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HARNESSING CLICK CHEMISTRY AND LIGHT: FROM NANOMATERIAL FUNCTIONALIZATION AND BIOMEDICAL APPLICATIONS TO PHOTO-INDUCED METAL CATALYSIS

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THESIS OVERVIEW

During my three years of doctoral studies, I had the opportunity to develop three research projects focused on the preparation of organic compounds for specific applications. Although these projects covered completely different areas of organic chemistry, they were unified by a common goal: employing key enabling techniques to address recurring challenges, simplify synthetic procedures to make them easily reproducible in any setting, reduce waste, and prioritize greener methodologies over classical approaches. At the same time, each project aimed to fill gaps in the literature and tailor the synthesized products to their intended applications. Across all three projects, click chemistry and photochemistry played a central role, and in one project these two techniques were even combined.

In chapter 2 is reported the synthesis of organic custom-made ligands that can be covalently bound to the surface of NPs, using photo-click-chemistry. The goal of the project was to drastically change the surface properties of NPs for the preparation of magnetorheological electrolytes (MRE) to be applied in the REMAP (Reusable Mask Patterning) project. REMAP is a project funded by the European Union that wants to revolutionize the microfabrication sector (e.g. for advanced photovoltaic applications) by developing a greener surface patterning technique. The crafted MRE properties are exploited to form infinitely reusable masks that would represent a huge step forward in the field of microfabrication with respect to standard lithography, in a period in which, more than ever, the interest in the reduction of pollution and waste, and in the displacement of fossil fuels is high.

The use of photochemistry continues in Chapter 3, focusing in detail on the novel field of light-induced transition-metal catalysis. A new type of isocyanide-insertion reaction was developed to prepare amides in a single step from alkyl iodides, isocyanides, and water through a multicomponent light-activated palladium-catalysed coupling reaction. Despite being among the most used tools in the hand of synthetic chemist, palladium catalysed reactions have some limitations. The most relevant is its incompatibility with alkyl electrophiles. Visible light-induced transition metal catalysis has emerged as an innovative approach to overcome those constraints. In these transformations a transition metal complex plays a dual role by harvesting photon energy and enabling bond forming/breaking reactions.

Lastly, chapter 4 reports the design and characterization of amphiphilic dendrimers as potential building blocks for self-assembled nanostructures (micelles or vesicles) suitable for drug delivery and nanomedical applications. Dendrimers are highly branched, tree-like macromolecules whose structure and surface chemistry can be precisely controlled. Their unique architecture allows them to mimic biological polymers such as proteins, making them attractive candidates for biomedical use. The project involved the stepwise synthesis of a second-generation dendrimer using a convergent approach, combining a hydrophilic polyamide dendron with a hydrophobic D-mannitol-based core through a Cu-catalysed azide-alkyne cycloaddition ("click") reaction.

SCIENTIFIC OUTPUT OF THE THESIS

Chapter 2: Smart material: the surface functionalization of nanostructured magnetorheological electrolytes with the use of click-chemistry

From the work carried out, two papers were written and are ready for submission. They focus on the experimental results described, ranging from organic synthesis to analytical results.

Chapter 3: Visible light induced Pd catalysed isocyanide insertion into inactive iodides

The project described led to this publication: Messina, A.; Monticelli, F.; Miroglio, T.; Gagliardi, A.; Viviani, I.; Banfi, L.; Riva, R.; Moni, L.; Basso, A.; Lambruschini, C. Photoexcited Palladium Complex-Catalyzed Isocyanide Insertion into Inactivated Alkyl Iodides. *Molecules* **2025**, *30* (12), 2584. <https://doi.org/10.3390/molecules30122584>.

Chapter 4: Preparation of novel amphiphilic dendrimers for biomedical applications through the use of click-chemistry

The work reported in this chapter represents only a preliminary investigation of a much larger project. In the future, further analyses will be performed, and new amphiphilic dendrimers will be synthesized.

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LIST OF USED ABBREVIATIONS

135-DEPT	Distortionless Enhancement by Polarization Transfer
ALK-X	Alkyl halide
APTES	3-aminopropyltriethoxysilane
ATR-IR	Attenuated Total Reflectance - Infrared
BET	Brunauer–Emmett–Teller
BHT	Butylhydroxytoluene
BiOS	Biologically Oriented Synthesis
BippyPhos	5-(Di-tert-butylphosphino)-1', 3', 5'-triphenyl-1'H-[1,4']bipyrazole
BOC ₂ O	Di-tert-butyl dicarbonate
B.P.	Boiling Point
CBD	Condition-Based Divergence
CDI	1,1'-carbonyldiimidazole
CNP	Conjugated Nanoporous Polycarbazole
CNRS	Centre National de la Recherche Scientifique
COSY	Correlated Spectroscopy
CPU	Central Processing Unit
CuAAC	Copper catalysed Alkyne-Azide Cycloaddition
DAD	Diode Array Detector
DCM	Dichloromethane
DEG(-OMe)	Diethylen glycol (mono methyl ether)
DFT	Density Function Theory
DIPEA	N,N-diisopropylethylamine
dG _n	n-generation dendron
DLS	Dynamic Light Scattering
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
DMPA	2,2-dimethoxy-2-phenylacetophenone
DMSO	Dimethylsulphoxide
DOS	Diversity-Oriented Synthesis
DOSY	Diffusion-Ordered Spectroscopy
dppb	1,4-Bis(diphenylphosphino)butane
dppe	1,2-Bis(diphenylphosphino)ethane

dppf	1,1'-Bis(diphenylphosphino)ferrocene
DSC	Differential Scanning Calorimeter
EA	ethyl acetate
EDA complex	Electron Donor-Acceptor complex
EDCI	hydrochloride 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EnT	Energy Transfer
ESI	Electrospray Ionization
FC	Flash Chromatography
FT-IR	Fourier Transform Infrared spectroscopy
G _n	n-generation dendrimer
GC-MS	Gas Chromatography- Mass Spectrometry
HAT	Hydrogen Abstraction
HB	Random hyperbranched polymers
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HOBT	1-hydroxybenzotriazole
HOMO	Highest Occupied Molecular Orbital
HPLC	High Pressure Liquid Chromatography
HPLC-ESI-MS	High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry
HRMS	High-Resolution Mass Spectrometry
INL	International Iberian Nanotechnology Laboratory
INN-D	Institute Nanoscience and Nanotechnology Demokritos
IC	Internal Conversion
ISC	Intersystem Crossing
LED	Light Emitting Diode
LMCT	Ligand To Metal Charge Transfer
LUMO	Lowest Unoccupied Molecular Orbital
MEMS	Microelectromechanical systems
MF	Magnetic Field
M.P.	Melting Point
MPTES	3-mercaptopropyltriethoxy silane
MRE	Magnetorheological Electrolytes
MRF	Magnetorheological Fluid

MRS	Modular Reaction Sequences
N _A	Avogadro's number
NMR	Nuclear Magnetic Resonance
NP (s)	Nanoparticle(s)
OL	Optical Lithography
OPL	Optical Projection Lithography
PC	Photocatalyst
PE	Petroleum Ether
PET	Photoinduced Electron Transfer
PG	Protecting Group
REMAP	Reusable Mask Patterning
SAR	Structure-Activity Relationship
SET	Single Electron Transfer
S _N	Nucleophilic Substitution
S _N 2	Nucleophilic Substitution type 2
SRR	Single Reactant Replacement
TBAB	N-tetrabutylammonium bromide
TBS	Tert-butildimethylsilyl-
TEG(-OMe)	Tetra ethylene glycol (mono methyl ether)
TEM	Transmission electron microscopy
TEMPO	2,2,6,6-tetramethylpiperidine 1-oxyl
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl-
Unige	University of Genoa
UniLux	Université du Luxembourg
UV	Ultraviolet
XantPhos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene
Y BRSM	Yield Based on the Recovered Starting Material

Chapter 1

GENERAL INTRODUCTION

Since 1828 when Wohler achieved the synthesis of urea from inorganic compounds, which is recorded as the birth of organic chemistry, this science has evolved, becoming one of the widest fields of chemistry able to comprehend many subgenres. The innovation brought by researchers led to the development of new strategies and methodologies that enabled the efficient preparation of new drugs, powerful agrochemical compounds, innovative materials and many other specialty chemicals.

Over time, synthetic chemists have learned to integrate traditional techniques with multidisciplinary approaches to meet the growing demand for new substances, materials, and devices. However, crafting the right molecule for a specific application remains a time-consuming and complex task. It largely relies on trial-and-error processes and is often compared to an art form. This challenge also results in the accumulation of significant chemical waste. Today, this bottleneck can be addressed through the development of new synthetic methods that allow researchers to rethink and streamline synthetic routes^{1,2}.

The key enabling techniques are the strategies employed to overcome these limitations. Biocatalysis, photochemistry, catalysis, electrochemistry, flow chemistry, advanced organic synthesis, and high-throughput experimentation are among the methods considered³.

Over the three years of the PhD, a multidisciplinary approach was applied to develop three distinct research projects, making extensive use of key enabling techniques to achieve cleaner, faster, and greener synthetic routes. Among the various available methodologies, click chemistry, multicomponent reactions, and photocatalysis were specifically employed.

1.1 Click-chemistry

One of the most complex and articulated branches of organic chemistry is the synthesis of natural products. Molecules produced by living organisms have always fascinated and inspired researchers. A real competition emerged between research groups to be the first to accomplish the synthesis of the most diabolically complex natural substances. These achievements typically involved low-yielding, multi-step syntheses, often exceeding twenty steps, in which the most advanced and sophisticated reactions and methodologies were employed.

Primary and secondary metabolites have long astonished chemists. How can living organisms produce such complex organic compounds with apparent easiness. Moreover, the biosynthesis of primary metabolites demonstrates how exceptional living organisms are as combinatorial chemists: starting from only a few monomers and oligomers, they are able to generate the central macromolecules essential for life processes⁴.

In 2001, Sharpless introduced the concept of *click chemistry*. It was inspired by nature's strategy: the use of simple reactions and small building blocks connected through heteroatom linkages. Since one of the major challenges in modern advanced organic synthesis is the intrinsic complexity of the reactions and the specialized techniques they require, click chemistry proposes the opposite approach, making synthesis straightforward, robust, and accessible to virtually anyone in any setting.

Sharpless defined specific criteria for a reaction to qualify as a click reaction: it should be quantitative or high-yielding; generate no, or only harmless, by-products that can be removed without chromatography; require simple setup and conditions; be ideally insensitive to water and oxygen; use no solvent or a solvent that is easily removed and prefer the use of water when possible; and be stereospecific⁵.

This new philosophy of synthesis rapidly became popular and was adopted across research laboratories worldwide, since it applies not only to organic chemistry but also to materials science, engineering, and many other fields.

In 2022, the Royal Swedish Academy of Sciences awarded Barry Sharpless the Nobel Prize for the *click approach to organic synthesis*.

1.1.1 Thiol-ene click reaction

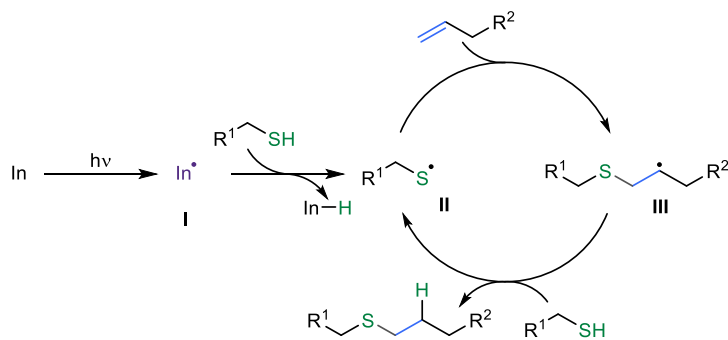
Thiol reactivity has been known since the beginning of the 20th century, and the interactions between thiols and alkenes were thoroughly investigated in the past, especially because these compounds could be easily obtained from petroleum. The specific reactivity of thiols and alkenes was recognized in 2008 by Killops as a click reaction, and the thiol-ene reaction rapidly became one of the most widely used click transformations in materials science.

The thiol-ene radical reaction is known for its reproducibility and efficiency. It displays fast kinetics, can be performed under neat conditions, and shows high regioselectivity in favour of anti-Markovnikov addition across the C=C bond. Furthermore, this transformation tolerates the presence of oxygen and water, does not require any metal catalyst, and is compatible with a wide variety of functional groups, making it highly orthogonal and eliminating the need for protecting groups. Another significant advantage of the thiol-ene reaction is the strength of the C-S covalent bond formed in the product⁶.

Historically, the thiol-ene reaction was carried out thermally, whereas recently, with the advancement of photochemistry, the light activated thiol-ene reaction became popular among chemists. Therefore, it is necessary to distinguish between thermal thiol-ene and photochemical thiol-ene. In the thermal version the use of a radical initiator is essential to activate the radical mechanism, instead in the light activated one this component could be omitted. However, in the latter case are required photons which wavelength is between 200 and 300 nm. Such electromagnetic radiations are so energetic that many functional groups and even portion of molecules could be degraded and homolytically cleavage, leading to side reactions and

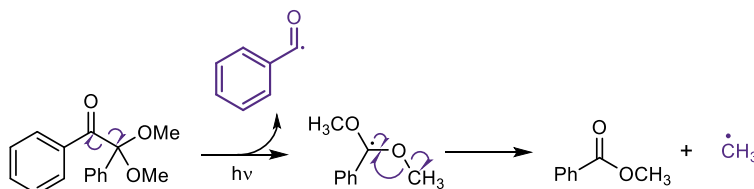
byproducts. Therefore, the possibility to use a radical initiator sensitive to radiation close to visible light becomes the first choice. The thermal thiol-ene is still used in polymer chemistry (for example in the reticulation processes) even if it is lower yielding than the photochemical version⁷.

The photoactivated thiol-ene reaction mechanism is reported in *Scheme 1.1*. The radical mechanism starts with the light activation of the radical initiator, which upon excitation undergoes a homolytic cleavage generating two radicals (*Scheme 1.2*). The radical initiator (**I**) abstracts a hydrogen atom from the thiol group generating a radical thiol (**II**). This step is highly selective because the hydrogen abstraction is guided by the relative pKa of the different hydrogen atoms, and the thiol group is the most acid in the reaction mixture. Intermediate **II** performs a high regioselective addition reaction to the alkene generating a secondary alkyl radical (**III**). The anti-Markovnikov regioselectivity of the addition is guided by the relative stability of the radical formed. Primary alkyl radicals are more unstable than secondary alkyl radicals. To conclude the radical mechanism, the intermediate **III** abstracts a hydrogen atom from another thiol in the reaction mixture regenerating the radical thiol (**II**) which can continue the radical mechanism^{6,7}.



Scheme 1.1: the radical mechanism of the thiol-ene reaction

In the specific application of the thiol-ene reaction described in Chapter 2, the radical initiator chosen was 2,2-dimethoxy-2-phenylacetophenone (DMPA, also known as Irgacure 651). Its photochemical degradation pathway is shown in *Scheme 1.2*. The use of this initiator allows the reaction to be carried out with LED light at 365 nm, instead of the typical 250 nm irradiation required for thiol-ene reactions performed without a radical initiator.



Scheme 1.2: photochemical degradation pathway of DMPA (2,2-dimethoxy-2-phenylacetophenone)

The thiol-ene click reaction finds many uses in material science. For example, Crowe *et al.*⁸ in 2005 used this click reaction to modify the wettability of different material covered in poly(vinylmethylsiloxane). The use of photochemical thiol-ene reaction gave to the researcher the possibility to covalently bind to the surface thiols with different neutral alkyl chain (like dodecane thiol) and anionic alkyl chain bearing a carboxylate end terminal. The successful modification of

the surface properties was tested by contact angle which revealed the formation of superhydrophobic surfaces (using neutral alkyl thiols) and super hydrophilic surfaces (using anionic alkyl thiols).

In 2008, Killops *et al.*⁹ efficiently prepared a family of fourth-generation dendrimers using the radical thiol–ene reaction. The dendrimers were synthesized in a divergent manner by alternating thiol–ene reactions with condensation steps. The dendrimer core, 2,4,6-triallyloxy-1,3,5-triazine, was functionalized with thioglycerol under 365 nm UV irradiation in the presence of DMPA. The first dendrimer generation was obtained quantitatively, notably without the need for any solvent. This first generation was then subjected to a quantitative esterification using 4-pentenoic anhydride, enabling the first branching step of the structure. By repeating these simple transformations, the researchers efficiently prepared a homogeneous fourth-generation dendrimer. Finally, the dendrimer surface was modified with various thiols to tailor its final properties.

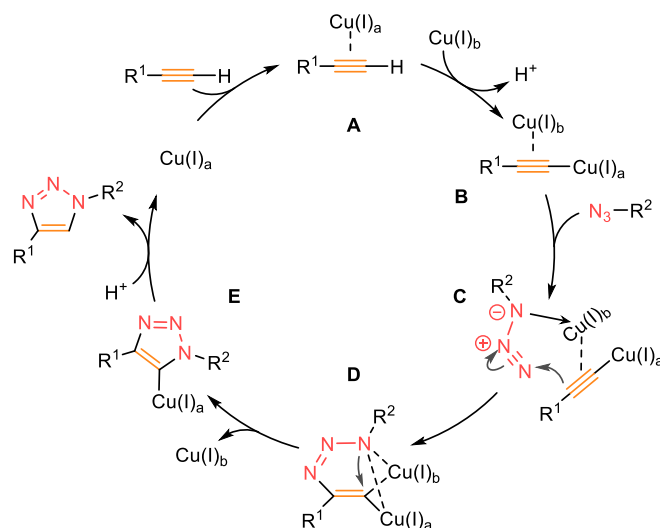
1.1.2 Copper catalysed Alkyne Azide Cycloaddition reaction (CuAAC)

The Copper catalysed Alkyne Azide Cycloaddition reaction (CuAAC) is probably the most recognizable click reaction, and it gained major attention in 2001 thanks to the work of Meldal and Sharpless. The origins of the copper free cycloaddition between azides and alkynes are dated back to the beginning of the last century¹⁰ and this type of cycloaddition reaction was elucidated by R. Huisgen only in 1960¹¹, who coined the term 1,3-dipolar cycloaddition. Today, the name Huisgen cycloaddition is still referred to this transformation. The real revolution happened in 2001 when Meldal and Tornøe proposed to add a catalytical quantitative of Copper (I) in the reaction crude¹². The benefits were independently recognized by Meldal and Sharples noting an increase in reaction rates by seven times and perfect regioselectivity, leading to the formation of 1,4-disubstituted 1,2,3-triazole ring in almost quantitative yield respect to mixture of 1,4-disubstituted and 1,5-disubstituted products that was usually obtained in the thermal uncatalyzed version^{12,13}.

What made the CuAAC reaction successful is its combination of reliability, selectivity, insensitivity and simplicity. The triazole that can be obtained almost quantitatively is essentially chemically inert to reactive conditions and perfectly biocompatible. CuAAC tolerates a vast range of functional groups and is largely insensitive to solvent or pH. Normally, CuAAC reaction is performed in mild conditions, and the suitable solvent is water, eventually mixed with alcohols, THF or dioxane, making it a perfect green reaction¹⁴. This click-reaction found application in biomolecular ligations, targeting (also in vivo conditions) and many more related biological applications because neither azides nor alkynes typically occur in biological systems, and they can be introduced selectively.

The mechanism of the CuAAC reaction is reported in *Scheme 1.3*. The final mechanism was elucidated only in the 2010's by the combination of crystallographic experiments, kinetic studies and DFT calculations¹⁵. Copper (I) has a crucial role since the beginning of the mechanism, because it easily coordinates in a di- π -to fashion the alkyne interacting with its π -orbitals (**A**).

Kinetic measurements in intermediate concentrations indicated that the reaction was at least second order with respect to the concentration of Cu(I) and alkyne. In fact, DFT calculations proved that copper complexation enhances the acidity of the alkyne making the deprotonation reaction easy, thus favoring the interaction with a second copper atom (**B**). These calculations are supported by structural database entries showing stable complexes formed by di- or tri-coordinated copper atoms bound to a single alkyne. The second copper atom coordinated to the Cu-acetylide complex helps in the mechanism by coordinating at the same time the azide (intermediate **C**), which now can be easily attached by the secondary carbon of the alkyne made more nucleophile by the double coordination, giving intermediate **D**. The formation of the triazole is completed by the addition of the nucleophilic nitrogen atom of the azide to the double coordinated terminal carbon of the alkyne (intermediate **E**). Intermediate **E** was isolated and characterized exploiting the enhanced stability given by the use of special N-heterocyclic carbene as ligand for copper catalyst¹⁶. Finally, the H⁺ liberated initially accelerates the dissociation of Cu(I) from intermediate **E** giving the final triazole and restoring the catalyst.



Scheme 1.3: mechanism of the CuAAC reaction

To obtain a regioselective formation of 1,5-disubstituted 1,2,3-triazole is necessary to use fine-tuned Ru(II) complexes¹⁷, rare earth metal complexes¹⁸, or cooperative s-block bimetallics catalysis¹⁹.

Recently, it was discovered that the CuAAC can be performed in another type of green solvent: deep eutectic solvents. Those solvents are obtained combining two (in some cases three) chemical substances which are able to form a new eutectic mixture (with a melting point below that of its individual components) through the formation of a hydrogen-bond network. Deep eutectic solvents are recognized as green solvents because the components are non-toxic, inexpensive and the original mixture can be reused many times without losing performances.

