triazole), 128.90 (2C, ph^3, ph^5), 123.11 (1C, 1,2,3-triazole), 78.43 (1C, $COH(CH_3)_2$), 56.52 (1C, $(C_6H_5)COCH_2N$), 31.41 (2C, $COH(CH_3)_2$)

6.2.6 Synthesis of 5-(dimethylamino)-N-(prop-2-yn-1-yl)naphthalene-1-sulfonamide



700 mg of dansyl chloride (2.604 mmol, 1 eq) and 0.167 ml of propargylamine (2.604 mmol, 1 eq) were dissolved in 10 mL dried CH_2Cl_2. After 10 minutes stirring 0.361 ml of triethylamine (2.604 mmol, 1 eq) were added. The solution immediately turned from dark yellow to fluorescent. Reaction progress was monitored via TLC in CH:EE = 1:1. After 24 hours, it was quenched with water (pH 7) and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure.

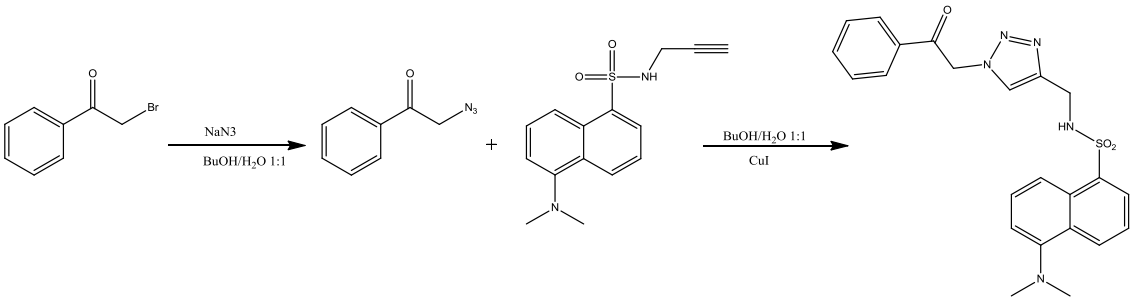
yield: 736 mg (98%)

TLC: R_f = 0.66 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.55 (d, 1H, J = 7.76 Hz), 8.27 (t, 2H, J = 7.76 Hz), 7.56 (quintet, 2H, J = 7.31 Hz), 7.21 (d, 1H, J = 7.48 Hz), 4.82 (s, 1H, NH), 3.77 (d, 2H, J = 2.52 Hz, N-CH_2), 2.89 (s, 6H, N-$(CH_3)_2$), 1.92 (t, 1H, J = 2.50 Hz, CCH)

^{13}C-NMR: (δ, 20°C, $CDCl_3$, 75.53 MHz): 152.0 (1C, naph), 144.1 (1C, naph), 134.1 (1C, naph), 130.8 (1C, naph), 129.8 (1C, naph), 128.5 (1C, naph), 126.1 (1C, naph), 123.1 (1C, naph), 118.5 (1C, naph), 115.2 (1C, naph), 77.6 (1C, CCH), 72.6 (1H, CCH), 45.4 (2C, N-$(CH_3)_2$), 32.9 (1C, N-CH_2)

6.2.7 Synthesis of 5-(dimethylamino)-N-((1-(2-oxo-2-phenylethyl)-1H-1,2,3-triazol-4-yl)methyl)naphthalene-1-sulfonamide



100 mg of 2-bromo-1-phenylethanone (0.50 mmol, 1 eq) and 36 mg of sodium azide (0.55 mmol, 1.1 eq) were dissolved in 2 mL *tert*-BuOH/H_2O 1:1 and stirred over night at room temperature. The reaction progress was monitored via TLC in CH:EE = 1:1.

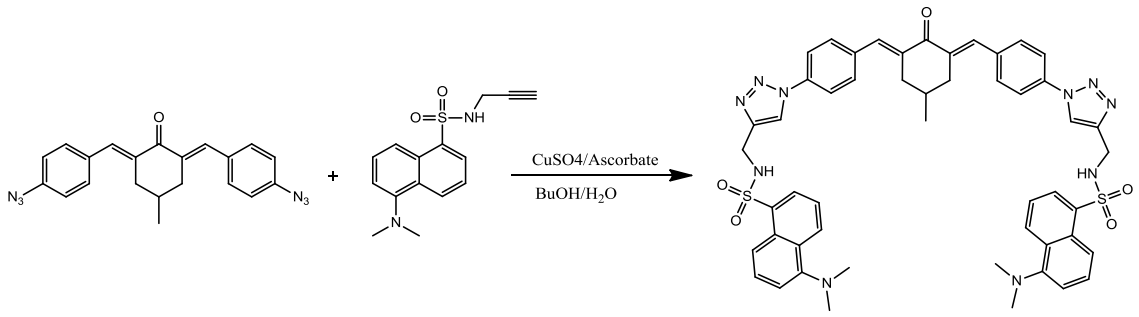
The next morning the reaction was finished and to the yellow solution was then added 144 mg of dansyl propargylamine (0.50 mmol, 1 eq). The temperature was increased to 60°C and stirred until everything was solved. Now 5mg of copper (I) iodide (5 mol%) were added and the reaction was stirred over night again at 60°C. Afterwards solids were removed by filtration and the solvent was evaporated. The residue was taken up in about 100 mL CH_2Cl_2 and extracted with saturated $NaHCO_3$ solution 4 times, brine solution 2 times followed by H_2O dist. three times. The organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

yield: 200 mg (89%)

TLC: R_f = 0.76 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.28 (d, 1H, naph), 8.22 (d, 1H, naph), 8.01 (d, 1H, naph), 7.89 (t, 2H, ph), 7.54 (m, 6H, ph, naph, triazole), 6.92 (d, 1H, naph), 5.71 (t, 1H, NH) 4.55 (s, 2H, $(C_6H_5)COCH_2N$), 4.02 (d, 2H, CH_2NH), 2.83 (s, 6H, $N(CH_3)_2$)