1.2 An introduction to photochemistry and photocatalysis

Light is everywhere around us, from simple light bulbs and LED lights to the sun. Light has been used for chemical reactions since the beginning of life on Earth, and bacteria and plants are the most expert in doing so. For a photochemical reaction to occur, either in a bacterium, a plant or in a laboratory, the Grotthuss-Draper Law, or First Law of Photochemistry, must be satisfied²⁰. The light must be absorbed by a species participating in the process, typically either one of the substrates or a photocatalyst.

Physics helps us to understand from an algebraic point of view the process of absorption, describing it with the Lambert-Beer equation. The intensity of the absorption of a specie in solution at a given wavelength is quantified by the *molar attenuation coefficient* ϵ [$\text{L mol}^{-1} \text{cm}^{-1}$], which is correlated to the absorbed quantity of light (I/I_0), the concentration C [mol L^{-1}] of the solution and the length of the cell l [cm] (A and T are called *absorbance* and *transmittance*):

$$A = -\log(T) = -\log\left(\frac{I}{I_0}\right) = \epsilon \cdot l \cdot C$$

The absorption of light promotes an electron from the ground state to the excited state, and it can happen only when the energy of the incident photons perfectly matches the energy gap between the two orbitals. Physically, this is rationalized using the Planck equation ($E = h\nu$, E : energy of the photons, h : Planck's constant, ν : frequency of the beam of light).

During excitation, the two interested orbitals are the HOMO (highest occupied molecular orbital) and the LUMO (lowest unoccupied molecular orbital) and the promoted electron must retain its spin giving a singlet state (the direct formation of triplet states is forbidden and unlikely to happen, but still possible through an intersystem crossing (ISC)²¹), and without structural reorganization of the nuclei). The molecular phenomena following absorption are usually depicted and described through the *Jablonski diagram* (Figure 1.1).

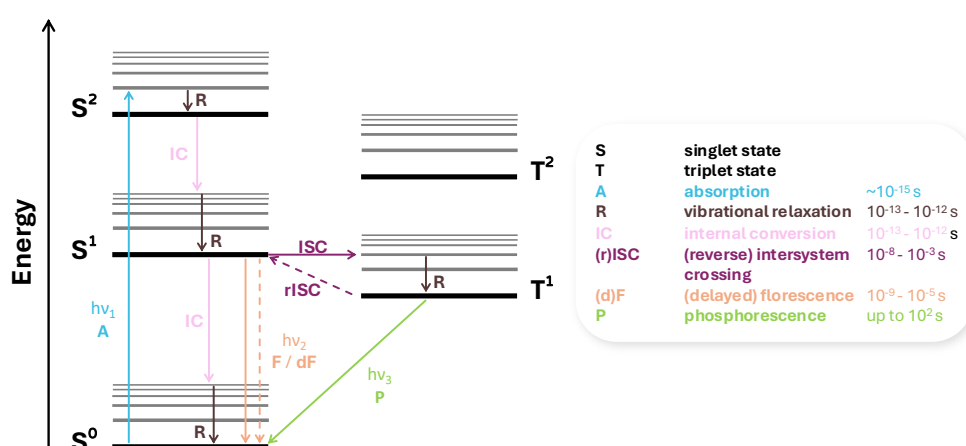


Figure 1.1: typical Jablonski diagram reporting the possible processes taking place in an excited state molecule.

Upon partial dissipation of excess energy through vibrational relaxation and internal conversion (thermal, non-radiative processes), a molecule reaches the lowest excited electronic state. From this state, the molecule can either return to the ground state by emitting a photon (fluorescence)

or undergo a change in spin multiplicity to form a triplet state via intersystem crossing (ISC). The triplet state may subsequently deactivate through radiative emission (phosphorescence).

ISC and phosphorescence, which involve changes in spin multiplicity, are particularly important in systems where radical processes are involved. Although spin-forbidden, these processes can occur due to spin-orbit coupling, which mixes singlet and triplet states. The probability of ISC is enhanced in the presence of heavy atoms or external magnetic fields, which perturb the orbital motion of electrons and increase spin-orbit interactions.

On a molecular level the absorption process is guided by the presence of specific functional groups called *chromophores*. The vastity of the conjugation of the system and the potential presence of other functional groups (*auxochromes*) affects the intensity (*hyperchromic* [$\epsilon\uparrow$] and *hypochromic* [$\epsilon\downarrow$] effect) and the wavelength of the absorption band (*bathochromic* [$\nu\downarrow$, $\lambda\uparrow$] and *ipsochromic* [$\nu\uparrow$, $\lambda\downarrow$] effect, or red and blue shifts respectively).

The photochemical activation of a molecule is independent of the temperature, thus being orthogonal to the commonly exploited thermal processes. Moreover, the free energy variation in a photochemical process can be either negative or positive, opening up reaction pathways that are unfeasible in the mere thermal domain. These are the main reasons for the huge success that photochemistry found among synthetic organic chemists²².

The activation of organic reactions with light can be achieved in different ways, the first one to be studied was the, so called, “direct photochemistry”. In the direct form, the light is used to excite directly the organic molecules. Most organic compounds are colourless or slightly pale yellow, therefore the range of wavelengths studied is in the ultraviolet region and lower frequencies are not sufficiently energetic to promote electronic transitions. The UV radiation is very energetic, and it is able to excite several functional groups. The typical electronic transitions observed during a direct photochemical reaction are the $\pi\text{-}\pi^*$ and the $n\text{-}\pi^*$ transitions, which are the only ones falling into synthetically useful areas of the near-UV region (Figure 1.2).

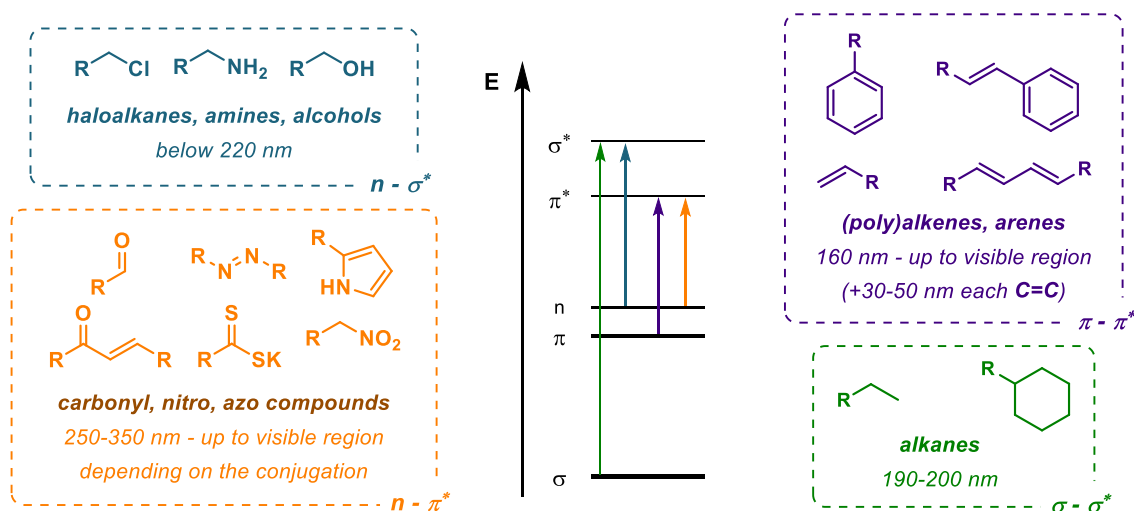


Figure 1.2: Typical electronic transitions and molecule classes associated to them ($\sigma\text{-}\pi^*$ and $\pi\text{-}\sigma^*$ are symmetry-forbidden)²³

Despite being synthetically useful, the UV radiation is not the preferred one. The range of wavelength used (150 nm to 350 nm) is so energetic that in many situations, it favours the formation of huge quantity of side-reactions and, consequently, side-products. Moreover, the possibility of using visible light is more appealing, thanks to a major safety and lower prices since the commercialization of strong and durable LEDs²⁴.

1.2.1 The vast world of photocatalysis

Photocatalysis (PC) recently emerged as a novel type of catalysis and immediately gained a lot of attentions, outrunning all together the direct photochemistry, the introduction of dyed auxiliary and the formation of EDA complexes (here not described). Photocatalysis can open new reactions mechanisms, speed up reactions and let the use of visible light.

A photocatalyst is a foreign species in solution able to absorb light and transfer the energy gained upon excitation to one or more molecules in solution.

How photocatalysts change the reaction path is represented in *Figure 1.3* in comparison with a classical thermal reaction (*Figure 1.3-a*) and a direct photochemical reaction²² (*Figure 1.3-b*).

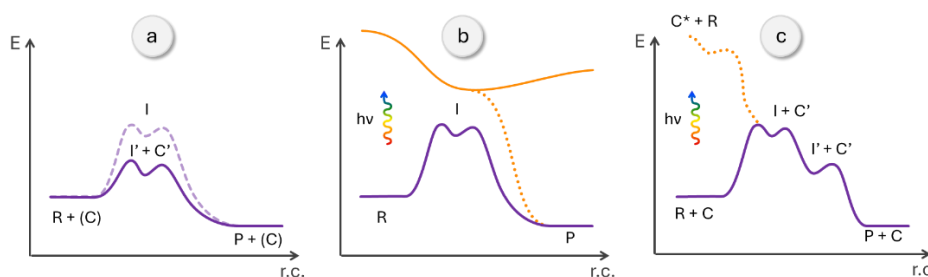


Figure 1.3: Differences between: (a) catalysed thermal reaction (solid line) and a thermal reaction (dotted line) (b) photochemical reaction via direct excitation of the substrate and (c) photocatalyzed process. (Reproduced from Ravelli et al. Chem. Soc. Rev. 2009, 38(7),1999-2011).

In a thermal reaction, the reagent (R) is converted into the product (P) through one or more transition states with possibility to form one (or more) intermediates (I) between two transition states (*Figure 1.3-a*). Whenever the activation energy is too high for the reaction to occur smoothly, the presence of a catalyst (C) might help lowering the energy of both the transition states and the intermediate. When light is used to excite one of the reagents, it reaches the excited state potential energy surface (yellow solid line). In this state, it possesses an energy higher than the transition state's one, allowing it to turn into the products easily (*Figure 1.3-b*). If none of the reagents can absorb light, the use of a photocatalyst (C) becomes essential. It absorbs light, gets excited (C^*) and then transfer the energy acquired to the reagent (R) in its ground state. Several intermediates are formed and evolved toward the final product, and the catalyst, if not yet in the ground state, is restored by a turnover event (*Figure 1.3-c*).

Homogeneous photocatalysts can be labelled in two different ways based on the activation pathway of the substrate. *Photoredox catalysts* are those species that mainly activate the reagent via photoinduced electron transfer (PET) mechanism, while *photosensitizers* are those that mainly

operate via energy transfer (EnT) mechanisms. Practically, most photocatalysts may operate through both mechanisms, therefore it is important to give the right label based on the specific mechanism of the reaction²⁵. It is possible to perform photocatalysis in heterogeneous conditions, in this scenario the activation mechanism of the substrate is completely different and works like a semiconductor.

Homogeneous photocatalysts can be organic molecules and transition metal complexes like is shown in *Figure 1.4*. The most appealing compounds among the two are the transition metal complexes and, generally, Ir and Ru are the most broadly used metals over all the others. The main advantage against the organic compounds is the tunability, by simply modifying the electronic properties of the ligands is possible to change the redox potential of the complex. On the other hand, they are less appealing from an environmental point of view, being also more expensive due to the low availability of the metal ore from which they derive. Moreover, a great part of the excitation energy is lost thermally, and dissipated by the solvent, while reaching the synthetically useful triplet state²⁶.

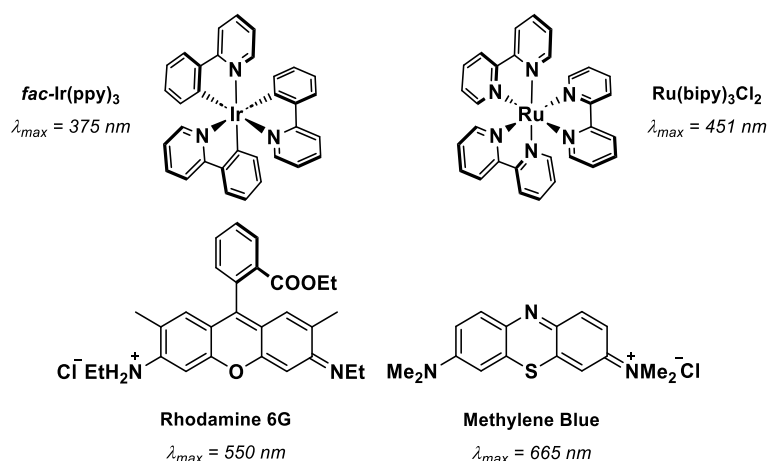


Figure 1.4: Examples of various photoredox catalysts with their absorption wavelengths

As anticipated, one of the biggest advantages of photocatalysis is the possibility to shift the operative wavelength to the visible region, which offers a milder, safer, and more selective alternative. The visible light-activated reactions often proceed at ambient temperature, harsh reagents are not necessary, and visible light is abundant, inexpensive, and environmentally friendly, making this technique aligned to the principles of green chemistry. Furthermore, the development of efficient photocatalysts enables new reactions mechanism, and to speed up reactions.

In traditional photocatalysis, the *photoredox catalysts* can operate through either photoinduced electron transfer (PET) mechanism or energy transfer (EnT) mechanisms (in this case it is called *photosensitizer*). Once the substrate is excited or activated, the entire reaction mechanism can proceed. In this case, the catalyst itself does not directly participate in the reaction mechanism (*Figure 1.5A*)²⁷.

In cooperative catalysis, two transition metals are used. One acts as the transition metal catalyst (TM) responsible for the reaction, while the other serves as the photocatalyst (PC) (*Figure 1.5B*). Again, the PC can operate via electron or energy transfer to the TM, exciting it. The TM then becomes the only species that directly participates in the reaction mechanism. This strategy can accelerate the reaction, combining the benefits of both catalytic systems; however, the need to employ two transition metals in a single reaction (which increases costs) limits the applicability of this approach²⁸.

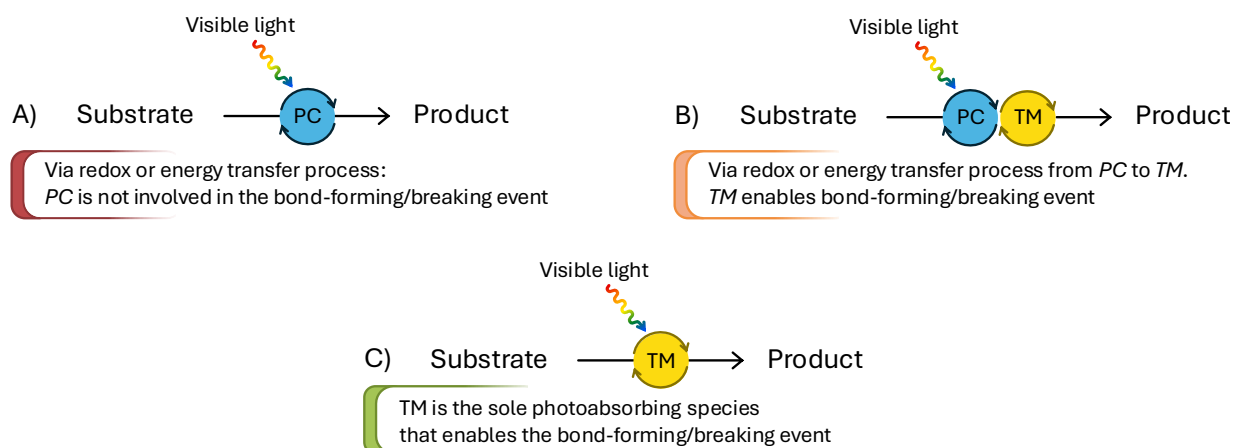
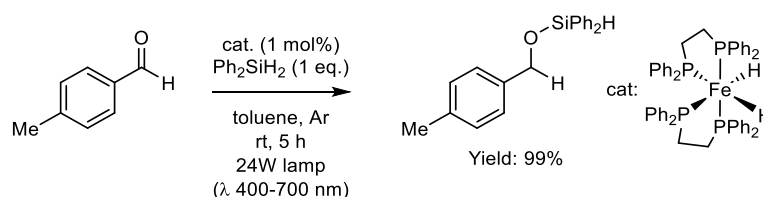


Figure 1.5: Representation of the different photocatalytic systems. A) traditional photocatalysis, B) cooperative photocatalysis, C) transition metal photocatalysis

Transition-metal photocatalysis is an emerging approach in which only one transition metal complex is used. These transformations operate via single catalytic cycle, where the metal complex acts both as a PC and as a TM catalyst (*Figure 1.5C*). It gets excited upon light absorption and can transfer the acquired energy as a photoredox catalyst or photosensitizer. When it returns to the ground state, it can actively catalyse the reaction. Therefore, these transition metal complexes play a simultaneous dual role. These catalysts are TM complexes (Co, Fe, Cu, Pd, Pt, Au) possessing typical phosphine-, amine-, oxime-, or acetylides, ligands that are usually employed in conventional catalytic transformations²⁹. This approach reduces operative costs, as it avoids the use of rare transition metals; in particular, commonly used iridium- and ruthenium-based photocatalysts are expensive due to the scarcity of the metal ores and the complexity of the bipyridyl ligands employed. Transition-metal photocatalysis also enables the use of visible light, since the majority of transition-metal complexes exhibit intense absorption bands in the visible region.



Scheme 1.4: innovative photoactivated hydrosilylative reduction using Fe(II)

In 2011 Darcel and Sartais reported an innovative hydrosilylative reduction of conjugated ketones and aldehydes using Fe(II) catalyst in presence of diphenylsilane under the irradiation of blue light³⁰. The strength of this transformation was the photo activation of a classical transition metal complex and subsequent studies proved how this combination was essential for having the desired products in quantitative yield. Further studies proved that the temperature enhances greatly the kinetic of the reaction making it faster. Over time, the authors have expanded their study, and many methods were developed on the reduction of esters, amides, and imines via hydrosilylation with a diverse set of Fe-catalysis under visible light irradiation.

An in-depth analysis of this type of catalysis is presented in chapter 3.

1.3 Multicomponent reactions

Multicomponent reactions (MCRs) are a class of reactions of great interest in synthetic organic chemistry. A multicomponent reaction is defined as a transformation where three or more components react in a single process either through a concerted or multi-step mechanism affording a product containing all the essential structural elements of the starting compounds³¹. The first reported multicomponent reaction dates back to 1850, when Adolph Strecker discovered the one-pot synthesis of α -aminoacids that is named after him³². Although the strength of MCRs, this field started to grow only a century later following the publication of Passerini's three-component reaction and Ugi's four-component reaction³³. Ugi was the first one to recognize the huge potential of this approach in synthetic chemistry, proposing a series of analogues of a local anaesthetic, xylocaine, through a combinatorial approach.

Multicomponent approaches typically allow to prepare in a single step structurally complex and poly-functionalized molecules with potentially high molecular weights. Furthermore, by just changing the nature of the substituents in the starting materials is possible to synthesize an entire library of compounds rapidly. Notably, the side reactions are usually condensation of some components generating small molecules that can be easily removed by purification, making these transformations extremely advantageous in terms of step and atom economy.

The strong chemoselectivity of MCRs avoids extended studies of protecting strategies and recently the implementation of asymmetric or enzymatic catalysis allowed to partly overcome the common lack of stereo selection that these transformations often display³⁴.

Being extremely robust and well-known metal cross-couplings, alkylations, and amidations reactions are extensively used in drug research. However, most active molecules possess linear shapes and sp^2 character, and are part of only a small portion of the entire chemical space³⁵. Although the success of the mentioned reactions in the preparation of active small molecules for their reliability and modularity, MCRs can be used to access a wide range of molecular scaffold in the sp^3 rich area and can be used for installing privileged motifs such as cyclopropane rings and spiranes with ease. For this reason, MCRs found large application in biologically oriented synthesis

(BiOS) and diversity-oriented synthesis (DOS), where the investigation on structure-activity relationships (SARs) plays a prominent role³⁶.

The key strength of MRC is the modularity, notably most functional groups are tolerated and need no protection, thus MCRs products can be directly functionalized to achieve diversification and structural complexity. Since MCRs is a relatively new research field and it started to be embraced and used by research groups recently, the novelty can be introduced by engineering the existing processes via several procedures. The most common ones are the single reactant replacement (SRR), the design of modular reaction sequences (MRS), the modification of some reaction parameters to achieve condition-based divergence (CBD) or the direct combination of multicomponent reactions (MCR²)³¹.

All the advantages of MCRs described above satisfy several principles of green chemistry (*Figure 1.6*) and of the ideal synthesis³⁷, making MCRs an ever-growing field of studies.