6.2.8 Synthesis of 5-(dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide

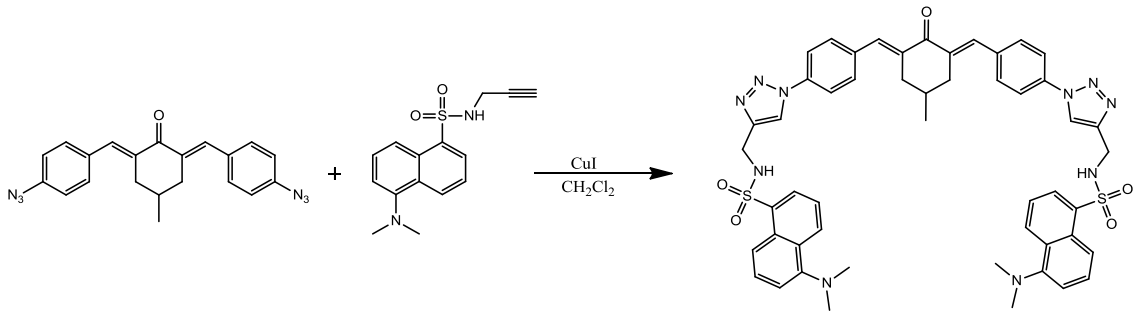


20 mg of 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone (0.069 mmol, 1 eq) and 26 mg of dansyl propargylamine (0,069 mmol, 1 eq) were dissolved in 200 µl *tert*-BuOH/H_2O 1:1 (0.4 M) and stirred for 10 minutes at room temperature. Subsequently 0.9 mg of copper(II) sulfate (0,003 mmol, 5 mol%) and 1.4 mg ascorbate (0.007 mmol, 10 mol%) were added to the yellow solution. The reaction progress was monitored via TLC in CH:EE = 1:1. There was no detectable reaction.

yield: 0 mg (0%)

TLC: R_f = 0.61 (CH/EE, 1:1, (v:v))

6.2.9 Synthesis of 5-(dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide



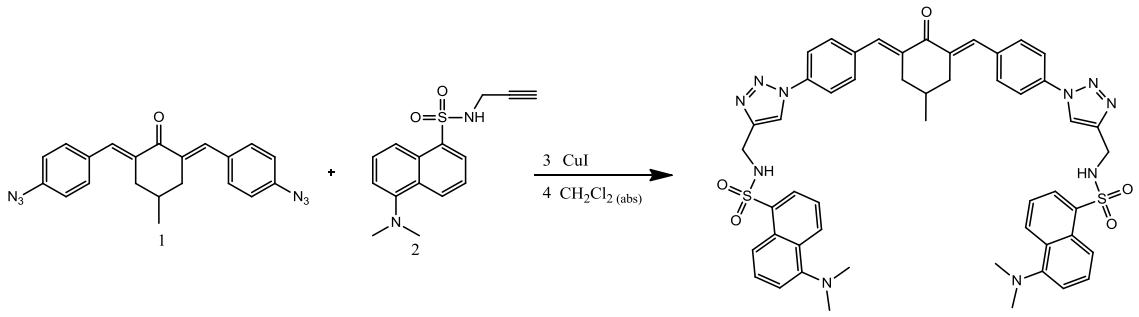
35.5 mg of 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone (0.122 mmol, 1 eq) and 50 mg of dansyl propargylamine (0.184 mmol, 1.5 eq) were dissolved in 1 mL CH_2Cl_2 and stirred for 10 minutes at room temperature. Subsequently 3 mg of copper (I) iodide (0.061 mmol, 0.5 eq) were added to the yellow solution. Immediately the solution became a little cloudy. The reaction progress was monitored via TLC (CH:EE = 1:1). After 24 hours, the reaction was quenched with water (pH 7) and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure.

yield: 39.9 mg (44%)

TLC: R_f = 0.79 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.56 (d, 2H, naph), 8.29 (t, 4H, naph), 7.80 (s, 2H, 1,2,3-triazole), 7.58 (m, 13H, ph, naph, (ph)CH(cy)), 7.17 (d, 3H, naph), 5.40 (m, 2H, NH), 4.34 (d, 4H, $NHCH_2$-triazole), 3.04 (d, 2H, cy), 2.85 (s, 12H, $N(CH_3)_2$), 2.56 (d, 2H, cy), 2.17 (m, 1H, cy), 1.15 (d, 3H, cy-CH_3)

6.2.10 Synthesis of 5-(dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide



100 mg of 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone (0.344 mmol, 1 eq) and 190 mg of dansyl propargylamine (0.688 mmol, 2 eq) were dissolved in 10 mL dried CH_2Cl_2 and stirred for 10 minutes at room temperature. Subsequently 8.5 mg of copper (I) iodide (0.172 mmol, 0.5 eq) were added to the yellow solution and heated to 30°C. The reaction progress was monitored via TLC (CH:EE = 1:1). After 24 hours, it was quenched with water (pH 7) and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure.

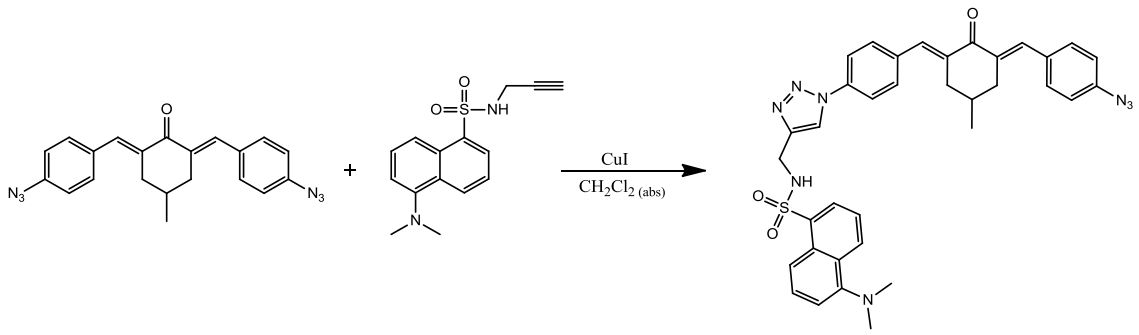
yield: 222.4 mg (87%)