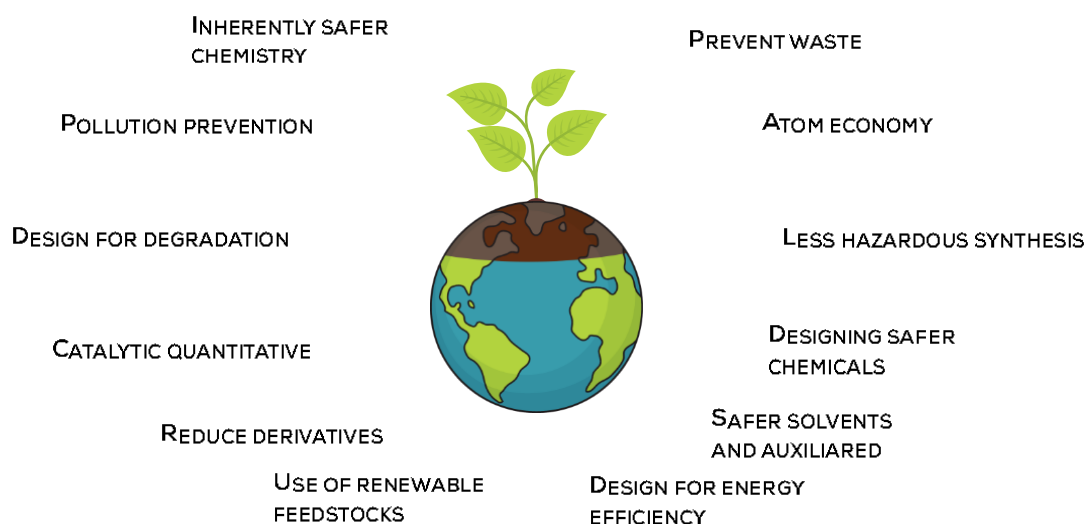


Figure 1.6: twelve principles of green chemistry

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Chapter 2

SMART MATERIAL: THE SURFACE FUNCTIONALIZATION OF NANOSTRUCTURED MAGNETORHEOLOGICAL ELECTROLYTES WITH THE USE OF CLICK-CHEMISTRY

2.1 Brief introduction to nanomaterials and magnetorheological fluids

Nanomaterials are defined as materials with at least one external dimension in the size range from approximately 1-100 nanometres. Instead, nanoparticles are objects with all three external dimensions at the nanoscale.

Nanoparticles (NPs) that are naturally occurring (e.g., volcanic ash, soot from forest fires) or are the incidental byproducts of combustion processes (e.g., welding, diesel engines) are usually physically and chemically heterogeneous and often termed ultrafine particles. Engineered nanoparticles are intentionally produced and designed with very specific properties related to shape, size, surface properties and chemistry. These properties are reflected in aerosols, colloids, or powders. One of the most fascinating properties is that the behaviour of nanomaterials may depend more on the surface area than particle composition. This is due to the greater ratio of the surface to the bulk. The relative-surface area is one of the principal factors that enhance NPs reactivity, strength, and electrical properties. Some examples of engineered nanomaterials include carbon buckyballs or fullerenes, carbon nanotubes, metal or metal oxide nanoparticles, quantum dots etc.

Nowadays is possible to find the presence of nanoparticles in every aspect of chemistry, starting from the application in drug delivery, bioimaging and organic synthesis (development of organo-heterogeneous catalysis), to application in manufacturing and materials, in the environment and energy harvesting fields and so on¹.

The preparation of magnetorheological fluids (MRFs) is one of the most peculiar applications of nanoparticles. MRFs are a group of fluids whose viscosity increases in the presence of an external magnetic field (MF). The extent of this change is such to convert the MRF from a liquid with a linear viscosity to a semi-solid state with very high viscosity. Rheology is the science that studies the deformations and flows of materials, both solids and fluids, under the influence of an external force. The name magnetorheological fluids is a direct consequence of the observed properties.

MRFs are suspensions of magnetizable micro- and nanoparticles dispersed in a carrier fluid. These kinds of fluids were first discovered by Rabinow in 1948² and the composition was 90% w/w of carbonyl iron and 10% w/w of silicone oil, petroleum oil or kerosene. MRFs behave as Newtonian fluids when the MF is absent. When the MF is applied, the response occurs within milliseconds and the particles present in the MRF are joined together in long chains, causing abrupt and significant

changes in fluid rheological properties³ (Figure 2.1). The materials whose properties vary consistently in the presence of an external force are commonly known as “Smart Materials”. To date different smart materials have been developed, some of them are magnetic suspensions (magnetorheological fluids and ferrofluids), electrorheological fluids, piezoelectric materials and shape-memory alloys. In recent decades, due to the development of magnetorheological technology and its applications in various industries, many studies have been performed on magnetorheological fluids. The development of MRFs lies between material science and organic/inorganic/physical chemistry.

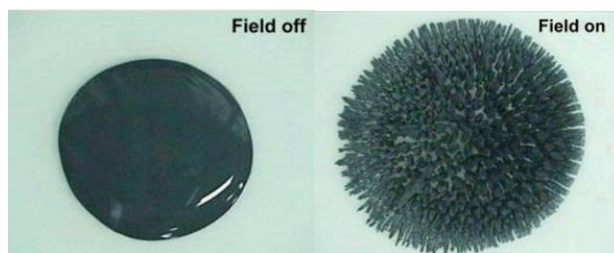


Figure 2.1: behavior of MRFs in the absence (left) and presence (right) of M_s

MRF consist of three constituents: magnetic particles with a volume percentage from 20% to 45%, a carrier fluid, and a set of additives. By selecting properly these three components it is possible to control the mechanical properties of the fluid such as the viscosity, the maximum shear yield stress, the resistance against the sedimentation, and the operating temperature range. The magnetic NPs are the core of the fluid and are the greatest part, they can be many metals, alloys and ceramics compositions; some of these are ferrite-polymer, iron–cobalt alloy, carbonyl-iron (one of the most used) and other magnetizable materials. The diameter of the magnetic core is normally between 5-20 nm mostly dependent on synthetic method and the future applications. The carrier fluid (or base fluid) plays the role of a liquid in which magnetic particles are suspended; commonly it can be a hydrocarbon oil or a silicone oil, but now a days the use of ionic liquids⁴ is becoming more popular, as well as the use of aqueous media. Various types of additives are used in the formulation of MRFs and their purposes are to prevent the sedimentation and accumulation of particles in the carrier fluid, enhance the fluid stability, reduce the amount of corrosion between the particles, and prevent the oxidation and loss of particles.

Being able to adapt to different operating systems and shaped coils, moreover, taking advantage of different magnetic circuits, this fluid can be used in various sectors, for example, shock absorbers⁵, vibrating dampers and earthquake vibration dampers⁶, clutches, fences and magnetorheological electrolytes (MR electrolytes or MRE)⁷.

2.2 The REMAP project

REMAP (Reusable Mask Patterning) is a four-year European founded project started in 2022 from the idea of Professor Diego Colombara (Unige) to revolutionize the micro-fabrication sector. REMAP is a multidisciplinary project that involved the knowledge of many different research group all around Europe, from magnetism, surface functionalization and electrochemistry of Unige and

CNRS (Nice, France), to theoretical studies of INN-D (Athens, Grece), and micro-fabrication, material science and engineering of INL (Braga, Portugal) and Uni.Lu (Luxembourg, Luxembourg)⁸.

Human activities strongly rely on micro-fabrications, and the request for manufactured products is constantly rising, since they are so widespread all around us⁹. To cite some of the different applications of micro-fabrications: integrated circuits and flat-panel displays (phones, laptops...), microsensors (smart houses, healthcare...), optoelectronics (LED lights, communications, ultrarapid internet access...)¹⁰. A specific definition to “microfabrication” does not exist, but generally, it is the collection of techniques used to fabricate devices in the micrometre range¹¹.

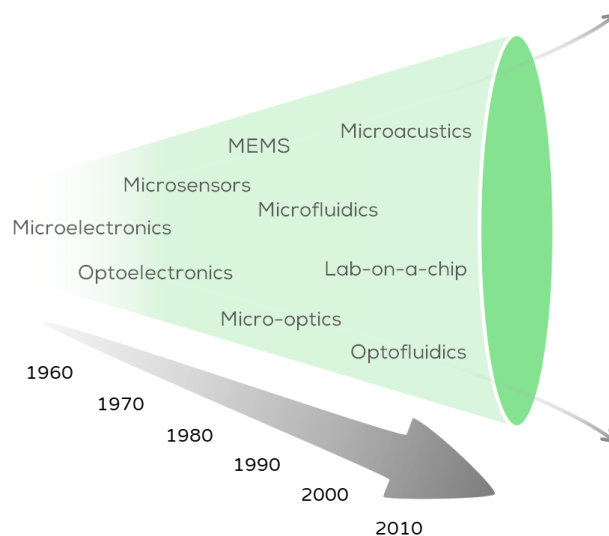


Figure 2.2: schematic representation of the evolution of microfabrication and MEMS devices

Historically the breakthrough in the microfabrication was the production of the first ever transistor in 1947, born from the fusion of solid-state physics and radar technology. Slowly techniques evolved and assembly of transistors was created until reaching the first ever printed circuit. Finally, a new type of material was created: microelectromechanical systems (MEMS) with all their subgenres, which embrace any type of application from microfluidics to DNA nanoarrays¹¹. An idea of the vastity of applications of MEMS is reported in *Figure 2.2*.

Microfabricated product are three-dimension objects, with width that can vary from 0.02 to 100 μm and thickness that ranges from 0.01 to 10 μm . However, even for those objects whose dimensions are not micrometric, like solar cells, the crucial microstructure is similar to those in microdevices.

Unfortunately, micro-fabricated products are still designed according to old and conservative principles, where most of the material and energy end up as waste. Given the global relevance of micro-fabricated products and MEMS, this industrial sector faces some of the highest economic and environmental costs, standing in contrast to the United Nations’ Sustainable Development Programme¹². This issue is becoming even more pressing today, as the demand for printed circuits continues to rise to support the development of artificial intelligence, the mass production of

electronic devices, and the growing need for more efficient energy sources, with innovative solar cells leading the way. The REMAP concept focuses on the latter: reducing costs and making solar cell production greener by minimizing waste.

The basic idea is to avoid the use of optical projection lithography because it is not economical and sustainable to produce micro-electrical circuits for solar cells and, instead, to use MRFs, in particular magnetorheological electrolytes (MREs). Briefly, common lithography requires non-reusable and expensive material for the creation of a mask for future etching and electroplating of semiconductors. Furthermore, this procedure requires many steps to complete the entire micro electrical circuit and therefore more waste. Moreover, optical projection lithography (OPL) has a spatial resolution that is way higher than the required to produce solar cells, thus it is overstated for this purpose.

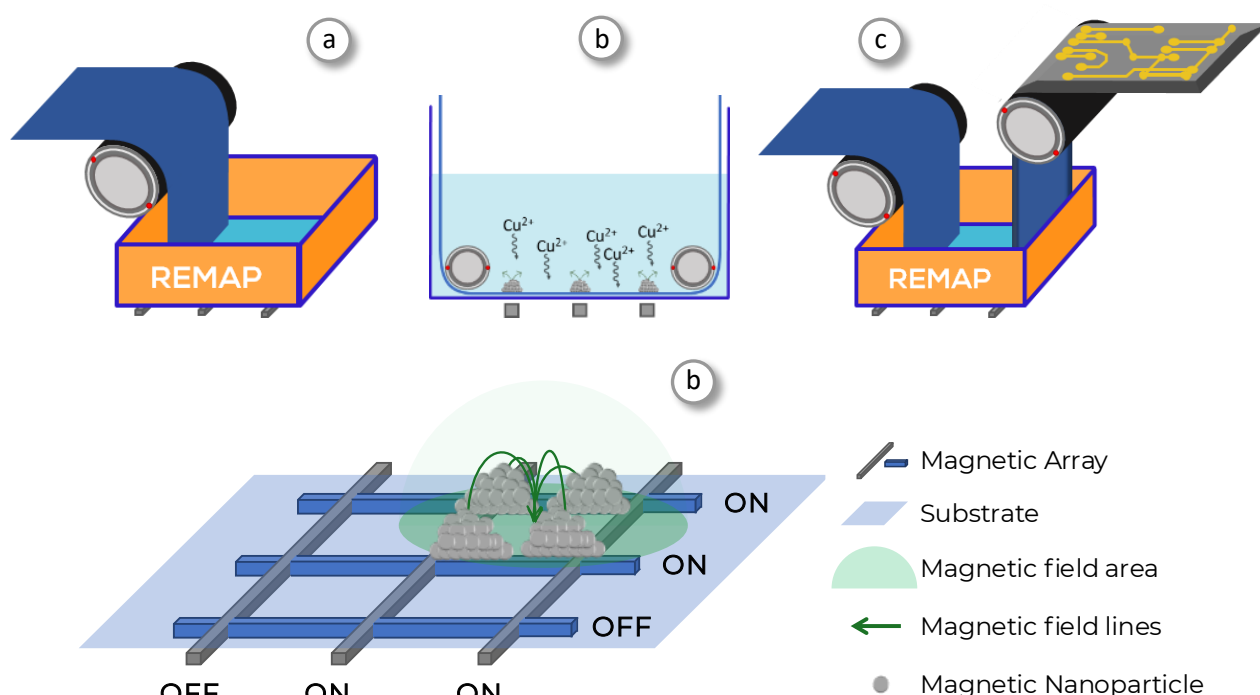


Figure 2.3: schematic representation of how REMAP should work. a. immersion of the substrate in the magnetorheological electrolyte suspension; b. creation of the mask pattern with the NPs and subsequent electrodeposition of the metal; c. Removal of the patterned substrate and reparation of the electroplating solution

How MREs should work in this type of system is shown in Figure 2.3. MREs can be used in REMAP because the application of a magnetic field in precise areas, using magnetic circuits, will cause aggregation of the nanoparticles (Figure 2.3b) for the creation of a sort of mask for the electroplating with copper or other metals. To make this procedure reversible and repeatable is fundamental that, once the magnetic field is removed, the nanoparticles will quickly redistribute in the carrier fluid without creating aggregates and working against the force of gravity (Figure 2.3c). The most critical part is the redispersion of NPs, once aggregation happened, as it is known in the literature¹³. Another fundamental aspect is masking efficiency because NPs must block all the

electrolytes (cations of the metal used in the electroplating process) without letting them pass through and plate the substrate (*Figure 2.3b*).

To address these issues different strategies can be used: i) the coating of magnetizable particles, ii) the use of spherical nanoparticles¹⁴ iii) the use of gels or other polymeric fluids¹⁵ and iv) the use of stabilizing additives¹⁶.

In the literature, there are studies on how the coating can affect the physical-chemical properties of the material. The “shell” part, for example amorphous silica, can efficiently prevent aggregation of particles and protect core materials from external degradation effects; for example Ding *et al.*¹⁷ studied magnetic NPs of Fe₃O₄ covered with a layer of silica with a thickness of approximately 12–15 nm, they demonstrated that this has enhanced the dispersion stability and also insulated the redox activity of the iron-core, without compromising the magnetorheological properties. In alternative, the coating can be made with organic compounds, as reported by Wang *et al.*¹⁸, where the coating was made directly on the iron-core of the NP using APTES ((3-aminopropyl)triethoxysilane) and the result is a mitigation of the sedimentation by reducing the density difference between the base fluid and the particles. Another type of organic coating is a polymeric coating¹⁹, but in many cases achieving a stable covalent functionalization of the surface is challenging.

The use of organic ligands can also enhance the magnetic properties of nanoparticles. Since, the surface-to-volume ratio in nanoparticles is high, the nature of the surface strongly influences many of their properties. The surface structure is typically disordered, with randomly missing bonds and incomplete atomic coordination compared with atoms in the core or in the bulk material. This disorder increases the magnetic anisotropy and consequently decreases the saturation magnetization. However, this effect can be mitigated by covalent surface functionalization: the binding of ligands to the surface helps restore coordination, reducing disorder and creating an environment more similar to the bulk state²⁰.

Thanks to the great advantages of using a surface coverage, for the REMAP application it was chosen to synthesise non-commercial and custom-made organic compounds and attach them covalently onto the surface of the magnetic NPs, by the use of click chemistry. The nature of the ligands was designed based on the final properties that the NP should have to perform as a good MRE (i.e. fast response to the MF, fast redispersion in the absence of the MF and good masking efficiency). During the three years interesting aspects were investigated, in particular, how the ligands interact and modify the surface properties of nanoparticles, and how they alter the magnetic properties.

2.2.1 The Optical Projection Lithography (OPL)

Lithography was invented in 1796 in Germany from the ideas of Alois Senefelder which desired a fast, reliable and efficient method to print both musical and theatre scores. Lithography is a planographic method of printing, that originally was based on the use of stone, paper, water,

greasy substances and ink. The name of this technique was created after the material used, in fact, it is the combination the Greek words *lithos* (stone) and *gráphō* (write).

Originally, the design was drawn on smooth stone plate using waxes and oils and the uncovered surface of the plate was made hydrophilic by the application of gums and moisture. Later, an oil-based ink was applied on the greasy part, where it could stick, and, after, it was transferred on a sheet of paper, producing a printed page. This simple technique spread rapidly in Europe and later in every part of the world, becoming the reference method to produce geographical maps and cheap paintings²¹.

From 1796 to the present, lithography has continually evolved and refined, giving rise to a variety of subgenres tailored to specific applications. Among these, optical lithography (OL) stands out as the most widely known and widely used²². The growth of printed circuits and semiconductor-based MEMS helped popularize this technique, as OL can produce highly accurate results thanks to its excellent spatial resolution²³.

In the contemporary photolithography, stone, paper, and wax are no longer used, instead silicon wafers and photosensitive polymers (the photoresist) are the common materials employed, and the ink was converted into a light beam. To perform a complete process of optical lithography are required many steps (*Figure 2.4*): substrate preparation, photoresist deposition, bake, alignment of the masks, exposure to light, mask removal, bake, etching or electrodeposition, photoresist removal, washing^{24,25}.

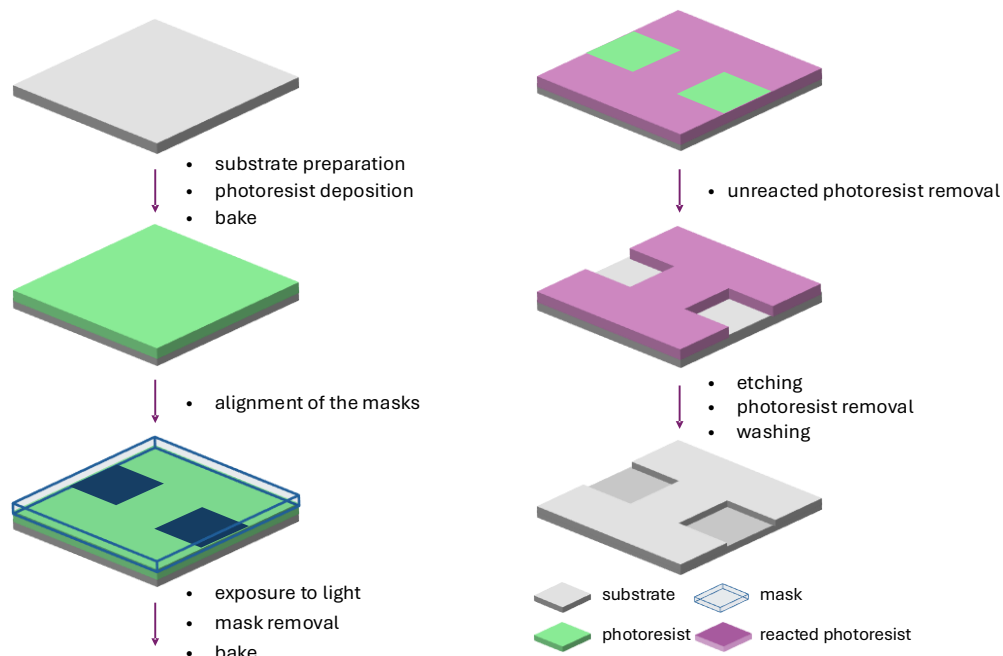


Figure 2.4: example of a typical sequence of lithographic processing steps

- The substrate preparation is a sequence of operation necessary to favour a perfect adhesion of the photoresist to the substrate and to eliminate any surface defect that could alter the final properties of the manufactured material. Generally, a multitude of washing and heating steps

are alternated to remove any possible impurity. Finally, the surface is finely polished until perfectly reflecting to ensure the absence of surface defects.

- The photoresist deposition is usually made by spin coating: the polymer, dissolved in a suitable solvent, is dispensed onto the rotating substrate to ensure a homogeneous distribution on the surface. Afterward, the solvent is gradually evaporated in a controlled environment and then in an oven. The baking process is necessary to remove any remaining solvent and to firmly anchor the photoresist to the substrate.
- On the covered substrate is perfectly aligned a mask, which is opaque to electromagnetic radiation. Then a strong light is shown on it. The mask is essential to ensure the correct formation of the pattern on the photoresist. Where the light interacted with the substrate, photochemical reactions that changed locally the chemical-physical properties of the polymer took place. The irradiated photoresist is now more sensitive to specific reagents and solvents. Part of this process is the washing and final baking. In this way a specific pattern is impressed on the surface of the substrate that now is exposed only in precise areas.
- The etching process consists of a chemical and/or mechanical treatment of the exposed areas and is used to remove part of the material. Practically, this process is used to dig paths in the original substrate, which depth is in the range of few micrometres. The opposite of this process is the electrodeposition of a metal on the substrate. In this way a micrometric layer of conductive atoms is deposited on the surface of the unmasked wafer, sometimes this step is called doping if an atomic layer is deposited and then heated to blend it into the silicon. Finally, the material is washed, and the photoresist is completely removed.