TLC: R_f = 0.68 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.56 (d, 2H, naph), 8.29 (t, 4H, naph), 7.80 (s, 2H, 1,2,3-triazole), 7.58 (m, 13H, ph, naph, (ph)CH(cy)), 7.17 (d, 3H, naph), 5.40 (m, 2H, NH), 4.34 (d, 4H, $NHCH_2$-triazole), 3.04 (d, 2H, cy), 2.85 (s, 12H, $N(CH_3)_2$), 2.56 (d, 2H, cy), 2.17 (m, 1H, cy), 1.15 (d, 3H, cy-CH_3)

^{13}C-NMR: (δ, 20°C, $CDCl_3$, 75.53 MHz): 189.59 (1C, cy CO), 145.00 (2C, naph$CN(CH_3)_2$), 140.55 (2C, naphCSO_2), 136.32 (2C, phC-triazole), 136.29 (2C, cyCCH), 135.87 (2C, CH), 134.75 (2C, naph), 134.40 (2C, phCCH), 131.71 (2C, naph), 130.77 (2C, naph), 129.82 (2C, triazole-CH_2), 129.67 (2C, naph), 128.74 (2C, naph), 128.66 (2C, naph), 123.35 (2C, CH triazole), 120.13 (4C, ph), 118.81 (4C, ph), 115.39 (2C, naph), 45.50 (4C, $C(CH_3)_2$), 38.82 (2C, $NHCH_2$), 33.10 (2C, cyCH_2), 29.32 (1C, cyCCH_3), 21.73 (1C, CCH_3)

6.2.11 Synthesis of N-((1-4-((E)-3-(4-azidobenylidene)-5-methyl-2-oxocyclohexylidene)methyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(dimethylamino)naphthalene-1-sulfonamide



1 g of 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone (2.70 mmol, 10 eq) and 78 mg of dansyl propargylamine (0.270 mmol, 1 eq) were dissolved in 20 mL $CH_2Cl_{2\ (abs)}$ and stirred for 10 minutes at room temperature. Subsequently 38 mg of copper (I) iodide (0.135 mmol, 0.5 eq) were added to the yellow solution and heated to 30°C. The reaction progress was monitored via TLC (CH:EE = 1:1). After 120 hours, it was quenched with water (pH 7) and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The product was columned with CH/EE = 5 : 1.

yield: 160.5 mg (88%)

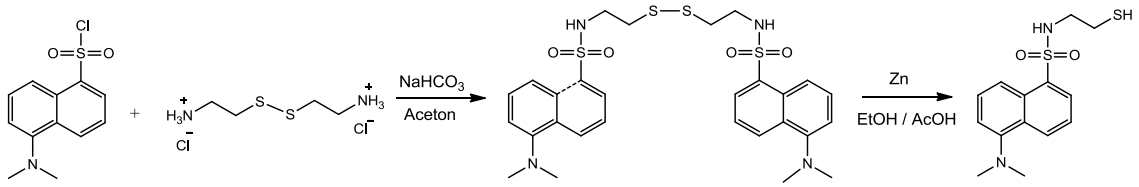
TLC: R_f = 0.49 (CH/EE, 1:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.49 (d, 1H, naph), 8.30 (d, 2H, naph), 7.75 (s, 2H, (ph)CH(cy)), 7.52 (m, 9H, ph, 1,2,3-triazole, naph), 7.15 (d, 1H, ph), 7.08 (d, 1H, ph), 5.86 (t, 1H, NH), 4.33 (d, 2H, $NHCH_2$-triazole), 3.02 (d, 2H, cy), 2.82 (s, 6H, $N(CH_3)_3$), 2.52 (m, 2H, cy), 2.04 (bs, 1H, cy), 1.11 (d, 3H, cy-CH_3)

^{13}C-NMR: (δ, 20°C, $CDCl_3$, 75.53 MHz): 189.73 (1C, cy CO), 144.83 (1C, naph$CN(CH_3)_2$), 140.66 (1C, naphCSO_2), 136.87 (2C, naph), 136.64 (2C, cyCCH), 136.25 (1C, phC-triazole), 135.35 (2C, CH), 134.85 (2C, phCCH), 134.76 (1C, naph), 132.69 (1C, triazole-CH_2), 132.25 (2C, ph), 131.68 (2C, ph), 130.82 (1C, naph), 129.99 (1C, naph), 128.80 (1C, naph), 123.45 (1C, CH triazole), 120.19 (2C, ph), 119.24 (2C, ph), 118.43 (1C, naph), 115.58 (1C, naph), 114.35 (1C, phC-N_3), 45.58 (2C, $C(CH_3)_2$), 38.90 (1C, $NHCH_2$), 36.65 (2C, cyCH_2), 29.83 (1C, cyCCH_3), 21.78 (1C, CCH_3)

6.3 Thiol-Alkene Labeling

6.3.1 Synthesis of 5-(dimethylamino)-N-(2-mercaptoethyl)naphthalene-1-sulfonamide



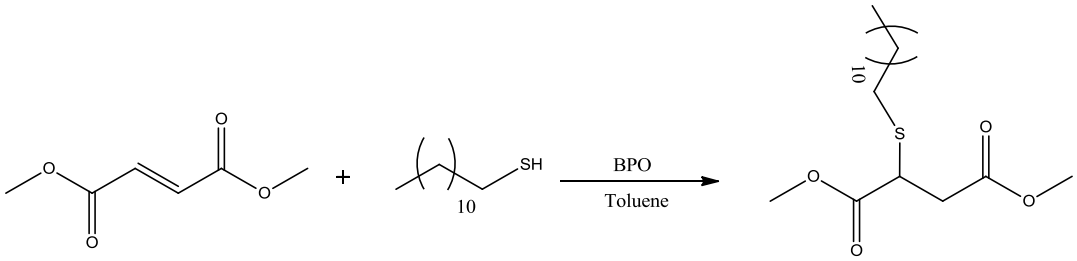
200 mg of dansyl chloride (0.743 mmol, 2 eq) were dissolved in 45 mL acetone and 1.5 mL H_2O. 84 mg cystamine dihydrochloride (0.370 mmol, 1 eq) was dissolved in 5 mL 1 M $NaHCO_3$ and added drop wise whereas pH value was kept to 7 - 8 by adding $NaHCO_3$ solution (controlled via pH indicator stripes). Reaction progress was monitored via TLC in CH_2Cl_2 : MeOH = 20:1 until no further dansyl chloride was detected. After 12 hours, 200 mL CH_2Cl_2 were added and the solution was extracted with saturated $NaHCO_3$ solution four times, followed by brine solution two times and distilled water two times. The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remaining product was dried, yielding 210 mg (91.3%) of a fluffy, crispy yellow solid. Didansyl cystamine was diluted in 52 mL EtOH. The yellowish solution turned greenish- blue after the alternating addition of 3.0 g Zn and 17.2 mL acetic acid. The reaction process was monitored via TLC in CH_2Cl_2 : MeOH = 20:1. The reaction was stirred overnight. Afterwards solids were removed by filtration and the solvent evaporated. The residue was redissolved in about 100 mL $CHCl_3$ and extracted with saturated $NaHCO_3$ solution 4 times, brine solution 2 times followed by H_2O dist. three times. Again, the organic layer was dried over Na_2SO_4 and solvent was distilled off under reduced pressure yielding a dark yellow film.

yield: 180,1 mg (86 %)

TLC: R_f = 0.74 (CH_2Cl_2/MeOH, 20:1, (v:v))

^{1}H-NMR: (δ, 20°C, $CDCl_3$, 300.36 MHz): 8.56 (d, 1H, $^3J_{HH}$ = 8.36 Hz, ph^9), 8.27 (d, 1H, ph^2), 8.25 (d, 1H, ph^4), 7.56 (m, 2H, ph^3, ph^8), 7.22 (d, 1H, $^3J_{HH}$ = 7.46 HZ, ph^7), 5.17 (t, 1H, $^3J_{HH}$ = 10.85 Hz, NH), 3.10 (dd, 2H, $HNCH_2CH_2SH$), 2.91 (s, 6H, $N(CH_3)_2$), 2.50 (dd, 2H, $HNCH_2CH_2SH$), 1.21 (t, 1H, CH_2SH)

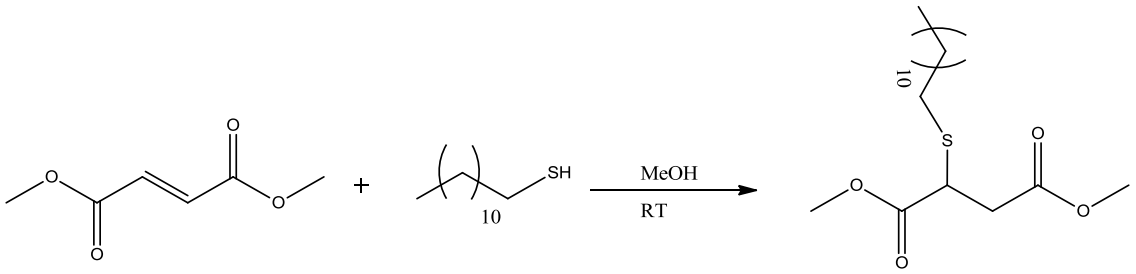
6.3.2 Synthesis of Ethyl 3-(phenylthio)propanoate



50 mg of dimethylfumarate (0.347 mmol, 1 eq) and 105 mg of dodecane-1-thiol (0.521 mmol, 1.5 eq) were dissolved in 2 mL toluene at 110°C. After 10 minutes 46 mg of benzoyl peroxide (0.5 eq) were added. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.47 (CH/EE, 1:1, (v:v)) (educt same as product)

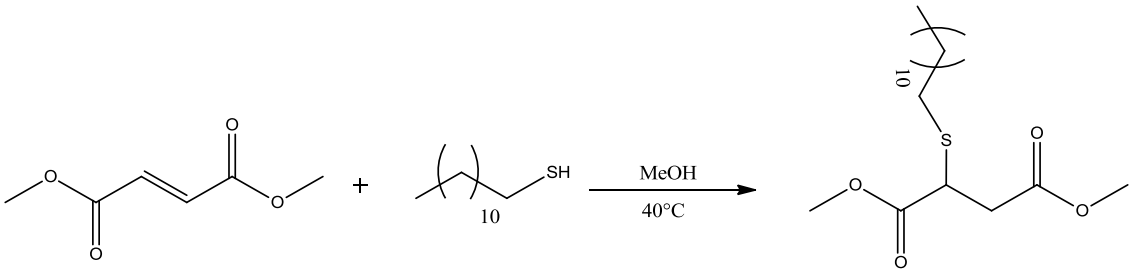
6.3.3 Synthesis of Ethyl 3-(phenylthio)propanoate



50 mg of dimethylfumarate (0.347 mmol, 1 eq) and 70 mg of dodecane-1-thiol (0.347 mmol, 1 eq) were dissolved in 2 mL methanol at room temperature. The reaction progress was monitored via TLC in CH:EE = 9:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.36 (CH/EE, 9:1, (v:v)) (educt same as product)

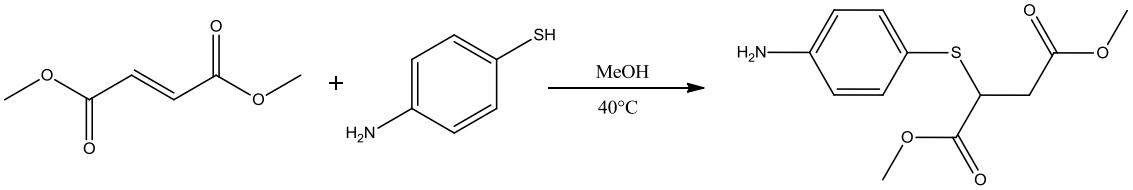
6.3.4 Synthesis of Ethyl 3-(phenylthio)propanoate