To build the extremely complex structures that make up a printed circuit, with transistors and interconnecting wires, the process previously described must be repeated 25 to 40 times. It is easy to understand how this makes fabrication time-consuming, expensive, and creates a lot of wastes. For example, the photoresist used in each cycle is disposable, and the polymer cannot be recycled. The most advanced CPU produced by Intel® in 1975 contained about 6000 transistors²⁶, whereas the most capable modern CPU for laptops contains around 100 billion transistors²⁷. For this manufactured materials, optical lithography (OL) no longer provides sufficient resolution and has been replaced by the more advanced optical projection lithography (OPL). OPL works similarly to OL, but instead of using opaque masks, a highly collimated beam of light directly prints the desired pattern onto the photoresist, achieving resolutions in the range of tens of nanometres²⁸. To reach this level of precision, OPL requires specially designed ultrapure photosensitive polymers (which are neither reusable nor recyclable), and the machinery must be installed in a cleanroom. A cleanroom is a space engineered to keep out dust, airborne organisms, and vaporised particles, protecting whatever materials are processed inside. In addition, the lighting is designed to filter out wavelengths that would be absorbed by the photoresist.

REMAP basic idea is to simplify the process, making it faster and greener. The possibility to use magnetic nanoparticles should combine in only one step the use of masks (or collimated light beams), photoresist, several washing phases, pre and post baking. The biggest advantage of REMAP is the possibility of modelling the aggregated particles for the creation of infinite reusable masks for the electrodeposition of conductive metals on the surface of the substrate.

2.3 Design of the organic ligands

As anticipated before the focus of this project is on the organic part, namely the synthesis of the ligands that will be anchored to the surface of the magnetic nanoparticles. To covalently anchor an organic chain to the surface of a nanoparticle, the use of a bifunctional linker bearing a siloxane group and an additional functional group (FG_1) is necessary. The siloxane moiety is able to form a layer covalently bound to the bare NP, while the additional functional group (FG_1) is used as a handle for further chemical transformations. The common methodology for surface functionalization of NPs consists of condensation reactions. Typically, high temperatures and harsh conditions are needed. The novelty in the approach studied is the possibility to use click chemistry to overcome all the issues that are normally found. Click-reactions are highly selective and orthogonal reactions that yield a very little amount or no side products (that can be easily removed without chromatography) and give heteroatom-linked molecular systems in mild conditions. Click-reactions can produce a wide variety of ligands in a short amount of time. An important aspect of these reactions is the insensitivity to oxygen or water and sometimes they can be even carried out without solvents²⁹. In literature are well known different strategy to use the click-chemistry to link the organic moiety of the ligand covalently to the surface of a nanoparticles exploiting the presence of bifunctional linkers (some of the different possibilities are shown in Figure 2.5).

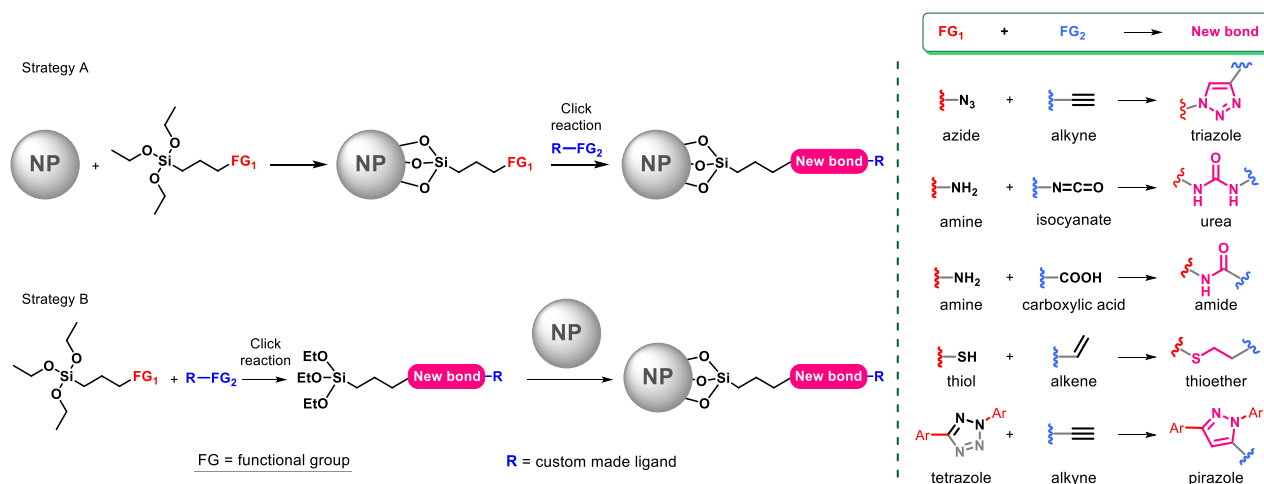


Figure 2.5: Possible strategies and click reactions that can be used for the surface functionalization of NPs

The most common approaches are depicted in Figure 2.5. The Strategy A consists, as first step, in the binding of a silane compound to the surface of the nanoparticle (or to an external SiO₂ coating). Then, the designed ligands bearing the FG_2 (functional group complementary to FG_1) is

synthesised separately, and, only as last step, the click-reaction is performed. In contrast, the Strategy B, inverts the order of the procedure. Firstly, the click-reaction between the linker and the ligand previously prepared is done, and subsequently the binding to the surface is performed.

Both approaches have advantages and disadvantages. Strategy A is more versatile and divergent since a single batch of NPs can be split and coupled with different ligands using the same click reaction. If N ligands will be prepared the number of reactions needed is $N+1$, instead for Strategy B the number is $3N$. On the other hand, only in Strategy B the success of the click reaction can be easily monitored by NMR (Nuclear Magnetic Resonance).

Alternatively, the entire synthesis of the ligand can be done on the NPs. This approach has the main advantage of the easiness of the purification because nanoparticles can be washed with solvents and blocked using a magnetic field. This is in accordance with the idea of “green chemistry”³⁰ of using less solvent for purifications. However, NMR cannot be used to monitor the correct formation of the bonds and the efficiency of reactions due to the magnetic properties of the NPs.

After a careful study of the pros and cons of every strategy the adoption of the “Strategy B” was considered for a specific reason: this strategy gives the possibility to check by NMR every compound synthesized. In this way it is possible to know exactly what is happening after every synthetic step except for the last one, due to the magnetic property of the nanoparticles.

In *Figure 2.5* are shown some of the different possible reactions to bond covalently the silane linker to the organic chain: one condensation reaction, the urea formation and three “true” click-reactions. The condensation is the synthesis of an amide and although being the easiest protocol, for its preparation requires harsh conditions (e.g. boiling in SOCl_2), thus in the last decades a plethora of coupling reagents compatible with many functional groups and conditions have been developed³¹. The main advantage of amides is that this type of covalent bond is strong, and the functional group obtained is stable. Nonetheless, despite the strength of the amidic bond, it was preferred to explore the use of click-chemistry and particularly the photoactivated thiol-ene click reaction and the CuAAC (Copper catalysed Alkyne-Azide Cycloaddition) click reaction. From preliminary results it was noticed that the triethoxysilane moiety is not compatible with the conditions required to perform the CuAAC (polymerization in water), thus it is not suitable for Strategy B. Consequently, it was decided to exploit the power of light working only with the photochemical thiol-ene reaction, for its advantages, the possibility to use neat conditions and to add novelty to the proposed work

After choosing the type of strategy and the suitable click reaction, the last parameter needed to be assessed: the type and nature of the organic chain and the terminal of the chain. The simplest approach was chosen as starting point, which consisted in the use of linear alkyl chains with the addition of three variables: the chemical-physical properties of the chain (hydrophobic or hydrophilic), the length of the chain (short or long), and the overall nature of the ligand (neutral,

positive or negative) (Table 2.1). A special attention was given to the terminal of the chain because it is the group that can interact with the carrier liquid and other electrolytes in solution. A cationic unit, for example $-^+NR_3$, in the applicative case of REMAP, will cause a Coulomb repulsion against the positively charged metal electrolytes letting them go only in the unmasked zone when the external magnetic field is turned on and preventing the diffusion of the electrolytes through the aggregated phase. An anionic functional group, like $-PO_3^{2-}$, could trap the metallic cation in solution, preventing infiltration of metallic electrolytes through the magnetorheological aggregate and avoiding deposition on the substrate. The presence of a charge, either positive or negative, should also enhance the dispersion properties of the NPs in the aqueous media. However, there could be an offset, because, if the surface of the nanoparticles is too positively/negatively charged the Coulomb repulsion could overcome the intensity of the magnetic field used and reduce the performance of the magnetic nanoparticles.

The nature of the chain, was another parameter important to consider, because it could have a different impact on the colloidal properties, stabilization of the nanoparticles and magnetic properties (Table 2.1). It is possible to operate with chains like hydrocarbons or with short polyethylene glycols that should give a more hydrophilic characteristic, to make NPs more compatible with the aqueous environment

All of this put together should give to the ligands the ability to prevent the migration of metal cations through the NPs when the magnetic field is active (masking efficiency) and favor a fast re-dispersion of NPs when the magnetic field is off.

Table 2.1: Different variables evaluated during the design of the ligands

Nature of the chain	Length of the chain		Nature of the ligand (ending group)		
	Short	Long	Neutral	Positive	Negative
Hydrophobic	Hydrocarbon C8	Hydrocarbon C16	H	NMe_3^+	PO_3^{2-}
Hydrophilic	Diethylene glycol	Tetraethylene glycol			

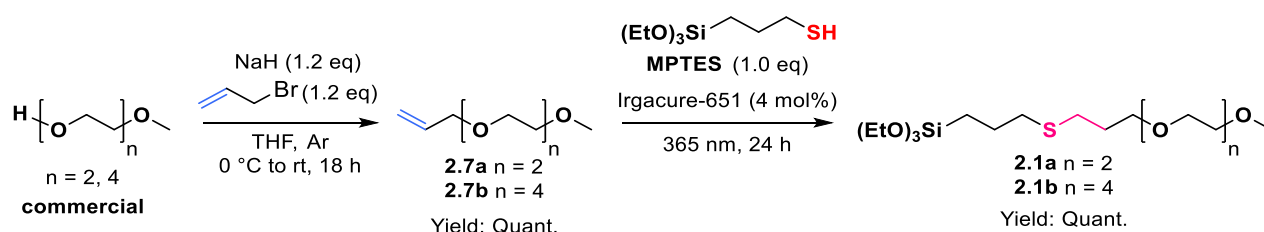
2.4 Synthesis of the organic ligands

2.4.1 Synthesis of the neutral ligands

During the preliminary design of the ligand synthesis, it was decided to start with the preparation of the short-chain neutral ligand **2.1a** from diethylene glycol mono methyl ether. Although, it was obtained in quantitative yield, even on a gram scale, from the preliminary results, it did not provide the desired physicochemical and steric properties required on the nanoparticle surface for the intended application. Therefore, it was decided to use the starting material and the intermediate as model compounds to test the reactions and optimize the conditions to achieve the highest possible yields. Diethylene glycol mono methyl ether was used as the model compound for the

preparation of the neutral hydrophilic ligand. Following the same idea 1-octanol was chosen as model compound for the preparation of the neutral hydrophobic ligand. These two compounds are commercially available, inexpensive, and closely mimic the chemical nature of their longer-chain homologues.

The synthesis of both short-chain and long-chain hydrophilic ligands **2.1a** and **2.1b** is reported in *Scheme 2.1*. The introduction of the allyl group was carried out by S_N with allyl bromide in the presence of NaH affording the desired product **2.7a** and **2.7b** in quantitative yield without the need for purification. Then the thiol-ene reaction was carried out, directly on the crude, using commercial MPTES (3-mercaptopropyltriethoxysilane) in the presence of catalytic Irgacure-651 (2,2-dimethoxy-2-phenylacetophenone) at 365 nm. Irgacure-651 is a radical photoinitiator that upon irradiation generates radicals, according to the mechanism reported in *Scheme 1.2*. The radical initiator gives the possibility to use less energetic photons, respect to UV. Ligands **2.1a** and **2.1b** were prepared on mg to g scale.

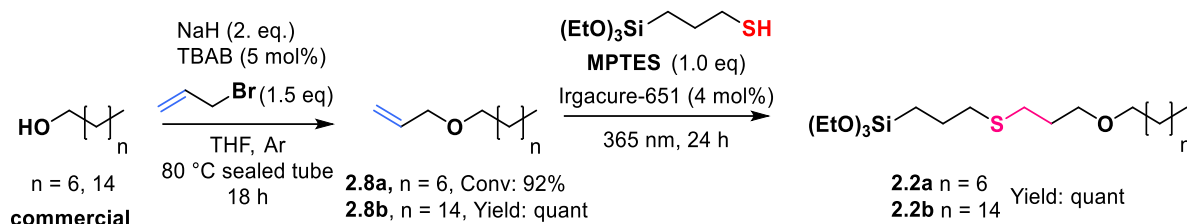


*Scheme 2.1: Synthesis of final ligands **2.1a** and **2.1b** from commercial DEG-OMe and TEG-OMe*

Afterwards, the work for the preparation of the neutral hydrophobic ligands started. As anticipated, the model starting material selected was 1-octanol ($n = 6$, *Scheme 2.2*), while the real ligand was based on cetyl alcohol ($n = 14$, *Scheme 2.2*). Unfortunately, it was discovered that intermediate **2.8a** was volatile, so it was decided to perform the optimization of the allylation reaction on cetyl alcohol. Starting with the allylation conditions used for **2.7a** and **2.7b**, described above, it was obtained only a 40:60 molar ratio of starting material/product (by ^1H NMR). Unsatisfied with these results it was performed an optimization of the reaction (*Table 2.2*). A higher excess of allyl bromide was beneficial to the starting material/product ratio, but the yield was not quantitative (*entry 1*). An increase in the temperature was detrimental if performed under reflux, probably due to the loss of allyl bromide (*entry 2*; THF boiling point [b.p.] = 66°C , allyl bromide b.p. = 71°C). The conversion value was restored using a sealed tube (*entry 3*). When TBAB (tetrabutylammonium bromide)³² was added, great improvements were observed (*entry 4*) and the reaction almost reached completion with 2 equivalents of NaH (*entry 5*, optimal conditions).

Scaling the reaction from mg to 1 g the desired complete conversion was obtained, and the yield was quantitative. Finally, the conditions found in *entry 5* were applied also to the allylation of 1-octanol, obtaining a conversion of 92%, despite that, the thiol-ene reaction was carried out on the unpurified crude. The thiol-ene reaction successfully delivered ligand **2.2a** in quantitative yield, the

same conditions were applied for the intermediate **2.8b** and ligand **2.2b** was obtained with complete conversion of the initial allyl ether, without requiring any purification.



Scheme 2.2: Synthesis of ligands **2.2a** and **2.2b** from 1-octanol and cetyl alcohol

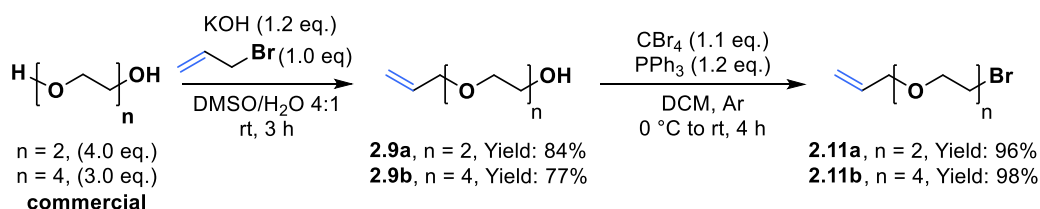
Table 2.2: parameter changed for the optimization of the synthesis of allyl ether **2.8b**

Entry	Conditions	Results
1	NaH (1.5 eq), AllylBr (1.5 eq.), THF, rt, 18 h	Starting/product 13:87 (NMR)
2	NaH (1.5 eq), AllylBr (1.5 eq.), THF, reflux, 24 h	Low conversion
3	NaH (1.5 eq), AllylBr (1.5 eq.), THF, 80 °C, sealed tube, 18 h	Starting/product 24:76 (GC-MS)
4	NaH (1.5 eq), AllylBr (1.5 eq.), TBAB (5 mol%), THF, 80 °C, sealed tube, 18 h	Starting/product 7:93 (GC-MS)
5	NaH (2.0 eq), AllylBr (1.5 eq.), TBAB (5 mol%), THF, 80 °C, sealed tube, 18 h	Starting/product 6:94 (GC-MS)

2.4.2 Synthesis of the negatively and positively charged ligands

Having in hand the first two class of ligands, it was started to study a way to obtain the anionic and cationic ligands. Using the literature and imaging a retrosynthetic approach it was found that the best way to obtain the desired products was to pass through a bromide as common intermediate (*Scheme 2.3 and Scheme 2.4*). This allowed to lower the number of required reactions and to maintain the overall synthetic path as easy as possible.

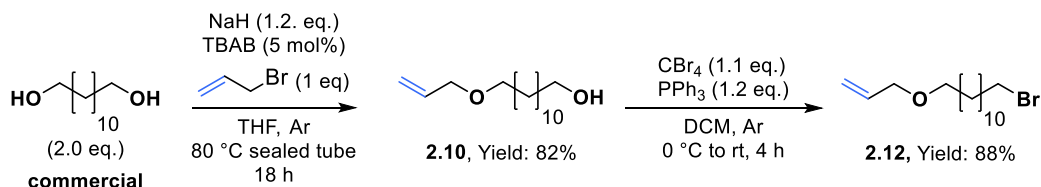
To obtain the common intermediates **2.11a** and **2.11b** it is necessary to perform a mono-allylation reaction and an Appel reaction. The first one is necessary to have an allylic function ready for the final thiol-ene reaction and the second one is fundamental for having in the organic chain a good leaving group necessary for the installation of the anionic or the cationic moiety.



Scheme 2.3: synthesis of **2.11a** and **2.11b**, common bromide intermediates for the hydrophilic ionic ligands (DMSO: dimethyl sulfoxide, DCM: dichloromethane)

When the mono-allylation of diethylene glycol (model compound chosen) was tried under the same conditions used in *Scheme 2.1* (reducing to 1 eq. the amount of allyl bromide), the desired product **2.9a** was obtained only in 20% yield. So, completely different conditions³³ were tried and,

this time, the allyl **2.9a** was obtained with 84% yield after chromatography (*Scheme 2.3*). It is important to notice that in this case diethylene glycol was used in large excess to avoid double allylation, and the chromatographic purification on silica was essential to eliminate the excess of unreacted diol. The same reaction performed on tetraethylene glycol gave **2.9b** in 77% yield. The lower yield of **2.9b** respect to product **2.9a** can be associated to a higher solubility of **2.9b** in water that gave more issues during the extractive work-up of the reaction.



Scheme 2.4: synthesis of the hydrophobic intermediate 2.12

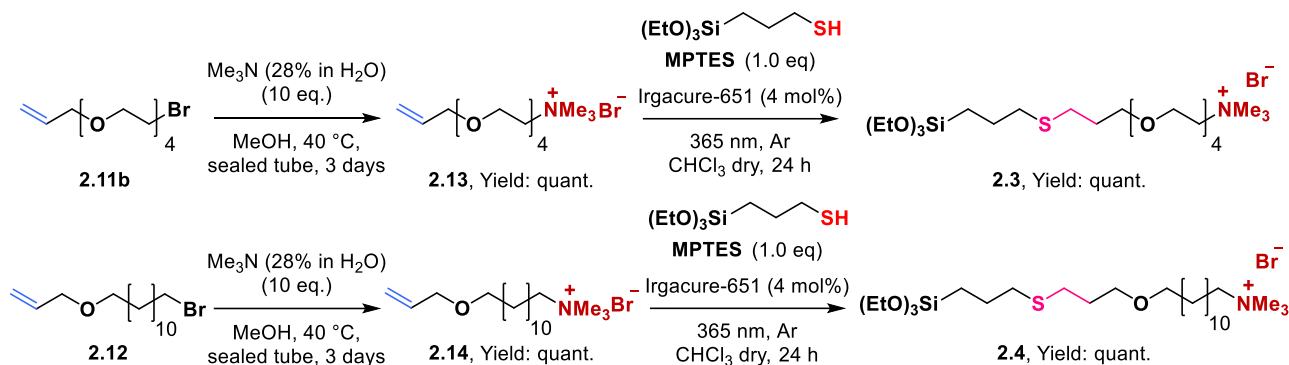
Table 2.3: optimization of the mono-allylation of dodecane diol (DMF: dimethylformamide)

Entry	Conditions	Results
1	KOH (1.2 eq), AllylBr (1 eq.), DMSO:H ₂ O (4:1), rt, 22 h	Yield: 38 %
2	NaH (2 eq), AllylBr (1 eq.), THF, rt, 15 h, Ar	Yield: 26 %
3	NaH (1.2 eq), AllylBr (1 eq.), DMF, 0 °C 30 min and rt 16h, Ar	Yield: 68 %
4	KOH (1.2 eq), AllylBr (1 eq.), TBAB (5 mol%), DMSO:H ₂ O (4:1), rt, 16 h	Yield: 25 %
5	NaH (1.2 eq), AllylBr (1 eq.), TBAB (5 mol%), THF, rt for 5 min and 80 °C for 20 h, sealed tube, Ar	Yield: 75%

Following the same strategy of *Scheme 2.3*, the allyl ether having the long hydrophobic chain was prepared. In this case, some issues due to the insolubility of the 1,12-dodecandiol in the reaction solvents were experienced and the best yield obtained was 38% (*entry 1*). Although the mono-allylation reaction was requiring an optimization, the choice of avoiding the use of a model compound was forced by the insolubility of 1,12-dodecandiol, because all the other short-chain commercial diol are more soluble in common organic solvents. So, a short optimization was performed directly on 1,12-dodecandiol (*Table 2.3*). The allyl bromide/NaH method was tried, but the product was obtained in only 26% yield (*entry 2*). Starting from the procedure of *entry 1*, TBAB was added to see if the yield could be increased, but it was only of 25 % (*entry 4*). An improvement was obtained changing the solvent³⁴ (*entry 3*), but the best results were obtained using the condition with THF, a catalytic amount of TBAB and heating to 80°C in sealed tube (*entry 5*). Scaling up the reaction up to 1g the yield recorded was even higher: 82% (*Scheme 2.4*).