50 mg of dimethylfumarate (0.347 mmol, 1 eq) and 70 mg of dodecane-1-thiol (0.347 mmol, 1 eq) were dissolved in 1.5 mL methanol at 40°C. The reaction progress was monitored via TLC in CH:EE = 9:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.36 (CH/EE, 9:1, (v:v)) (educt same as product)

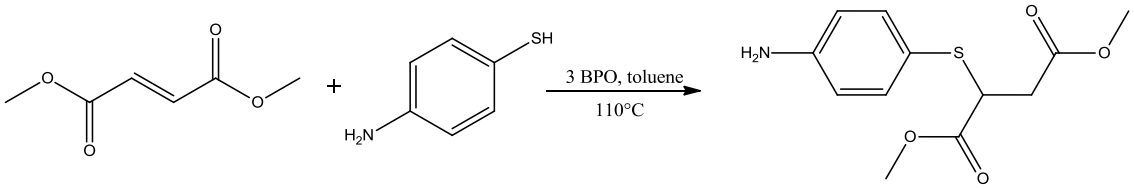
6.3.5 Synthesis of Dimethyl 2-((4-aminophenyl)thio)succinate



50 mg of dimethylfumarate (0.347 mmol, 1 eq) and 44 mg of 4-aminobenzenethiol (0.347 mmol, 1 eq) were dissolved in 1.5 mL methanol at 40°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.41 (CH/EE, 1:1, (v:v)) (educt same as product)

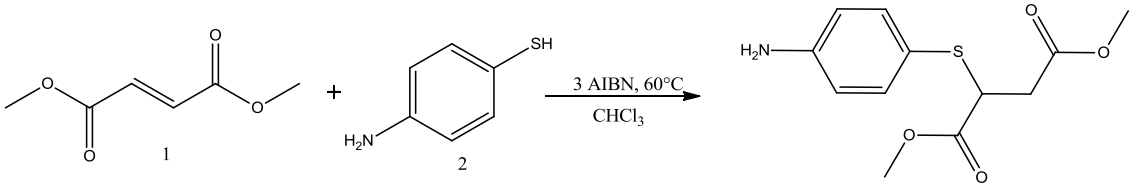
6.3.6 Synthesis of Dimethyl 2-((4-aminophenyl)thio)succinate



50 mg of dimethylfumarate (0.347 mmol, 1 eq) and 65 mg of 4-aminobenzenethiol (0.521 mmol, 1.5 eq) were dissolved in 2 mL toluene at 110°C. After 10 minutes 5 mg of benzoyl peroxide (0.5 eq) were added. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.40 (CH/EE, 1:1, (v:v)) (educt same as product)

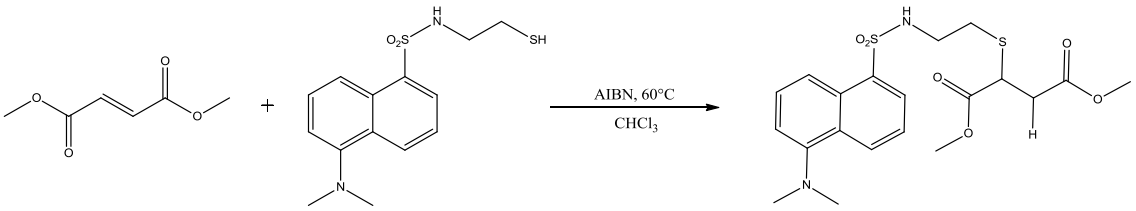
6.3.7 Synthesis of Dimethyl 2-((4-aminophenyl)thio)succinate



50 mg of dimethylfumarate (0.347 mmol, 1 eq) and 65 mg of 4-aminobenzenethiol (0.521 mmol, 1.5 eq) were dissolved in 2 mL $CHCl_3$ at 60°C. After 10 minutes 5 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.40 (CH/EE, 1:1, (v:v)) (educt same as product)

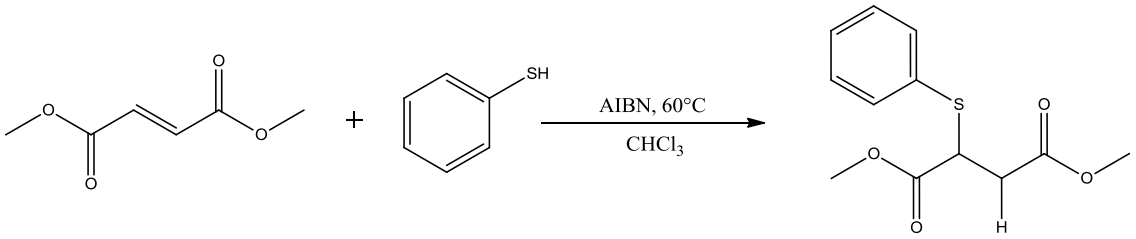
6.3.8 Synthesis of Dimethyl 2-((2-(5-dimethylamino)naphthalene-1-sulfonamido)ethyl)thio)succinate



50 mg of diemethyl fumarate (0.347 mmol, 1 eq) and 161 mg of 5-(dimethylamino)-*N*-(2-mercaptoethyl)naphthalene-1-sulfonamide (0.520 mmol, 1.5 eq) were dissolved in 6 mL $CHCl_3$ at 60°C. After 10 minutes 19 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 5:1. After 72 hours there was no detectable reaction.

TLC: R_f = 0.54 (CH/EE, 5:1, (v:v)) (educt same as product)

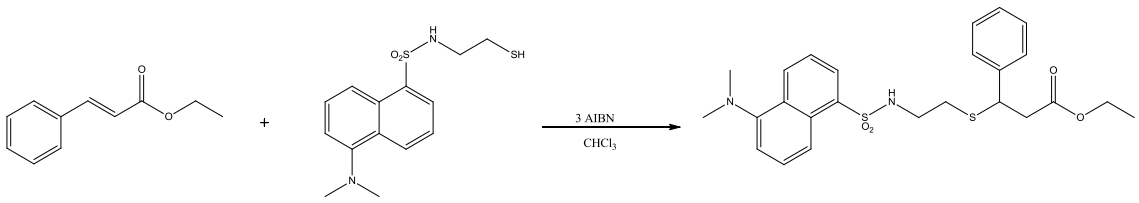
6.3.9 Synthesis of Dimethyl 2-(phenylthio)succinate



50 mg of diemethyl fumarate (0.347 mmol, 1 eq) and 57 mg benzenethiol (0.520 mmol, 1.5 eq) were dissolved in 7 mL $CHCl_3$ at 60°C. After 10 minutes 19 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C.The reaction progress was monitored via TLC in CH:EE = 5:1. After 60 hours there was no detectable reaction.