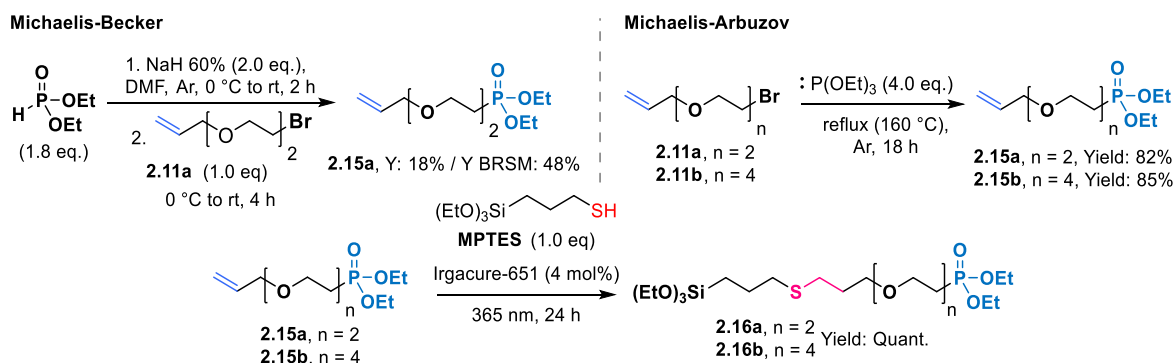
Finally, the alcohols (**2.10**, **2.9a** and **2.9b**, *Scheme 2.3*, *Scheme 2.4*) were converted into the bromides (**2.12**, **2.11a** and **2.11b**) by Appel reaction. In each situation the reaction gave the desired bromide smoothly in high yield. Unluckily, the Appel reaction always requires chromatographic purification on silica for the removal of the major side-product: triphenylphosphine oxide, but luckily it can be easily eliminated with this technique.

Having synthesized the bromide intermediates the synthesis of the cationic ligand was started. The cationic function can be easily installed with a simple S_N2 reaction, treating the bromide with aqueous Me₃N in MeOH at 40 °C for 3 days (Scheme 2.5)³⁵. The desired products were obtained in quantitative yield by evaporation of the solvents. Then the ammonium salt **2.13** and **2.14** were subjected to the thiol-ene reaction and the final products **2.3** and **2.4** were obtained in quantitative yield. It is worth noting that the thiol-ene reaction in Scheme 2.5 was the only one performed with solvent, this is due to the high viscosity of the quaternary ammonium salt that blocks the motion of the stirring bar.



Scheme 2.5: synthesis of ligand **2.3** and precursor of ligand **2.4**

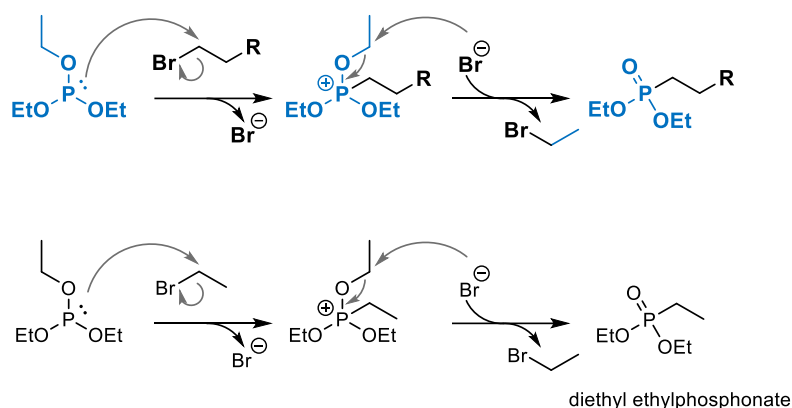
Terminated the synthesis of the cationic ligands, it was started the investigation for the preparation of the negative charged ligands. Initially, bromide **2.11a** was used as model compound. It was subjected to the Michaelis-Becker condition³⁶, but only 18% of the product was obtained. The Michaelis-Becker condition brought some issues: the DMF was not removed completely from the organic phase during the extraction, maybe due to its affinity with the compound, moreover, the residual solvent showed the same polarity (same R_f, retention factor) of the phosphonate ester in TLC, complicating the purification. After the chromatography, some unreacted starting material was also recovered (63%).



Scheme 2.6: Synthesis of the diethyl phosphonates **2.15a** and **2.15b** having the hydrophilic chain and the final ligand **2.16a** and **2.16b** (Y BRSM = yield based on the recovered starting material).

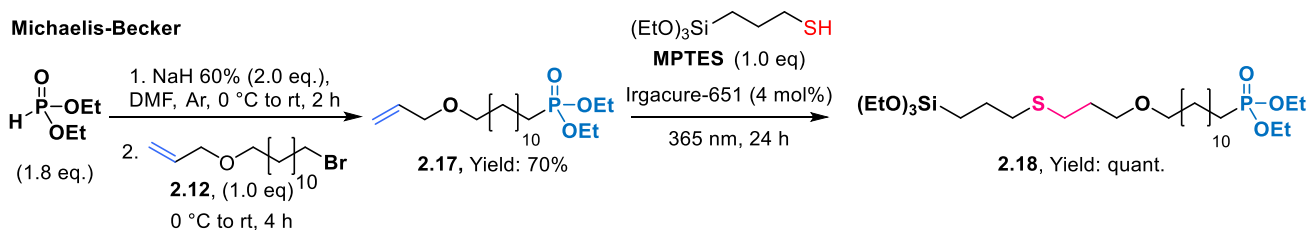
A better result was obtained adopting the Michaelis-Arbuzov conditions³⁷ (Scheme 2.6) and the product was isolated in excellent yield (82%). In this reaction, triethyl phosphite acts as both reagent and solvent, and high temperatures (reflux) are required to promote the “S_N2 type

mechanism” (Scheme 2.7). Due to the specific type of mechanism is always formed the diethyl ethylphosphonate as side product. This compound cannot be removed with the typical chromatographic purification, instead it is easily eliminated under high vacuum at 40 °C for at least 18 h. While working with the Michaelis-Arbuzov reaction it was noted that triethyl phosphite needs to be purified before use, by pre-treating it with Na and then distilling it under reduced pressure. It was stored under Ar, as a supplementary safety precaution. As final step the phosphonates intermediates **2.15a** and **2.15b** were used in the final thiol-ene reaction giving the protected ligands **2.16a** and **2.16b** in quantitative yield (Scheme 2.6).



Scheme 2.7: Michaelis-Arbuzov reaction mechanism (top); diethyl ethylphosphonate formation mechanism (bottom)

The hydrophobic alkyl bromide **2.12** was subjected to the Michaelis-Becker conditions and the phosphonate ester **2.17** was obtained in 70% isolated yield (Scheme 2.8). However, for having a total conversion of **2.12**, the diethyl phosphite must be freshly distilled. Otherwise, as proved many times, the reaction stops its conversion, luckily without creating side products and giving the possibility to recover all the unreacted starting material.



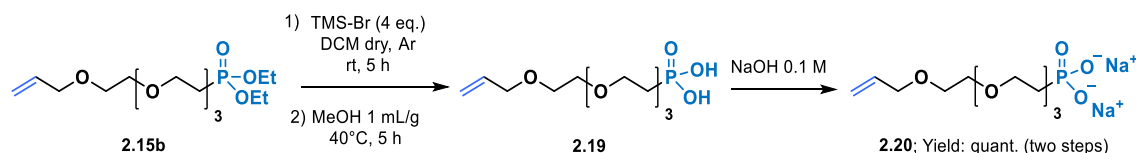
Scheme 2.8: Synthesis of the protected diethyl phosphonate ligand **2.18**

Then the thiol-ene reaction was carried out on allyl ether **2.17** and the expected product (**2.18**) was obtained in quantitative yield on a scale of a few hundred milligrams (Scheme 2.8). Ligands **2.16a**, **2.16b** and **2.18** are diethylphosphonate esters and are not anionic in this form. Unfortunately, it was discovered that the thiol-ene reaction, if performed directly on the phosphonic acid, does not work due to the sensitivity of the radical mechanism to acids.

Unfortunately, the dealkylation reaction to convert the phosphonate ester into the phosphonic acid cannot be done in the presence of an alkyl silane because the required conditions, like it is shown in Scheme 2.9, use methanol, which is a promoter of the silane polymerization. This would

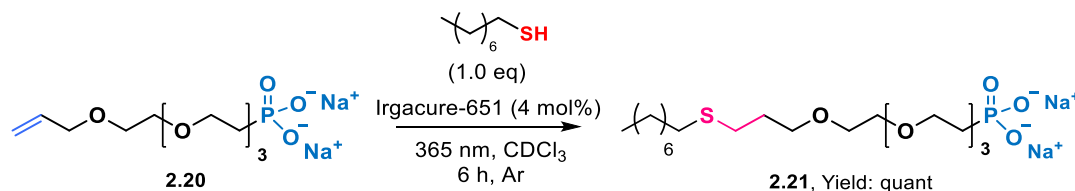
require performing the reaction only after the ligand is bound to the surface of the particle, albeit it is not the best option. It is possible that the dealkylating reagent (TMS-Br, trimethylsilylbromide) can undergo side reactions with the surface of the nanoparticle and with the siloxane bond between the ligand and the surface, furthermore it is not possible to use the NMR to follow the reaction, due to the magnetic core of the nanoparticles. Another possible way would be a hydrolysis with concentrated HCl³⁸, but again these reaction conditions are not tolerated by the bare ligand (water and H₃O⁺ promote polymerization) nor with the particles functionalized (evidence of the instability of maghemite NPs in HCl were found), moreover the direct hydrolysis with acid is reported to be very slow, more than 3 day are required, and sometimes the reported conversions are low³⁸.

To avoid this problem, performing the thiol-ene reaction using the sodium phosphonate salt was envisioned (Scheme 2.9), inspired by the work of Tucker-Schwartz et al.³⁹ and the successful result obtained with the preparation of the cationic ligand.



Scheme 2.9: synthetic procedure to obtain the phosphonate salt **2.20** (DCM: dichloromethane).

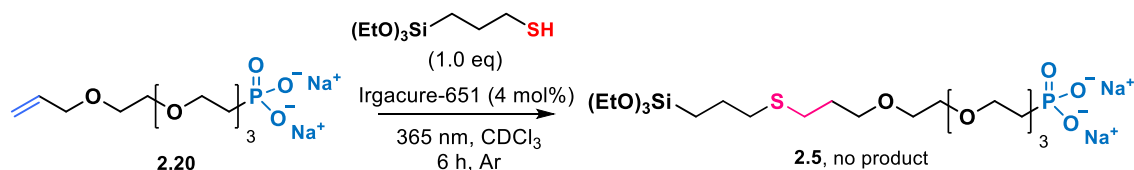
In the beginning, the dealkylation reaction of compound **2.15b** gave problems of irreproducibility and degradation of the starting material. Finally, it was discovered that the reaction is very sensitive to moisture and works only if the starting allyl ether **2.15b** is fresh or at maximum two months old. It was even tried to dry the starting material, **2.15b**, in the presence of P₂O₅ in high vacuum, but partial degradation of the organic compound was found. Furthermore, it was discovered that the first step of the reaction can be sped up by increasing the equivalents of TMS-Br: from 18 h with 2.3 eq³⁶ to 5 h with 4 eq. The following step of the synthetic path is a simple acid-base reaction with NaOH. To be sure that no inorganic salt was left in the crude a reversed phase chromatography was performed delivering pure **2.20** in quantitative yield over two steps.



Scheme 2.10: use of the allyl ether **2.20** in a model thiol-ene reaction with *n*-octanethiol

The purified product, **2.20**, was used in a model thiol-ene reaction using *n*-octanethiol, instead of MPTES. The crude of the click reaction was analysed after 6 h by ¹H-NMR. From the analysis it was possible to see the total conversion of the allyl ether (**2.20**) in **2.21** and a slight amount of thiol unreacted, mainly in the form of disulfide. To confirm this preliminary observation and to remove any impurities, the crude was purified by reversed phase chromatography. The only compound isolated matched the structure of compound **2.7** and its molecular mass was also confirmed by

HPLC-ESI-MS (High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectrometry) both using a positive and a negative ionization source. After the success in the synthesis of compound **2.21**, it was tried the same reaction with MPTES to obtain the final desired ligand **2.5** (Scheme 2.11). After 6 hours of reaction, the conversion of **2.20** was complete, while some unreacted thiol was present. The ^1H -NMR of the crude was complex, some characteristic signals of the product were present, but the integrations were not fully consistent with the desired molecule. Due to the chemical nature of **2.5**, no purifications could be carried out without obtaining polymerization.



Scheme 2.11: preparation of the final ligand **2.5** starting from MPTES and allyl ether **2.20**

Unfortunately, due to the need to deliver functionalized material to evaluate its performance for the final REMAP application, only the neutral and cationic ligands were considered, and the work on obtaining the anionic ligands was discontinued.

Notably, during the characterization of ligand **2.3**, a high tendency to trap moisture from air was noted, mainly due to the chemical-physical properties of the chain. In the attempt of drying it, the compound was desiccated over P_2O_5 in high vacuum. After 3 h, by ^1H -NMR it was noted that the compound was totally degraded (Figure 2.6). Surprised by this finding, the entire library of ligands was checked, to see if it was only an isolated case resulting from the use of P_2O_5 . Unfortunately, the same degradation process was found for all of them. Ligands **2.3**, **2.4**, **2.16** and **2.18** were totally degraded, instead ligands **2.1** and **2.2** were only partially degraded even if they were all stored in freezer at -20°C .

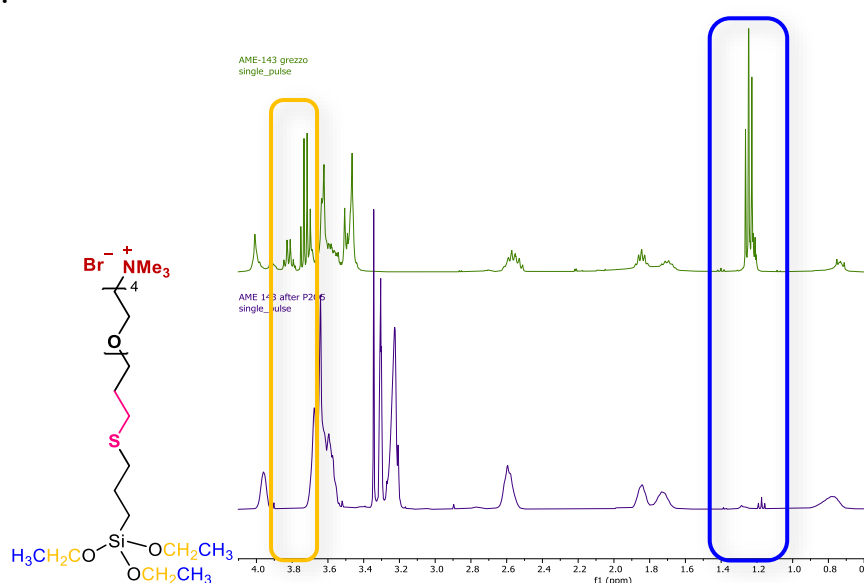


Figure 2.6: ^1H -NMR spectra of compound **2.3** before (in green) and after (in purple) the treatment with P_2O_5

The cause of the degradation can be explained considering the high tendency of siloxane groups to create polymers or oligomers due to the thermodynamic stability of the Si-O-Si bond. As can be seen by the ^1H -NMR spectra reported in *Figure 2.6* the degradation derives from the disappearance of the quartet at 3.9-3.8 ppm and the triplet at 1.2 ppm that are originated by the ethoxy groups.

At this point the entire library of compounds was synthesized again, but it was preferred to store them as allyl ethers (**2.7a**, **2.7b**, **2.8a**, **2.8b**, **2.13**, **2.14**, **2.15a**, **2.15b** and **2.17**) and not as triethoxysilyl compounds. The conversion to the final ligands by thiol-ene reaction can be done just before the coating experiments.

2.5 Functionalization of silica nanoparticles

After completing the preparation of the desired ligands, the surface functionalization experiments started. One of the biggest problems in this type of reaction is to find the right condition that can lead to the highest surface grafting (surface grafting = percentage of surface covered) and to achieve this in literature is possible to find a wide variety of procedures. As starting point, the neutral long-chained ligands (**2.1b** and **2.2b**) were used, believing that the absence of charges would have made the optimization process easier. The REMAP project is based on the use of functionalized magnetic nanoparticles, but while the production of NPs by REMAP's partners was still ongoing, it was decided to use silica nanoparticles as model substrate. As SiO_2 NPs a Merck product (product code: 637246) was selected, whose specifications reported by the vendor are: nanopowder (spherical, porous), 5-20 nm particle size (TEM) and surface area 590-690 m^2/g (TEM). Before performing our experiments, the material was characterized with in-house techniques: BET (Brunauer–Emmett–Teller) analysis and TEM (transmission electron microscopy) images.

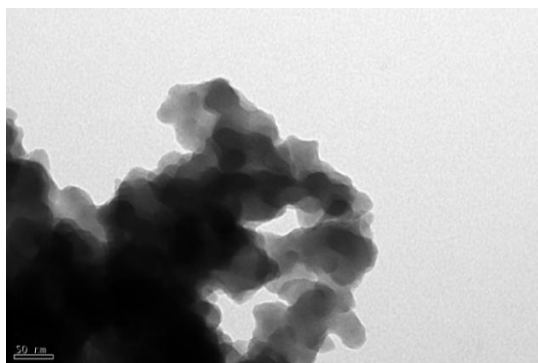
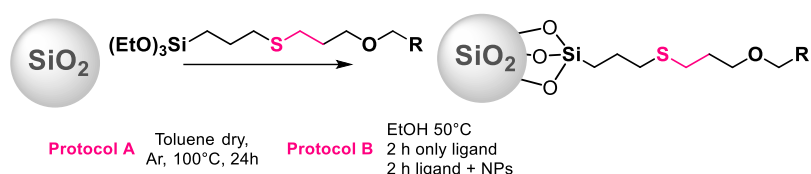


Figure 2.7: TEM image of the SiO_2 nanoparticles used (the scale of the magnification is reported in the bottom left)

The BET analysis returned a value of 659 m^2/g , which is perfectly in agreement with the vendor's value. However, the TEM analysis (performed in collaboration with Dr. Marco Piccinni) revealed that silica NPs presents as large aggregates that space out at least one micron. Particles on the edge of aggregates are the only ones recognizable, and they are shaped as round flat platelets ranging from 10 to 50 nm in width (*Figure 2.7*). Although the particle thickness is not quantifiable with TEM, the absence of ripples on the particles' surface and edges indicates their stiffness, which

is consistent with the 5-20 nm dimensions claimed by the supplier. However, the tendency of nanoparticles to aggregate is a potential hindrance to their complete functionalization. For those reasons the surface grafting was not calculated and only the TGA weight loss was considered.

During the design of the functionalization reactions, two types of protocols with totally different procedures, in terms of time, reaction temperature and solvent were considered to be used. To create the “Protocol A” the inspiration came from the articles published by Liu *et al.*¹⁸ where toluene at 100 °C for 24h was used, instead the articles by Gunatilake *et al.*⁴⁰ inspired the “Protocol B”, where ethanol was used; initially the ligand is “pre-hydrolysed” at 50°C for 2h, and then the nanoparticles are added and the functionalization reaction is carried out for 2h at the same temperature (Scheme 2.12).



Scheme 2.12: general reaction for surface functionalization of silica nanoparticles

The knowledge of the surface area of the NPs was necessary for the choice of the NPs/ligand ratio. In fact, knowing that maximum 4 ligands can be bound to one nm²,⁴¹ the surface area and the Avogadro’s number it is possible to calculate the amount of ligand per g of NP required to have a theoretical coverage of 100% (Equation 2.1). Practically, the functionalization reactions were operated using 2.5 times the value determined before, to be sure of reaching the value of total surface grafting.

$$\text{Theoretical surface grafting (mols/g}_{NP}) = \left(\frac{\text{Superficial Area } (\frac{m^2}{g})}{\text{Area occupied by 1 ligand (m}^2)} \right) \times \left(\frac{1}{N_A (\frac{1}{mol})} \right)$$

Equation 2.1: equation used to calculate the nanoparticles surface grafting

After the functionalization reaction the nanoparticles were washed to remove the ligand in excess and then dried for further analysis. Functionalized silica nanoparticles were analysed by thermogravimetric analysis (DSC-TG). Using this technique the ligand loading was quantified, by heating a crucible, loaded with the sample, inside an oven, following a thermal program. Thus, the weight loss of the NPs was recorded and correlated to the amount of ligand detached from the surface. This value is fundamental to calculate the percentage of grafted surface. In Table 2.4 are reported the result obtained from the TG analysis.

Table 2.4: TGA weight loss detected for the Functionalized silica nanoparticles

Entry	Protocol	Ligand	TGA data (% weight loss)	Ligand’s nature
1	“Protocol A”	2.1b	~21%	Neutral hydrophilic
2	“Protocol A”	2.2b	~39%	Neutral hydrophobic
3	“Protocol B”	2.1b	~55%	Neutral hydrophilic
4	“Protocol B”	2.2b	~23.5%	Neutral hydrophobic

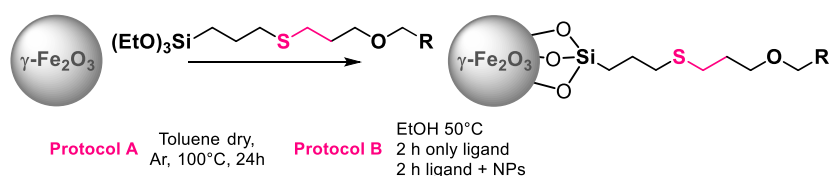
Comparing the experimental results, the “toluene protocol” (Protocol A) (*entry 1 and 2*) performed better for ligand **2.2b** (neutral hydrophobic), because a higher loading was reached. On the other hand, considering the “ethanol protocol” (Protocol B) (*entry 3 and 4*) the higher loading was reached with ligand **2.1b** (neutral hydrophilic). From this set of experiments, an interesting match between the chemical-physical properties of the solvent and ligand was found, hydrophobic ligands prefer “toluene protocol”, hydrophilic ligands prefer “ethanol protocol”.

After the experiment performed on SiO₂ nanoparticles, and with more experience gained, the surface functionalization reactions on maghemite NPs (γ -Fe₂O₃) started.

2.6 Functionalization of maghemite (γ -Fe₂O₃) nanoparticles

The REMAP project is based on the use of magnetic nanoparticles for the creation of infinitely reusable masks, and the ligands, as it is presented in the introduction, play a crucial role. Therefore, after the functionalization of model silica nanoparticles, maghemite nanoparticles (γ -Fe₂O₃) were used. The magnetic material was supplied by the REMAP French partners working in the CNRS of Nice. Maghemite was synthesized by coprecipitation method from Fe (II) and Fe (III) salts in presence of a strong base and then heated in presence of Fe(NO₃)₃ in acid environment to fully oxidize the magnetite core (Fe₃O₄) to maghemite (γ -Fe₂O₃). Once, the preparation was finished, the NPs were stabilized in aqueous solution at pH 1,42. The full characterization was performed, showing an average diameter of 10 nm (TEM), an average hydrodynamic diameter of 73 nm in EtOH (DLS) and 95 nm in H₂O (DLS) and a superficial area of 120 m²/g (BET)⁴². The BET analysis also showed that the nanoparticles have a low porosity.

For the functionalization reactions “Protocol A” and “Protocol B” previously described were replicated (*Scheme 2.13*). From *Equation 2.1* it was calculated a theoretical surface coverage of 0.7969 mmol_{ligand}/g_{NPs}. Practically, it was decided to operate with 2.5 times the value determined, to be sure of reaching the value of total surface grafting.



Scheme 2.13: general reaction for surface functionalization of maghemite nanoparticles

Before starting the actual functionalization reaction, a solvent exchange was necessary. The maghemite nanoparticles (NPs) were stored in an acidic aqueous solution, which is incompatible with the two protocols. Therefore, the particles were precipitated by titration using a 0.05 M aqueous solution of NH₄OH, adjusting the pH to 7. According to the literature, maghemite nanoparticles are colloidally stable only in acidic environments⁴³ (in absence of hydrogen chloride), their strong positive surface charge (+35 mV) causes the formation of intense Coulombic repulsion between nanoparticles. At neutral pH the surface charge decreases to approximately 0 mV, thus they lose their stability and precipitate because the residual Coulombic repulsion

between particles is no longer sufficient to counterbalance attractive forces. The titration procedure is reported in *Figure 2.9*, where the experimental setup is also shown. *Figure 2.8* illustrates the stability of the nanoparticles across different pH values.

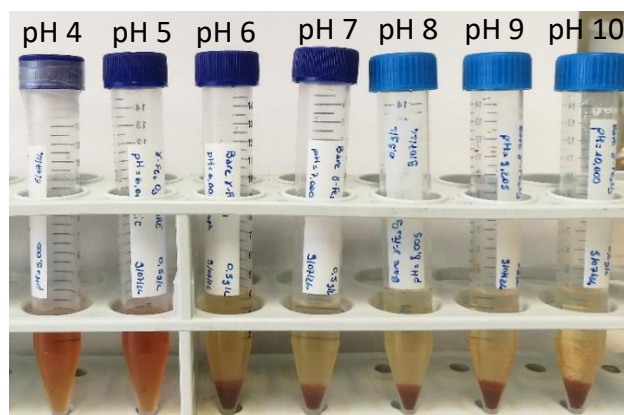


Figure 2.8: photos of bare maghemite suspensions in water at different pH. The pH was adjusted with different buffers (pH 4-5 citrate buffer, pH 6-8 phosphate buffer, pH 9-10 carbonate buffer).

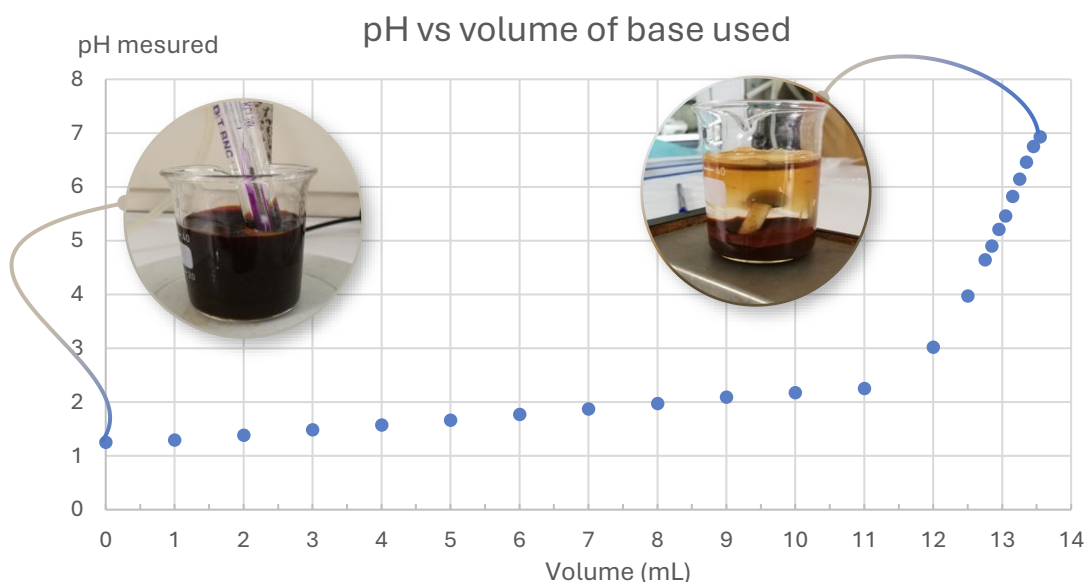


Figure 2.9: volume of base vs measured pH graph acquired during the precipitation titration of NPs. Photos of the beginning and the end of the titration are reported. Titration performed using 0.05 M aqueous solution of NH_4OH

Once the functionalization was accomplished, the functionalized nanoparticles were analysed using IR spectroscopy, the ligand loading was determined by TGA analysis, the hydrodynamic diameter and colloidal properties in different solvents were collected using a DLS and the magnetization of the NPs was measured.

2.6.1 Infrared absorption spectroscopy characterization

Infrared spectroscopy was chosen to fully characterize the functionalized nanoparticles for its qualitative properties. The ATR-IR evanescent wave used gives the possibility to analyse selectively the surface of the functionalized material, thanks to the use of low penetrative radiations. IR absorption spectrums of bare nanoparticles, ligands and functionalized nanoparticles were

recorded. The resulting absorption spectrums are superimposed (Figure 2.10, Figure 2.11, Figure 2.12, Figure 2.13) for a better visual comparison.

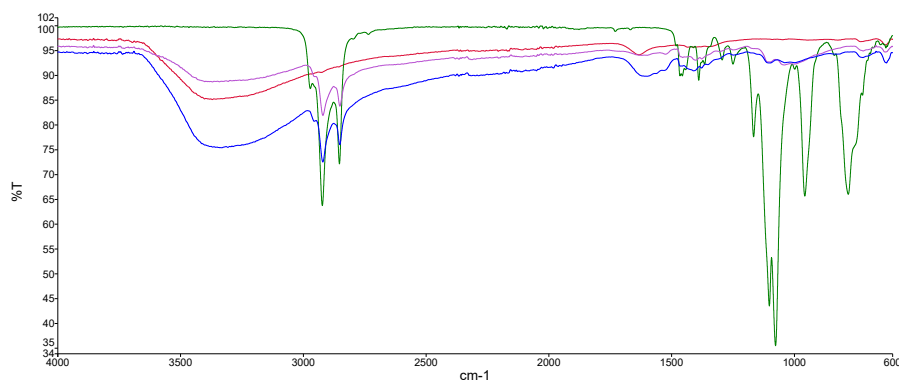


Figure 2.10: Superimposition of the ATR-IR absorption spectrums of the bare nanoparticles (red line), organic ligand (green line), $\gamma\text{-Fe}_2\text{O}_3@2.1b$ functionalized with method A (violet line), $\gamma\text{-Fe}_2\text{O}_3@2.1b$ functionalized with method B (blue line)

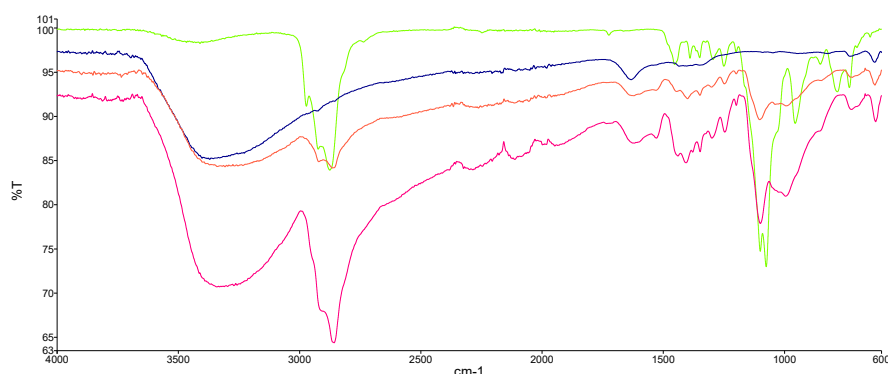


Figure 2.11: Superimposition of the ATR-IR absorption spectrums of the bare nanoparticles (blue line), organic ligand (green line), $\gamma\text{-Fe}_2\text{O}_3@2.2b$ functionalized with method A (orange line), $\gamma\text{-Fe}_2\text{O}_3@2.2b$ functionalized with method B (red line)

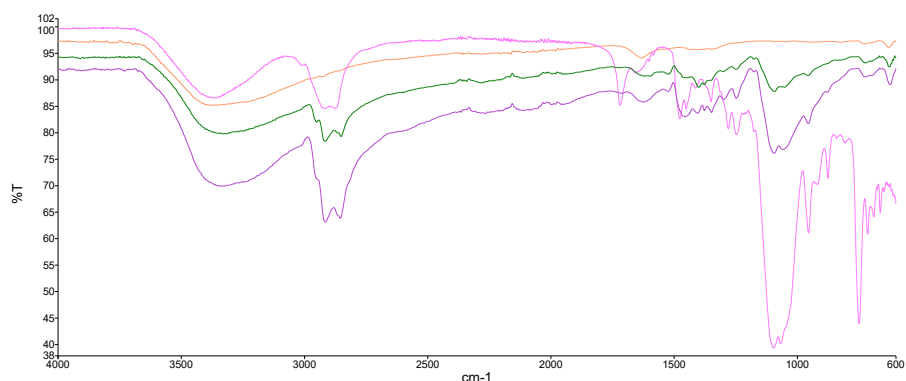


Figure 2.12: Superimposition of the ATR-IR absorption spectrums of the bare nanoparticles (orange line), organic ligand (pink line), $\gamma\text{-Fe}_2\text{O}_3@2.3$ functionalized with method A (violet line), $\gamma\text{-Fe}_2\text{O}_3@2.3$ functionalized with method B (green line)

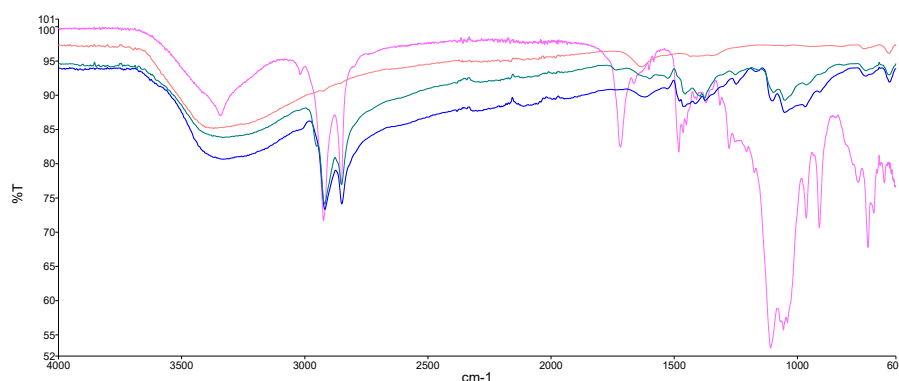


Figure 2.13: Superimposition of the ATR-IR absorption spectra of the bare nanoparticles (orange line), organic ligand (violet line), $\gamma\text{-Fe}_2\text{O}_3@2.4$ functionalized with method A (blue line), $\gamma\text{-Fe}_2\text{O}_3@2.4$ functionalized with method B (green line)

The spectrum of bare $\gamma\text{-Fe}_2\text{O}_3$ shows OH stretching and scissoring vibrations at 3373 and 1633 cm^{-1} , respectively. These signals can be attributed to the presence of free Fe–OH groups on the surface, typical of $\gamma\text{-Fe}_2\text{O}_3$, and to residual H_2O hydrogen-bonded to the surface. The water peak may originate from atmospheric moisture or residual from the precipitation process that was not fully removed under vacuum. Bands between 1433 and 1339 cm^{-1} are also observed, likely due to the out-of-plane bending of NH_4^+ salts⁴⁴, which may remain as residues from the precipitation step using a 0.05 M NH_3 solution (see section 2.6). Alternatively, these bands could arise from the asymmetric stretching of NO_3^- , originating from nitrate salts used during the synthesis of maghemite nanoparticles⁴². Finally, the bands at 728 cm^{-1} and 628 cm^{-1} correspond to the characteristic stretching vibrations of surface Fe–OH groups and Fe–O bonds deeper within the material, respectively. Additional diagnostic bands may be observed at wavenumbers below 600 cm^{-1} ⁴⁵.

The spectra of hydrophilic chain-functionalized nanoparticles show strong and broad OH stretching vibration bands at 3295 cm^{-1} , probably due to the presence of water. The same water could also account for the H–O–H scissoring vibration band at 1600 cm^{-1} . Even after extensive drying under high vacuum, these peaks remain, indicating that water is trapped by hydrogen bonds between the glycol chains⁴⁶. These signals are less intense but still detectable when a hydrophobic chain is attached to the surface, suggesting that in both cases complete surface coverage was not achieved, and H_2O can still be hydrogen-bonded to exposed areas. This conclusion is consistent with the TG–DSC results (see Section 2.6.2), where a weight loss between 90 °C and 150 °C is always observed. Due to the alkyl nature of the synthesized chains, strong absorption bands at 2921 cm^{-1} and 2856 cm^{-1} are always present, corresponding to $\text{C}(\text{sp}^3)\text{--H}$ stretching vibrations, along with a significant CH_2 bending vibration at 1522 cm^{-1} . Other important bands include the strong and broad absorption between 1100 and 1000 cm^{-1} , corresponding to Si–O stretching, and a weaker absorption near 1200 cm^{-1} , assigned to Si–C stretching vibrations.

A notable feature is the downshift and slight broadening of the Fe–O stretching vibrations corresponding to surface and core sites, now observed at 718 cm^{-1} and 624 cm^{-1} (in bare particles,

they appear at 728 cm^{-1} and 628 cm^{-1}). Another characteristic signal of the synthesized ligand is the weak C–S stretching vibration at 1200 cm^{-1} . Both ethylene glycol chains, neutral (**2.1b**) and cationic (**2.3**), exhibit an intense C(sp³)-O stretching vibration between 1200 and 1100 cm^{-1} . Since this absorption band is broad and poorly defined, it is not possible to determine the exact structure of the glycol chain; however, it is highly probable that it is amorphous, similar to that reported by Harder *et al.*⁴⁷. In contrast, the two ligands composed solely of hydrocarbon chains show a sharper CH₂ bending vibration at 1520 cm^{-1} . Lastly, the spectra of the two quaternary ammonium salt ligands (cationic hydrophilic and cationic hydrophobic) display a distinct C–N stretching absorption band at 980 cm^{-1} .

In conclusion, from IR spectroscopy is possible to see the presence of the ligand bound to the surface of the NPs. Although two different protocols were used, significant qualitative differences are absent between the IR spectrum of the functionalized nanoparticles with Protocol A and Protocol B, meaning that with both protocols is possible to covalently anchor the ligands to the surface of the particles.

2.6.2 Differential Scanning Calorimetry and Thermogravimetric Analysis (DSC-TGA)

The thermogravimetric analysis was performed in collaboration with Professor Delsante inside the laboratories of the Unige department of chemistry.

Before starting to analyse the ligand loading on maghemite NPs, firstly TGA was performed on the organic ligands and the bare NPs (*Table 2.5*) using two different gasses: air and Ar.

The final analysis showed important results. The weight loss of organic molecules was never of 100 % due to the presence of the triethoxysilane moiety in the ligands. This functional group at high temperature tends to form silicon dioxide, in fact, a powder was always found in the crucible after the analysis. When the TGA was run under air there was a complete combustion of the organic portion of the ligand, and a white powder was present in the crucible. SEM-EDX (scanning electron microscopy equipped with energy-dispersive x-ray probe) images of the powder were taken, and it was confirmed by elemental analysis that it is pure micrometric silica (*Figure 2.14*). When an inert atmosphere is used the combustion is not complete and some elemental carbon remains over the silica (black powder was found). This explains why the weight loss under Ar atmosphere is always lower than air.

Table 2.5: TGA of pure ligands and bare NPs. Data recorded from heating from 25°C to 1000°C

Sample	TGA data (% weight loss)	TGA data (% weight loss)
Ligand 1b	77%	90%
Ligand 2b	80%	88%
Ligand 5	73%	80%
Ligand 6	78%	84%
Bare NP (Batch 1)	0%	0%
	Data acquired from TGA performed in Ar atmosphere	Data acquired from TGA performed in air atmosphere

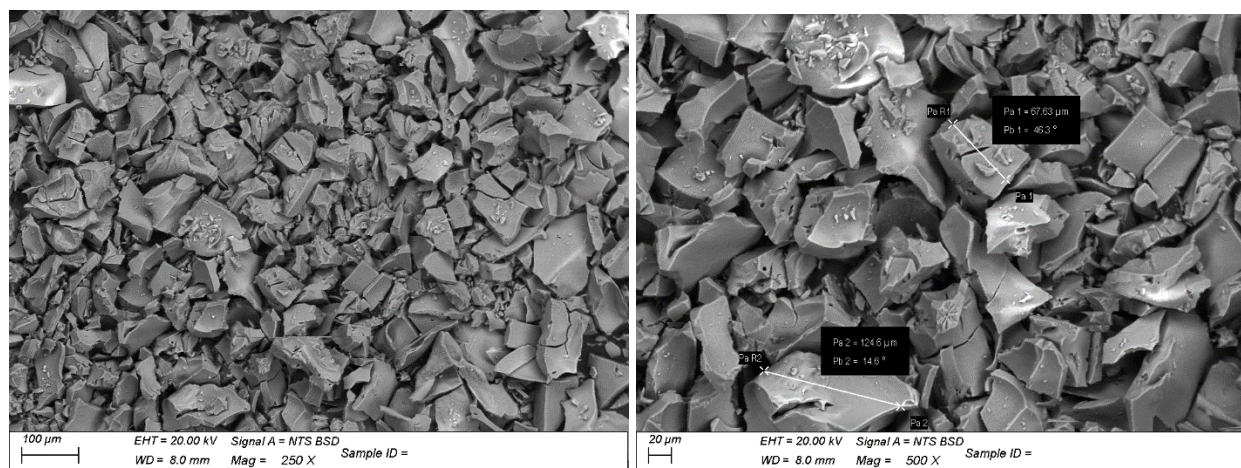


Figure 2.14: SEM images of the recovered solid from the crucible after TGA under air atmosphere

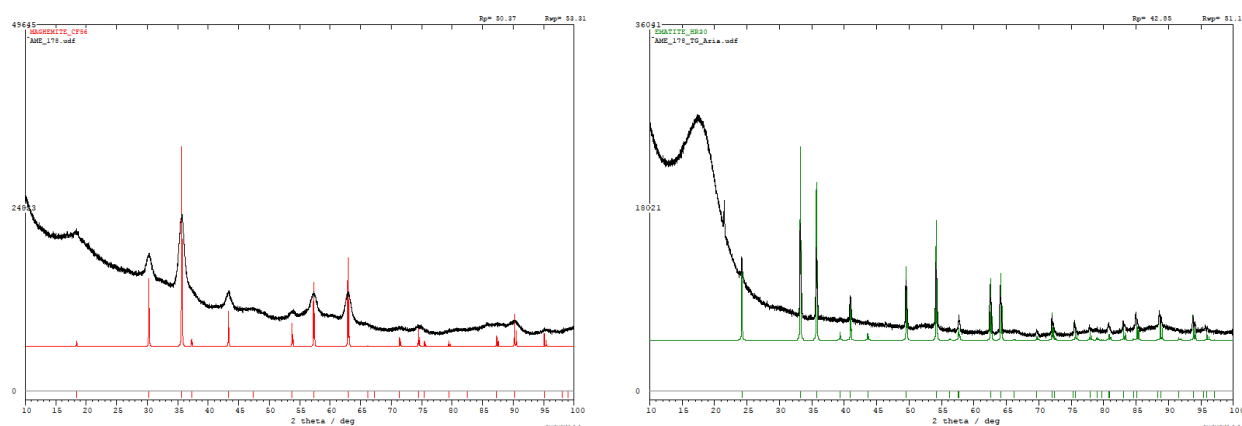


Figure 2.15: XRD spectrums of bare maghemite nanoparticles before the TGA (left) and after TGA (right). The broad peak in the right image correspond to the organic compound used to support the fine powder. The red diffraction spectrum is pure maghemite, the green diffraction spectrum is pure hematite

The TGA analysis of bare particles delivered the expected result both in air and Ar. Moreover, starting from 600°C, it was detected a strong exothermic peak in air, and weaker in Ar. Performing an XRD (X-ray diffraction) experiment on the powder recovered from the crucible it was demonstrated that the peak corresponds to a polymorphic transition of $\gamma\text{-Fe}_2\text{O}_3$ (maghemite) to $\alpha\text{-Fe}_2\text{O}_3$ (hematite) (Figure 2.15). In air the transition is complete, instead in Ar is partial.