TLC: R_f = 0.59 (CH/EE, 5:1, (v:v)) (educt same as product)

6.3.10 Synthesis of Ethyl-3-((2-(5-dimethylamino)naphthalene-1-sulfonamido)ethyl)thio)-3-phenylpropanoate



50 mg of ethylcinnamate (0.284 mmol, 1 eq) and 132 mg of 5-(dimethylamino)-*N*-(2-mercaptoethyl)naphthalene-1-sulfonamide (0.426 mmol, 1.5 eq) were dissolved in 8 mL $CHCl_3$ and stirred over night. The reaction progress was monitored via TLC in CH:EE = 5:1. In the next morning there was no detectable reaction. Hence 9 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 40°C.. The reaction progress was monitored via TLC in CH:EE = 5:1. After 72 hours there was no detectable reaction.

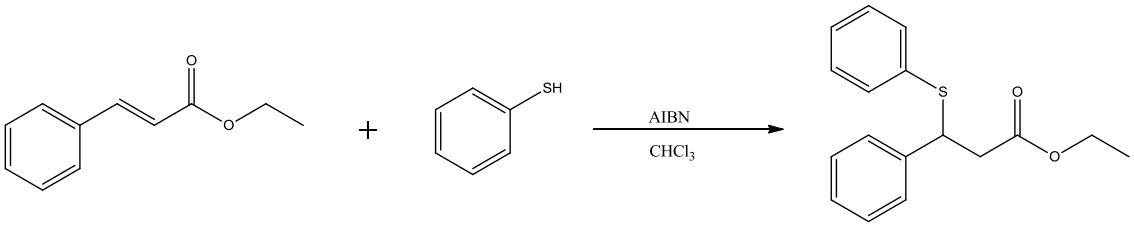
TLC: R_f = 0.67 (CH/EE, 5:1, (v:v)) (educt same as product)

6.3.11 Synthesis of Ethyl 3-(dodecylthiol)-3-phenylpropanoate

100 mg of ethylcinnamate (0.568 mmol, 1 eq) and 172 mg of dodecane-1-thiol (0.851 mmol, 1.5 eq) were dissolved in 5 mL $CHCl_3$ and stirred over night. The reaction progress was monitored via TLC in CH:EE = 5:1. In the next morning there was no detectable reaction. Hence 46 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 40°C.. The reaction progress was monitored via TLC in CH:EE = 5:1. After 72 hours there was no detectable reaction.

TLC: R_f = 0.96 (CH/EE, 5:1, (v:v)) (educt same as product)

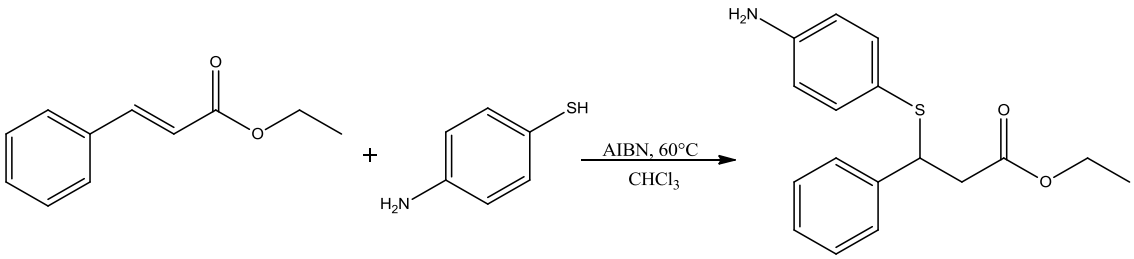
6.3.12 Synthesis of Ethyl 3-(phenylthio)propanoate



100 mg of ethylcinnamate (0.568 mmol, 1 eq) and 107 mg of benzenethiol (0.851 mmol, 1.5 eq) were dissolved in 3 mL $CHCl_3$ at 40°C. After 10 minutes 46 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 72 hours there was no detectable reaction.

TLC: R_f = 0.75 (CH/EE, 1:1, (v:v)) (educt same as product)

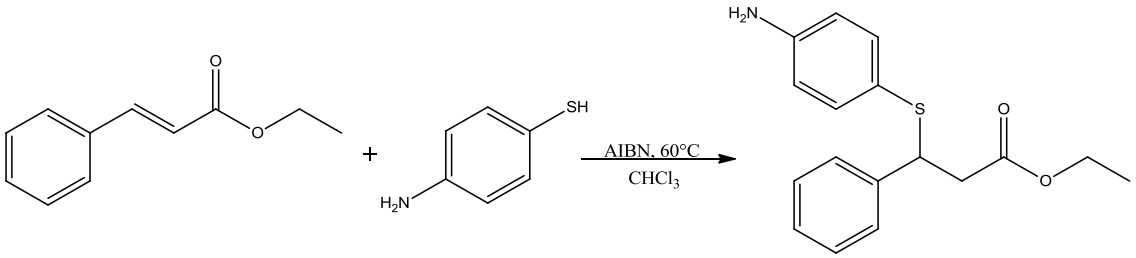
6.3.13 Synthesis of Ethyl 3-((4-aminophenyl)thio)-3-phenylpropanoate



50 mg of ethylcinnamate (0.284 mmol, 1 eq) and 53 mg of 4-aminobenzenethiol (0.426 mmol, 1.5 eq) were dissolved in 2 mL $CHCl_3$ at 60°C. After 10 minutes 5 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.44 (CH/EE, 1:1, (v:v)) (educt same as product)