After characterizing the organic ligands and bare maghemite by TGA, the functionalized nanoparticles were analyzed under the same conditions. The crucible in the TGA furnace was heated from 25 °C to 1000 °C to ensure complete combustion of all organic material, and each sample was measured twice, once under air and once under argon flow. After collecting and processing the data, the surface grafting percentage was calculated from the recorded mass loss using the equation:

surface grafting = (moles detached from the surface / theoretical moles required for 100% coverage) $\times$ 100.

Unexpected results were obtained. In several cases, the calculated surface grafting exceeded 100%. Furthermore, for all samples the mass loss measured under argon was higher than that

measured under air. This trend contrasts with previous results for the thermal degradation of simple organic chains. An example is reported in *Table 2.6*: for ligand **2.1b**, the calculated surface grafting was 108% in air and 112% under argon, and for ligand **2.4** the values were 97% in air and 125% under argon.

Table 2.6: results of TGA of functionalized NPs with ligands 2.1b and 2.4. Weight loss considered from 25°C to 1000°C

Ligand	Method	Weight loss %	Surface grafting	Weight loss %	Surface grafting
1b	Protocol A	28	108	29	112
4	Protocol B	34	97	44	125

Data acquired from **TGA** performed in **air atmosphere** Data acquired from **TGA** performed in **Ar atmosphere**

The thermogravimetric spectra were deeply investigated to understand the experimental results collected. A common trend was found in each analysis recorded independently from which type of gas was used. As an example, the TG spectrum of ligand-**2.3**@ γ -Fe₂O₃ in air is reported in *Figure 2.16*. The weight loss happens in three distinct stages: 40°C – 200°C, 200°C - 550°C and 650°C – 950°C (green solid line), and the result is confirmed by the DSC spectrum (blue solid line) that presents three exothermic peaks in the ranges previously reported. The last exothermic peak at 650°C is superimposed to the polymorphic transition of γ -Fe₂O₃ to α -Fe₂O₃, to which is not associated any weight loss, as it is reported in *Table 2.5*.

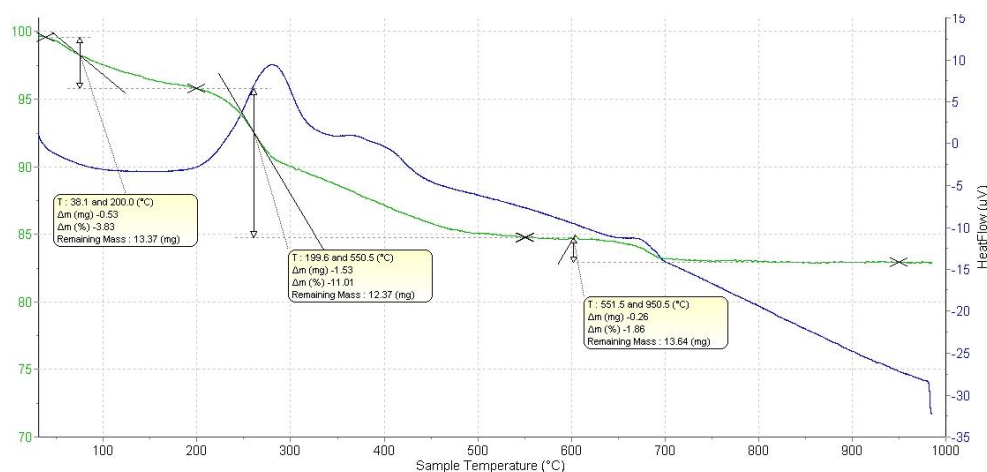


Figure 2.16: DSC-TG spectrum of 2.3@ γ -Fe₂O₃ in air. TG weight loss (green solid line), DSC graph (blue solid line)

The first stage, from 40 °C to 200 °C, corresponds to a weight loss of 3–7%. In this region, water and residual organic solvents can be thermally desorbed from the surface of the particles. This interpretation is consistent with both the relatively small mass loss, related to trace amounts, and the IR spectra. In those, broad absorption bands corresponding to adsorbed water on the particle surface, as well as water weakly bound to the organic chains, were always present. A similar weight-loss was observed in the TGA of the free organic chains, although it was less intense (2–4%, depending on the chemical nature of the chain).

The second stage, from 200 °C to 550 °C, corresponds to the thermal degradation of the ligand's organic chain, as also observed in the TGA profiles of the isolated ligands. No additional processes are expected to occur in this temperature range.

In the third temperature range (650 °C to 950 °C), a common trend was observed across all analyses. Under air flow, the weight loss in this region was 0.5–2%, whereas under argon it increased to 2–5%. Notably, this weight loss is completely absent in the TGA spectra of the free organic ligands and bare NPs.

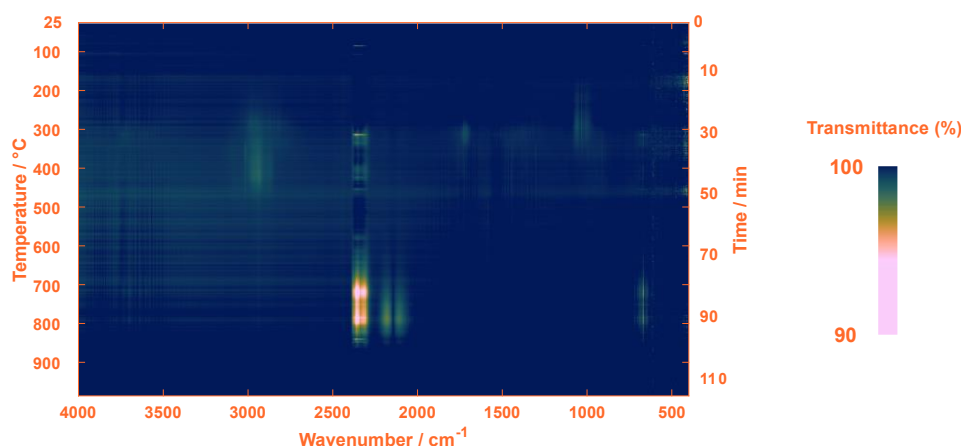


Figure 2.17: IR spectrum of evolved gas during the TGA of NPs functionalized with ligand **2.2** in Ar atmosphere

To investigate the origin of the high-temperature weight loss in more detail, a combined DSC–TG–IR experiment was carried out in collaboration with PhD student Michael Casale. The coupling with an infrared spectrometer enabled real-time analysis of the gases evolved during combustion in the instrument furnace. The IR spectra showed the evolution of CO and CO₂ in major quantities and short-chain alkyl compounds in traces over 600°C, particularly when Ar was used as gas (example reported in *Figure 2.17*). CO₂ can be identified by its single strong absorption band at 2400 cm⁻¹ and CO is characterized for two symmetric absorption bands at 2200 cm⁻¹. A possible explanation for the experimental data is the occurrence of a carbo-reduction process at the nanoparticle surface. Under Ar atmosphere carbonaceous residues accumulate on NPs surface in significant amount, and above 650°C redox reactions between Fe³⁺ and C may occur, yielding Fe²⁺ and oxidized carbon species (mainly CO, CO₂ and short-chain alkanes or alkenes). The same effect can be observed under air atmosphere, but with markedly lower intensity.

Considering all the results obtained and the explanations proposed, it was decided to take into account only the weight loss occurring between 200 °C and 600 °C under air. The DSC–TG measurements recorded under argon were discarded due to the incomplete combustion of the organic chain. This choice was expected to provide more accurate experimental data.

A new series of functionalization reactions was performed to evaluate possible effects related to different particle batches and reaction scales. The functionalization was carried out at the same scale as in the first set of experiments (100 mg) and was also increased to 1 g of NPs. All reactions

were conducted in parallel using a new batch of maghemite supplied by the French colleagues. Subsequently, the DSC–TG analyses under identical conditions were performed.

All the results gathered are reported in *Table 2.7*.

Table 2.7: TGA of functionalized maghemite under air flow. The weight loss considered is from 200°C to 600°C. Analysis performed on ≈12 mg of functionalized magnetic material

Entry	Weight loss %	Surface grafting	Weight loss %	Surface grafting
2.1b @γ-Fe ₂ O ₃			13.1 % (batch 1-O)*	50 %
	12.7 % (batch 1)	49 %	13.4 % (batch 1)	52 %
	11.1 % (batch 2)	43 %	19.7 % (batch 2)	76 %
			16.1 % (batch 2-1g)	62 %
2.2b @γ-Fe ₂ O ₃	14.6 % (batch 1)	51 %	10.2 % (batch 1-O)*	36 %
	14.1 % (batch 2)	49 %	9.9 % (batch 1)	35 %
	14.7 % (batch 2-1g)	52 %	8.9 % (batch 2)	31 %
2.3 @γ-Fe ₂ O ₃	21.1 % (batch 1)	61 %	15.8 % (batch 1)	46 %
	23.5 % (batch 2)	68 %	11.6 % (batch 2)	34 %
	27.7 % (batch 2-1g)	80 %		
2.4 @γ-Fe ₂ O ₃	17.2 % (batch 1)	49 %	22.2 % (batch 1)	63 %
	26.1 % (batch 2)	74 %	18.1 % (batch 2)	52 %
			17.7 % (batch 2-1g)	50 %

Protocol A

Protocol B

***Note:** orbital shaker used instead of magnetic stirring

A surface coverage between 50% and 80% was achieved. Interesting correlations between ligands and functionalization protocols were found: for ligand **2.1b**, Protocol B provided the highest coverage (in case of batch 2), whereas for ligands **2.2b** and **2.3**, Protocol A resulted in better grafting. For ligand **2.4**, however, the optimal protocol remained unclear, since the two nanoparticle batches showed opposite trends in surface coverage. A possible explanation lies in the chemical stability of the ligand. Compound **2.4** is the most unstable among the tested ligands and even traces of water can trigger rapid polymerization of its silane moiety. For the functionalization of nanoparticle batches 1 and 2, two separate batches of ligand **2.4** were used, each synthesized from different allyl ether precursor. For batch 1, allyl ether **2.14** was dried under high vacuum and subsequently stored; for batch 2, the allyl ether submitted to the thiol–ene reaction was freeze-dried for 36 h to remove all water traces. Moreover, the thiol–ene reaction for batch 2 was performed on a larger scale to provide sufficient ligand for both small- and large-scale functionalization. Consequently, in batch 1 the presence of residual water may have initiated premature polymerization of ligand **2.4** before the actual grafting step, partially suppressing its reactivity. Based on these considerations, Protocol A is likely the most suitable for ligand **2.4**. Nevertheless, all scale-up experiments were performed using the results obtained from the batch-1 functionalization, as previously discussed.

Along with the study of different protocols for the functionalization of maghemite nanoparticles, the possibility of using either classical magnetic stirring or mechanical stirring was evaluated. As a mechanical stirrer, an orbital shaker capable of controlling the reaction temperature was chosen. The experiment was carried out using ligands **2.1b** and **2.2b** to functionalize 100 mg of nanoparticles under the conditions of Protocol B. TGA analysis showed no significant difference between the two stirring methods, so magnetic stirring was selected for subsequent experiments. However, during larger-scale functionalization, the magnetic field generated by the stirring bar caused strong aggregation of the magnetic nanoparticles. This phenomenon may have reduced the functionalization efficiency by lowering the achievable surface coverage and could explain the lower grafting density achieved for ligand **2.1b**.

Overall, the two nanoparticle batches yielded comparable surface-grafting values, indicating that the developed protocols are reproducible.

The reproducibility of the TGA was tested using a sample of **2.1a** functionalized particles by doing 3 replicas in both atmospheres. It was performed by weighting in 3 different crucible the same mass of particles and heating them with the same thermal program in air and then it was repeated for Argon. The results, within the range from 13% to 15% weight loss, proved the precision of the technique and the uniformity of the coating in the chosen batch.

2.6.3 Colloidal properties studies

The behavior of the functionalized NPs in different solvents was tested to determine if the presence of the organic ligands has changed the surface properties. One useful technique to evaluate this parameter is DLS (dynamic light scattering), which is able to measure the hydrodynamic diameter of the particles in different solvents and determine its variation during time. This value is directly correlated with the aggregation of the particles that is the typical signal to recognize colloidal instability. REMAP application requires good colloidal stability in water, but it was decided to test also other solvents like toluene and THF (tetrahydrofuran) for other possible applications. Colloidal stability is related to electrostatic and steric repulsion among particles in a specific solvent. For this reason, Z-potential value of the surface of the particles was also measured. If the potential has an absolute value higher than 25–30 mV, it is generally accepted that the particles are electrostatically stable⁴⁸.

In *Table 2.8* are reported the experimental results. The bare particles are stable only in acidic water and the measured Z-potential proves this being +35.2 mV. The particles functionalized with ligand **2.1b** (hydrophilic neutral) showed good stability in deionized water for hours and months. The same hydrodynamic diameter was detected after 2 months with no precipitation (*Figure 2.18-a*). Ligand **2.1b** can stabilize the NPs in water by a synergic combination of weak electrostatic repulsion, steric hinderance and match between the chemical physical properties of it and water. In THF and toluene those functionalized particles showed stability only for few hours before precipitation occurred. However, the hydrodynamic diameter can be measured after 96 h because

a small portion of stable particles remained in suspension. The same observations were done for the particles functionalized with ligands **2.3** (hydrophilic cationic) and **2.4** (hydrophobic cationic). The positive charge of the ligands helps the stabilization of the particles in water by electrostatic repulsion. This hypothesis is proved by the zeta potential value of + 39 mV. On the other hand, these particles are unstable in THF and toluene, showing semi-total aggregation in few minutes for ligand **2.3** (*Figure 2.18-c*, particles aggregates visible by naked eyes: bigger at the bottom, smaller flocculate in the solvent) and hours for ligand **2.4**. The particles functionalized with ligand **2.2b** (neutral hydrophobic) showed instability in water: higher average hydrodynamic diameters (aggregation of the particle) and flocculation after few hours (*Figure 2.18-b*). The measurements performed after t_0 are correlated only to the smaller fraction of particles that were stable in suspension. After 96 hours total aggregation occurred. Ligand **2.2b** cannot stabilize the particles in water neither by electrostatic repulsion nor by steric hindrance. Due to the chemical physical properties of the chain, this ligand is the only one that gives a slightly longer stability to the particles in THF and toluene.

Table 2.8: hydrodynamic diameters and Zeta-potential measured for bare and functionalized nanoparticles. Solution concentration 0.5 g/L

Ligand	Hydrodynamic diameter in nm						Z-potential in H ₂ O
	Water		THF		Toluene		
Bare	t ₀	95.4	-		-		+ 35.2 mV
2.1b	t ₀	121.8	t ₀	463.0	t ₀	287.3	+ 21.8 mV
	t _{24h}	112.0	t _{24h}	381.0	t _{24h}	306.2	
	t _{96h}	106.8	t _{96h}	375.4	t _{96h}	225.4	
2.2b	t ₀	275.9	t ₀	211.9	t ₀	187.7	+ 7.9 mV
	t _{24h}	305.6	t _{24h}	215.1	t _{24h}	185.9	
	t _{96h}	269.2	t _{96h}	-	t _{96h}	-	
2.3	t ₀	94.2	t ₀	68.4	t ₀	224.3	+ 39.5 mV
	t _{24h}	88.7	t _{24h}	99.1	t _{24h}	-	
	t _{96h}	-	t _{96h}	-	t _{96h}	-	
2.4	t ₀	133.5	t ₀	171.0	t ₀	520	+ 39.9 mV
	t _{24h}	120.5	t _{24h}	206.6	t _{24h}	2440	
	t _{96h}	119.9	t _{96h}	-	t _{96h}	-	

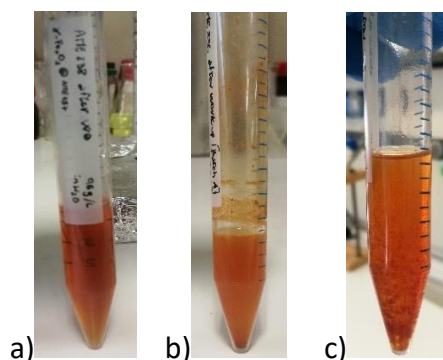


*Figure 2.18: a) ligand **2.1b**@ γ -Fe₂O₃ (neutral hydrophilic) at 0.5 g/L in deionized water; b) ligand **2.2b**@ γ -Fe₂O₃ (neutral hydrophobic) at 0.5 g/L in deionized water; c) ligand **2.3**@ γ -Fe₂O₃ (cationic hydrophilic) at 0.5 g/L in toluene*

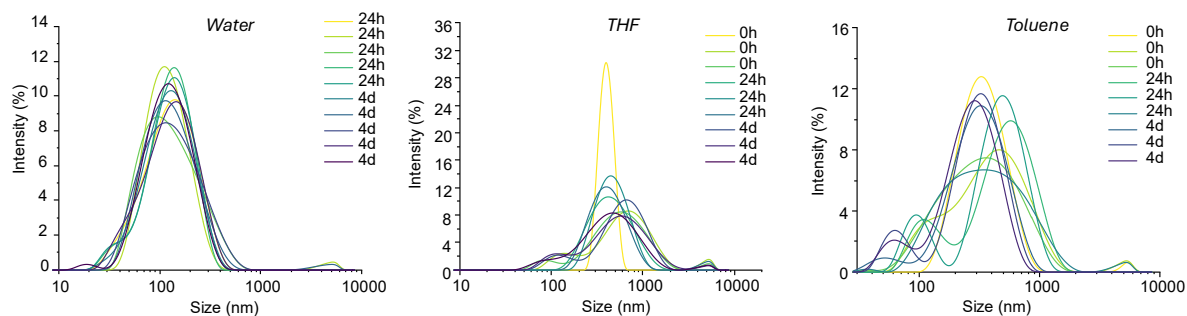


Figure 2.19: hydrodynamic diameter of **2.1b**@ γ -Fe₂O₃ (neutral hydrophilic ligand) at 0.5 g/L

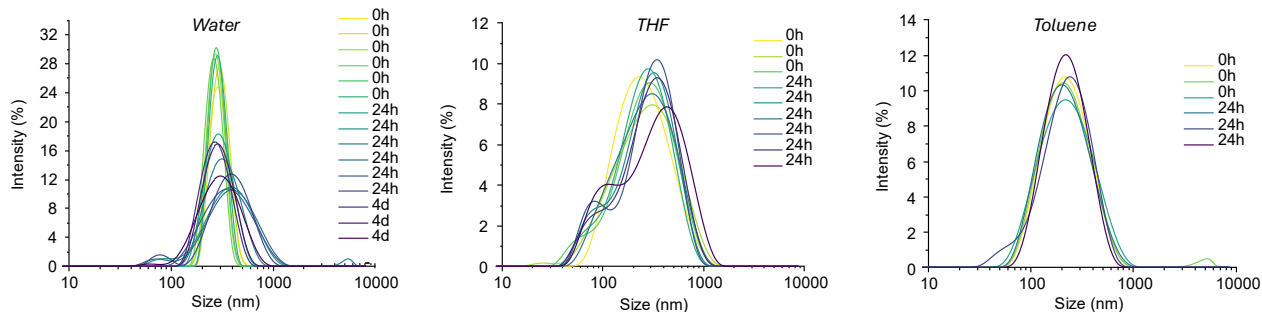


Figure 2.20: hydrodynamic diameter of **2.2b**@ γ -Fe₂O₃ (neutral hydrophobic ligand) at 0.5 g/L

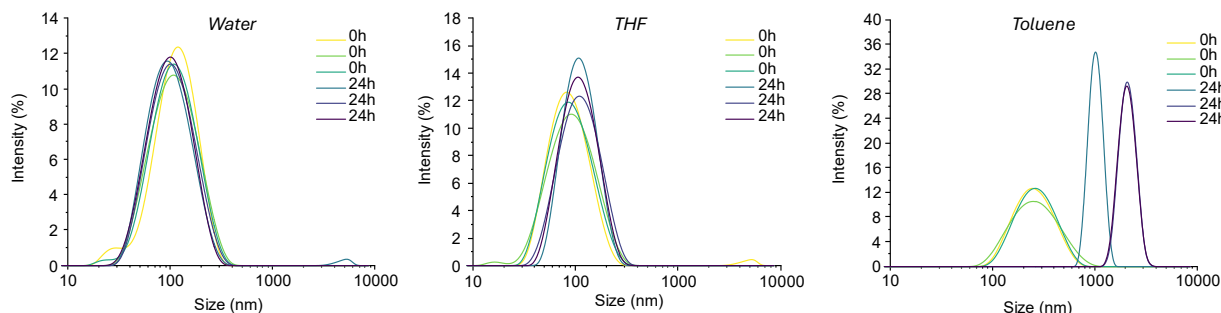


Figure 2.21: hydrodynamic diameter of **2.3**@ γ -Fe₂O₃ (cationic hydrophilic ligand) at 0.5 g/L

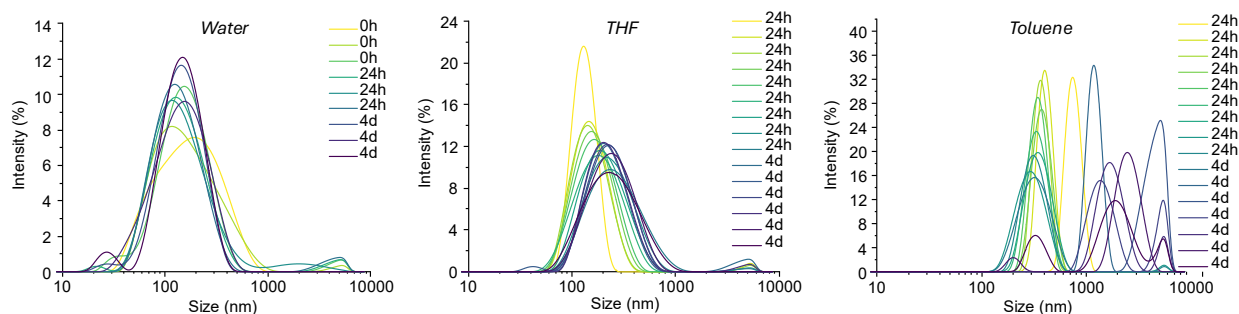


Figure 2.22: hydrodynamic diameter of **2.4**@ γ -Fe₂O₃ (cationic hydrophobic ligand) at 0.5 g/L

2.7 Conclusions

During the three years of PhD an important contribution was given to the REMAP project. Six novel organic ligands were studied. The synthetic approach designed is simple and uses easy to reproduce reactions, moreover each synthetic step was optimized to achieve quantitative or the highest possible yield. Chromatographic purifications were avoided where possible and other purification techniques were considered.