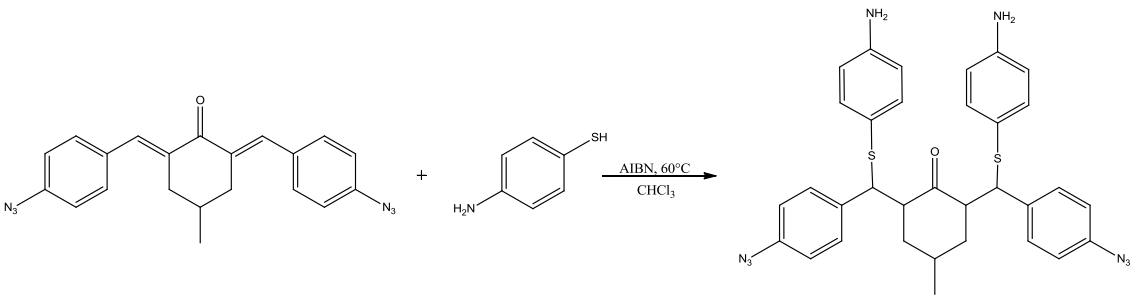
6.3.14 Synthesis of Ethyl 3-((4-aminophenyl)thio)-3-phenylpropanoate



50 mg of ethylcinnamate (0.284 mmol, 1 eq) and 35 mg of 4-aminobenzenethiol (0.284 mmol, 1 eq) were dissolved in 2 mL $CHCl_3$ at 60°C. After 10 minutes 5 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.41 (CH/EE, 1:1, (v:v)) (educt same as product)

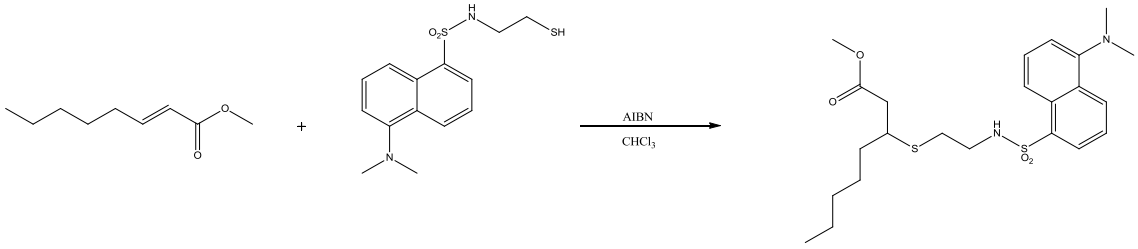
6.3.15 Synthesis of 2,6-bis(1-(4-azidophenyl)(4-aminophenyl)thio)-4-methyl-cyclohexanone



100 mg of 2,6-bis(4-azidobenzylidene)-4-methylcyclohexanone (0.270 mmol, 1 eq) and 101 mg of 4-aminobenzenethiol (0.810 mmol, 3 eq) were dissolved in 3 mL $CHCl_3$ at 60°C. After 10 minutes 8 mg of azobisisobutyronitrile (0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 96 hours there was no detectable reaction.

TLC: R_f = 0.42 (CH/EE, 1:1, (v:v)) (educt same as product)

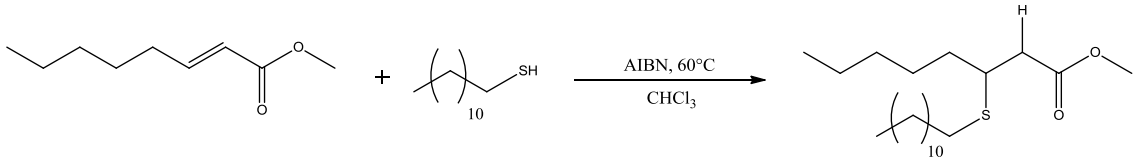
6.3.16 Synthesis of Methyl2-((2-(dimethylamino)naphthalene-1sulfonamido)ethyl)thio)-octanoate



50 mg of methyloctenoate (0.320 mmol, 1.1 eq) and 149 mg of 5-(dimethylamino)-*N*-(2-mercaptoethyl)naphthalene-1-sulfonamide (0.480 mmol, 1.5 eq) were dissolved in 5 mL $CHCl_3$ at 40°C. After 10 minutes 26 mg of azobisisobutyronitrile (0.160 mmol, 0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 72 hours there was no detectable reaction.

TLC: R_f = 0.80 (CH/EE, 1:1, (v:v)) (educt same as product)

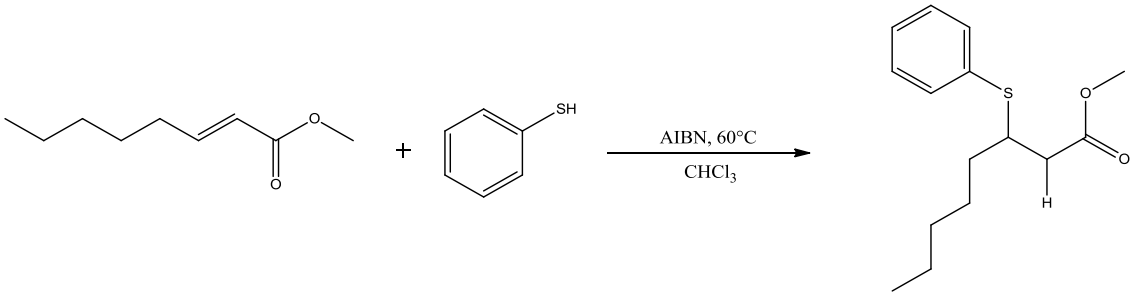
6.3.17 Synthesis of Methyl 2-(dodecylthio)octanoate



50 mg of methyloctenoate (0.320 mmol, 1.1 eq) and 97 mg of dodecane-1-thiol (0.480 mmol, 1.5 eq) were dissolved in 8 mL $CHCl_3$ at 60°C. After 10 minutes 26 mg of azobisisobutyronitrile (0.160 mmol, 0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 72 hours there was no detectable reaction.

TLC: R_f = 0.57 (CH/EE, 1:1, (v:v)) (educt same as product)

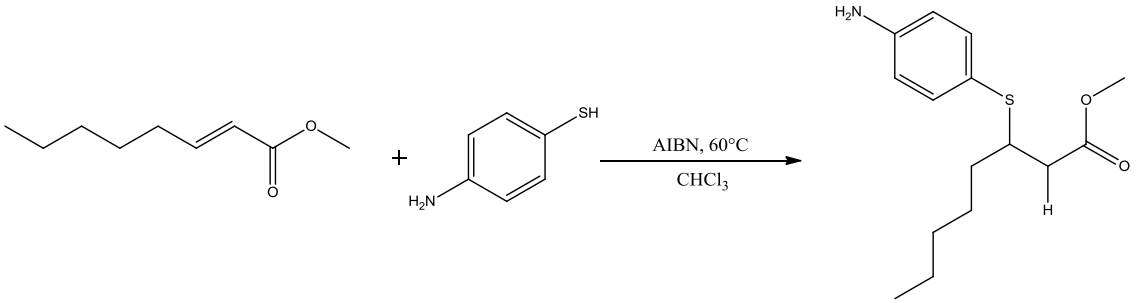
6.3.18 Synthesis of Methyl 2-(phenylthio)octanoate



50 mg of methyloctenoate (0.320 mmol, 1.1 eq) and 53 mg of benzenethiol (0.480 mmol, 1.5 eq) were dissolved in 7 mL $CHCl_3$ at 60°C. After 10 minutes 26 mg of azobisisobutyronitrile (0.160 mmol, 0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 60 hours there was no detectable reaction.

TLC: R_f = 0.65 (CH/EE, 1:1, (v:v)) (educt same as product)

6.3.19 Synthesis of Methyl 2-((4-aminophenyl)thio)octanoate



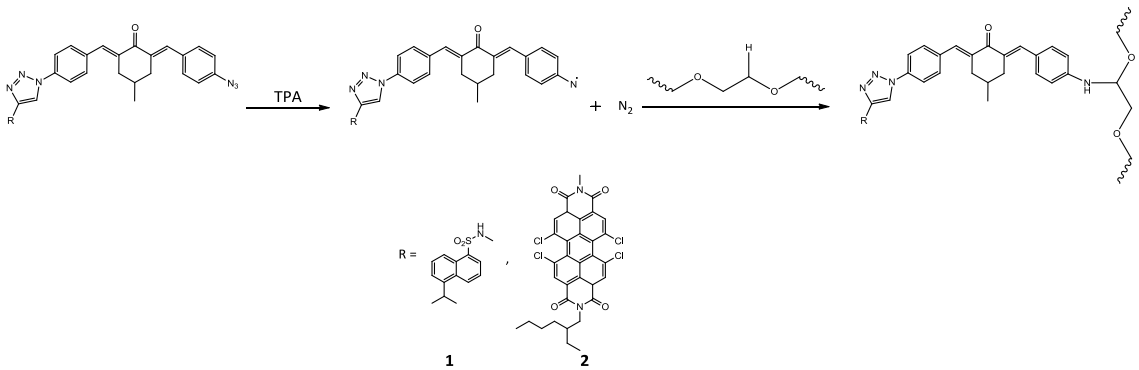
50 mg of methyloctenoate (0.320 mmol, 1.1 eq) and 60 mg of 4-aminobenzenethiol (0.480 mmol, 1.5 eq) were dissolved in 8 mL $CHCl_3$ at 60°C. After 10 minutes 26 mg of azobisisobutyronitrile (0.160 mmol, 0.5 eq) were added. The reaction was initiated by heating the solution to the boiling point with a heat gun. Then, the reaction mixture was kept at 60°C. The reaction progress was monitored via TLC in CH:EE = 1:1. After 60 hours there was no detectable reaction.

TLC: R_f = 0.65 (CH/EE, 1:1, (v:v)) (educt same as product)

6.4 TPA Experiments

All grafting experiments where performed at Vienna University of Technology. The laser used for this experimental setup was an all-diode-pumped Ti:Sapphire Laser from HIGH-Q LASER (Model Number IC-800-200 fs) with a pulse length of 160 fs and a wavelength of 810 nm. The pulse repetition rate was 73 Mhz. The near-gaussian beam profile allowed a line width of 14 nm. The constant output power was 200 mW, the operation temperature was kept to 18 °C by an external cooler. The inscribed structures had a size of 50 x 50 x 40 µm with a coating pitch of 0.5 µm.

6.4.1 Grafting of N-((1-4-((E)-3-(4-azidobenylidene)-5-methyl-2-oxocyclohexylidene)-methyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(dimethylamino)naphthalene-1-sulfonamide and 1,6,7,12-tetrachloroperylene-3,4,9,10-tetracarboxylic 2-(2-ethylhexyl)amine-8 ((1-(4-3-(4-azidobenzylidene)-5-methyl-2-oxocyclohexylidene)methyl)phenyl)-1H-1,2,3-triazol-4-yl)



For both cases a two weight percent solution in DMF was made. In the solution a hydrogel pet was put and expanded for about one hour. Now the pet was placed at the sample holder at the TPA-system. With the above mentioned adjustments the dyes where grafted on the polymer matrix. The reaction was characterized by LSM-microscopy. In case of perylene derivative there was no reaction detectable.

7 Appendix

7.1 List of Abbreviations

BAC-M	2,6-bis(4-azidobenzylidene)-4-methyl-cyclohexanone
TPA	two-photon absorption
CuAAC	Cu-catalyzed [3+2] azide-alkyne cycloaddition
ene	Carbon-carbon double bond
S	singlet state
T	triplet state
ISC	inter system crossing
I	transmitted intensity
z	propagation direction
α	absorption coefficient
ν	frequency
λ	wavelength
h	Planck constant
OPA	one-photon absorption
c	concentration
I_0	initial light intensity
β	TPA cross section
GM	Goeppert-Mayer
HOMO	highest occupied molecule orbital
LUMO	lowest unoccupied molecule orbital
eq	equivalent
Ph	phenyl
naph	naphthalene
RT	room temperature
DMF	2,5-Dimethylfuran

THF	tetrahydrofuran
CH	cyclohexane
EE	ethyl acetate

7.2 List of Tables

7.3 List of Figures

7.4 List of Schemes