Finally, four novel ligands were synthesized via click-chemistry, anchored to the surface of the particles and then the modification of the surface properties were investigated. The anionic ligands were not isolated; however, it was demonstrated that the thiol-ene reaction can be carried out in presence of anionic salts and the model product with octanethiol (**2.21**) was obtained in quantitative yield. By performing a further optimization of the reaction conditions will be possible to isolate the desired compounds.

DSC–TG and IR analyses demonstrated that the two proposed protocols can be used to anchor the custom-made ligands onto the surface of both silica and maghemite nanoparticles. For the model silica nanoparticles, a clear correlation was observed between the chemical-physical properties of the ligands and the nature of the protocol employed. In contrast, for the maghemite nanoparticles no such correlation was found; however, it is evident that selecting the appropriate protocol can increase the surface coverage by 20–30% in some cases. The reproducibility of both experimental protocols and analytical techniques was confirmed, further supporting the homogeneity of surface coverage within each batch of functionalized nanoparticles. Moreover, an extended study of the thermogravimetric behavior of the functionalized nanoparticles enabled to highlight a phenomenon that is rarely considered or evaluated in similar studies reported in the literature.

DLS analysis showed that the covalent surface functionalization of maghemite nanoparticles altered their surface properties, demonstrating that colloiddally stable suspensions in neutral water can be maintained for months when using the two cationic ligands or the neutral hydrophilic ligand. In contrast, the neutral hydrophobic ligand enabled only a short-term improvement of colloidal stability in THF and toluene.

The colleagues at Université du Luxembourg tested the performance of the grafted particles for the final REMAP application, evaluating their stability in electrolyte-containing solutions, their aggregation in the presence of a magnetic field (MF) and subsequent redispersion once the MF was removed, and finally their masking efficiency. The sample provided passed all tests, but the masking efficiency registered after the electrodeposition process did not match the REMAP final requirements. In the experiments carried out NPs functionalized with ligand **2.3** performed better, while particles functionalized with ligand **2.2b** were not used (not sufficient colloidal stability in aqueous solutions). The iron core of the particles was not inert under these conditions: traces of Fe were found plated on the substrate surface. A silica shell around the nanoparticles would likely have prevented these side processes and could have increased the grafting density, thereby improving the final properties of the functionalized material.

2.8 Experimental procedures

2.8.1 General Aspects

Solvents and reagents

Commercial solvents and reagents were used directly without any other purification. All non-aqueous reactions were performed under an inert atmosphere of argon or nitrogen. Inert gases were passed over a U-tube of silica and activated 3 Å molecular sieves. When inert atmosphere was used, commercially available dry solvents were employed, and every possible precaution was taken.

Routine procedure

Every piece of glassware was dried and stored in oven at 100°C. Analytical TLC was performed on Merck silica gel 60 F254 0.25 mm TLC glass plates and visualized by UV (254 nm). TLC plates were also stained by dipping and heating with Hanessian stain (CAM, 21 g (NH₄)₄MoO₄·4H₂O and 1g Ce(SO₄)₂·4H₂O in 31 mL of H₂SO₄ and 469 mL of H₂O), KMnO₄ (1,5 g of KMnO₄, 10 g of K₂CO₃, 1 mL of 10% aq. NaOH in 200 mL of deionized water). R_f were measured after an elution of 7–9 cm. After extractions, the aqueous phases were always re-extracted three times with the appropriate organic solvent, and the organic extracts were always dried over Na₂SO₄ and filtered before evaporation to dryness. Concentration under reduced pressure was performed by rotavapor at 40 °C. Chromatographic purification was performed as flash chromatography (FC) on MERCK Geduran® Si 60 Å (40–63 µm, 230–400 mesh) under positive pressure and the crude mixtures were loaded as solid absorbed on silica or as solution in the eluent. Petroleum ether (PE) is 40–60 °C. Purified compounds were dried further under high vacuum (~10⁻² torr).

All products were characterized by ¹H, ¹³C, IR, GC-MS and HPLC-ESI-MS.

Nuclear magnetic resonance spectroscopic analysis (NMR)

¹H and ¹³C NMR spectra were recorded on a JEOL 400 (at 400 MHz and 101 MHz respectively). NMR spectra were recorded using residual solvent and TMS as the internal standard ¹H NMR: TMS = 0.00, CDCl₃ at 7.26 ppm for ¹H and 77.16 ppm for ¹³C, DMSO-D₆ at 2.50 ppm for ¹H and 39.52 for ¹³C and CD₃OD at 3.31 ppm for ¹H and 49.00 for ¹³C). Data for ¹H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, integration, and coupling constants (Hz). Data for ¹³C NMR spectra are reported in terms of chemical shift (δ ppm). Interpretation of spectra has been made also with the aid of COSY, HSQC and HMBC experiments. The following abbreviations are used to indicate the multiplicity in NMR spectra: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and their combinations; the b in front of the multiplicity stands for a broad signal. For some compounds the extra peaks are due to residual undefined impurities (<5%) due to difficulties in the purification. All the NMR spectrums were analysed using the software MestReNova (Mestrelab Research® v. 14.2).

Infrared absorption spectrums (IR)

Fourier transform infrared (FTIR) spectra were recorded on a Perkin Elmer Spectrum 65 (Perkin Elmer, Waltham, MA, USA) instrument, equipped with a universal attenuated total reflectance (ATR) sampling. IR peaks are reported as decreasing wavenumber ($\bar{\nu}$, cm^{-1}). All the spectrums were elaborated using the software Spectrum (PerkinElmer® v.10.03). Each absorption spectrum of organic molecule was processed with a 10% smooth algorithm. Each absorption spectrum of nanoparticles was processed with a 10% smooth algorithm, ATR correction and baseline correction.

Gas chromatography Mass spectrum coupled (GC-MS) analysis

GC-MS analyses were performed on Shimadzu GC-MS-QP2010 SE with an AOC-20i Plus auto-injector mounting an Avantor Hichrom HI-5 MS column (internal diameter 0.25 mm, film thickness 0.25 mm, length 30 m). A Mass Analyzer Metal quadrupole mass filter with pre-rods was used and the ionization was obtained through Electronic Impact operating with an ionization potential of 70 eV. He was used as gas carrier, flux 1.0 mL/min, V_{inj} is 1 μL , the split ratio used is 1:10. Samples were prepared as EtOAc solutions at 100 ppm. Solvent delay = 2.5 min, mass range 35-400, T_{inj} = 250 °C, T_{detector} = 250 °C, T_{MS} = 200°C.

The method used for the analysis is: Starting temperature 100 °C, starting time 3 min, temperature gradient 25 °C/min, final temperature 300 °C.

The analysis was processed using the software GCMS LabSolution Shimadzu (v. 4.52) and the data are reported as follow: retention time (t_{R} , min), m/z (threshold 5%) and relative abundance

High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) analysis was performed on Agilent HP 1100 equipped with a DAD detector (220 nm) and column Synergi Hydro-RP 80A column (150 × 3 mm, 4 μ) at 26 °C with flow = 0.5 mL/min and gradient $\text{H}_2\text{O}/\text{CH}_3\text{CN}$, (A = H_2O +1% HCOOH , B = MeOH +1% HCOOH), 0 min A = 90%, 30 min B = 0%.

Differential Scanning Calorimetry and Thermogravimetric Analysis (DSC-TGA)

Differential Scanning Calorimetry and Thermogravimetric Analysis (DSC-TGA) was performed using a LabsysEvo 1600–Setaram (Thermo Fisher Scientific). Approximately 12 mg of sample was weighted in an open alumina crucible and heated from 25°C to 1000°C, at 10 °C/min under an Ar or air atmosphere (20 ml/min). To determine surface grafting the weight loss between 200 °C and 600°C was considered.

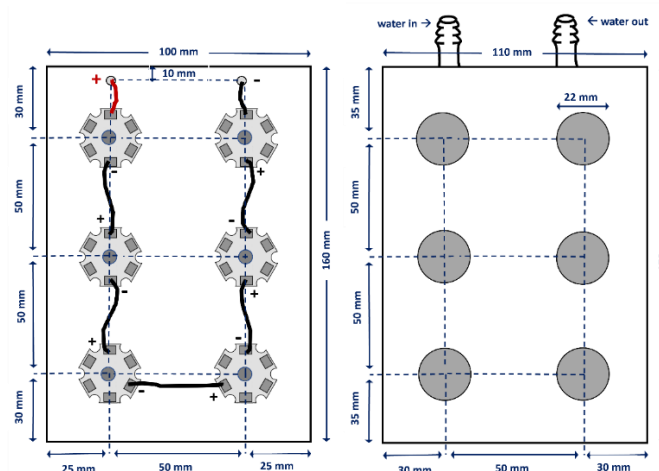
Dynamic Light Scattering DLS

Colloidal properties were assessed by Dynamic Light Scattering (DLS) and zeta potential (ζ) measurements, using a Malvern Analytical Zetasizer Nano ZS 90 system (Malvern Instruments, UK). Functionalized MNPs were dispersed at a concentration of 0.5 g/L in deionized water,

tetrahydrofuran (THF) and toluene, representing non-polar organic solvents. DLS measurements were carried out using a glass cuvette, while ζ measurements employed a DTS1070 cell with a fixed scattering angle of 90°. All experiments were conducted at a constant temperature of 21 °C. Each sample was measured at least three times every 20 min, and measurements were performed after preparation and after storage for 24 and 96 h at room temperature (without agitation). The Z-average hydrodynamic diameter (d_h) and polydispersity index (PDI) were calculated as the average over the replicates at each time point.

Photochemical Apparatus

Batch reactions were conducted in a dedicated apparatus consisting of an aluminum cooling plate (LWH 160x100x25 mm) with 6 OSRAM® Oslon SSL 80 LDCQ7P (nominal 450 nm, royal blue) LEDs mounted in series powered by a MeanWell® LPC-20-700 constant current power supply (700 mA) and a water-cooled aluminium vessel holder. The vessel holder (LWH 170x110x38 mm) holds the sealed vials 10 mm over the LEDs in a fixed position while cooling both the LED plate and the vial, keeping the temperature of the latter below 20°C. Both the LED plate and the water-cooled vessel were custom built, while the vials were purchased from Wicom International Co. (WIC43005, 5 mL crimp top vial 38.5x22.0 mm; WIC44510 20 mm crimp caps with 3.0 mm PTFE septum).



2.8.2 Surface grafting procedures

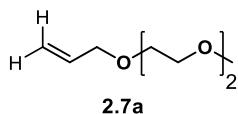
Protocol A: In a glass vial containing the precipitated γ -Fe₂O₃ NPs, small amount of toluene is added. A sonication for 10 minutes at max power follows to disperse the nanoparticles. Then, the ligand (2.5 eq. respect to the theoretical amount to have a 100% superficial coverage) is added as a solution in toluene. Final concentration of the dispersion is 40 mgNP/mL. A drop of distilled water is added. The vial is closed with a screw cap and heated to 100°C for 24 h keeping a constant stirring of 550 RPM. After the reaction, the stir bar is removed and the solvent decanted keeping the magnetic particles still with a strong magnet. The NPs are transferred in a Falcon using maximum 10 mL of a mixture of 1:3 EtOH:Acetone, they are sonicated at max power for 10 minutes and

centrifugated at 3144 RCF/7500 RPM for 30 minutes. The washing is repeated other 2 times. Finally, to the NPs, EtOH is added until a stable suspension is obtained (concentration 5 mg/mL).

Protocol B: In a flask, equipped with a magnetic stir bar, the custom-made ligand (2.5 eq. respect to the theoretical amount to have a 100% superficial coverage) is weighted and later solubilized in EtOH (approximately the 5/6 of total EtOH). The flask is closed and heated at 50°C for 2h with a constant magnetic stirring of 550 RPM. After 2 h, the solution is cooled to rt and a suspension of maghemite NPs in EtOH is added (for the suspension is used the remaining 1/6 of the total EtOH for a concentration of 50 mg/mL). Before the addition, the nanoparticles were sonicated for 1h at maximum power. The final reaction concentration is 8.8 mgNP / mLsolvent. The NPs suspension and the ligand are heated at 50°C for 2h. Then, the flask is cooled and all the functionalized particles suspended in EtOH are transferred into a new falcon. To wash the NPs a mixture of 1 (hexane): 4 (acetone): 1 (EtOH) is used and centrifuge is used to aggregate the solid material (3144 RCF/7500 RPM for 30 min). Every time the suspension is sonicated for 10 minutes at max power. The process is repeated 4 times. Finally, the NPs are suspended in EtOH (final concentration of 5 mg/mL).

2.8.3 Preparation of neutral hydrophilic ligands **2.1a** and **2.1b**

Synthesis of 3-(2-(2-methoxyethoxy)ethoxy)prop-1-ene (**2.7a**)



NaH (60% in mineral oil, 400 mg, 9,99 mmol) was suspended in dry THF (48.5 mL) under Ar atmosphere. The reaction was cooled to 0°C and tetraethylen glycol monomethyl ether (0,98 mL, 8,3 mmol) was slowly added. After 30 minutes at 0°C allyl bromide (0,86 mL, 9,99 mmol) was very slowly injected. After 10 minutes the reaction was let to warm to room temperature. After 16 hours at rt, s.s. NH₄Cl was added and the organic solvent was partially removed. The mixture was diluted with AcOEt and transferred in a separatory funnel. The organic phase was extracted 3 times with s.s. NH₄Cl, washed with brine, dried (Na₂SO₄), filtered and evaporated to dryness. The crude allylether (1,329 g, colourless oil) was sufficiently pure to be used in the next step without further purification.

Molecular weight: 160.21 g/mol (C₁₂H₂₄O₅)

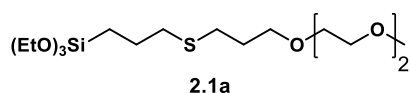
TLC: R_f 0,75 (EA, CAM weak, KMnO₄ basic)

¹H NMR (400 MHz, CDCl₃) δ 5.92 (ddt, *J* = 17.2, 10.4, 5.7 Hz, 1H, CHH=CHCH₂O), 5.32 – 5.23 (m, 1H, CHH^[cis]=CHCH₂O), 5.17 (dq, *J* = 10.4, 1.3 Hz, 1H, CHH^[trans]=CHCH₂O), 4.02 (dt, *J* = 5.7, 1.4 Hz, 2H, CHH=CHCH₂O), 3.69 – 3.64 (m, 4H, 2x CH₂), 3.64 – 3.60 (m, 2H, CH₂), 3.56 (dd, *J* = 5.6, 3.7 Hz, 2H, CH₂), 3.38 (s, 3H, OCH₃).

GC-MS: Rt= 4.475 min; m/z (Relative abundance %) 39 (21), 41 (92), 42 (6), 43 (23), 44 (3), 45 (43), 55 (8), 56 (5), 57 (5), 58 (28), 59 (100), 60 (4), 71 (7), 73 (22), 84 (5), 85 (5), 89 (6), 101 (4), 103 (16)

All the data are in accordance with literature

Synthesis of 16,16-diethoxy-2,5,8,17-tetraoxa-12-thia-16-silanonadecanesilapentacosane (**2.1a**)

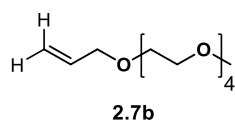


In a vial for photochemical reactions allyl ether (**2.7a**) (121 mg, 0.755 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 182 μ L, 0.755 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 651, 7.7 mg, 0.0300 mmol) were added and then mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (301 mg, yellowish viscous oil) was obtained. The crude ligand was sufficiently pure to be used in the next step without further purification.

Molecular weight: 398.63 g/mol ($C_{17}H_{38}O_6SSi$)

^1H NMR (400 MHz, $CDCl_3$): δ 3.86 – 3.78 (m, 6H, 3x CH_3CH_2-O-Si), 3.67 – 3.62 (m, 4H, 2x CH_2), 3.61 – 3.58 (m, 2H, CH_2), 3.57 – 3.52 (m, 4H, CH_2 and $S-CH_2CH_2CH_2-O$), 3.38 (s, 3H, $-OCH_3$), 2.57 (t, J = 7.3 Hz, 2H, $S-CH_2CH_2CH_2-O$), 2.55 – 2.50 (m, 2H, $Si-CH_2CH_2CH_2-S$), 1.85 (p, J = 6.5 Hz, 2H, $S-CH_2CH_2CH_2-O$), 1.69 (p, J = 7.6 Hz, 2H, $Si-CH_2CH_2CH_2-S$), 1.22 (1.25 – 1.20 (m, 9H, 3x CH_3CH_2-O-Si), 0.77 – 0.70 (m, 2H, $Si-CH_2CH_2CH_2-S$).

Synthesis of 2,5,8,11,14-pentaoxaheptadec-16-ene (**2.7b**)



NaH (60% in mineral oil, 698 mg, 17.5 mmol) was suspended in dry THF (48.5 mL) under Ar atmosphere. The reaction was cooled to 0°C and tetraethylen glycol monomethyl ether (2.9 mL, 14.6 mmol) was slowly added. After 30 minutes at 0°C allyl bromide (1.5 mL, 17.5 mmol) was very slowly injected. After 10 minutes the reaction was let to warm to room temperature. After 16 hours at rt, s.s. NH_4Cl was added and the organic solvent was partially removed. The mixture was diluted with AcOEt and transferred in a separatory funnel. The organic phase was extracted 3 times with s.s. NH_4Cl , washed with brine, dried (Na_2SO_4), filtered and evaporated to dryness. The crude allylether (3.605 g, yellowish oil) was sufficiently pure to be used in the next step without further purification.

Molecular weight: 248.32 g/mol ($C_{12}H_{24}O_5$)

TLC: Rf 0.27 (1:2 PE:EA, CAM weak, $KMnO_4$ basic)

^1H NMR (400 MHz, $CDCl_3$) δ 5.98 – 5.85 (m, 1H, $CHH=CHCH_2-O$), 5.27 (dq, J = 17.2, 1.5 Hz, 1H, $CHH^{(trans)}=CHCH_2-O$), 5.18 (dd, J = 10.4, 1.3 Hz, 1H, $CHH^{(cis)}=CHCH_2-O$), 4.02 (dt, J = 5.7, 1.2 Hz, 2H, $CHHCHCH_2-O$), 3.69 – 3.53 (m, 16H, 8 CH_2), 3.38 (s, 3H, CH_3).

^{13}C NMR (101 MHz, $CDCl_3$) δ 134.9 ($CHH=CHCH_2-O$), 117.2 ($CHH=CHCH_2-O$), 72.4 ($CHH=CHCH_2-O$), 72.1 (CH_2), 70.8 (5 CH_2), 70.7 (CH_2), 69.6 (CH_2), 59.2 (CH_3).

GC-MS: Rt= 9.710 min; m/z (Relative abundance %) 39 (7), 40 (2), 41 (76), 42 (10), 43 (73), 55 (45), 56 (17), 57 (100), 58 (50), 67 (9), 68 (10), 69 (35), 70 (16), 71 (75), 81 (7), 82 (20), 83 (34), 84 (11), 85 (41), 95 (6), 96 (11), 97 (31), 98 (7), 99 (11), 111 (16), 125 (8)

IR wavenumber (cm^{-1}): 2864, 1646, 1456, 1350, 1293, 1248, 1199, 1101, 1030, 995, 924, 851, 731

$$\text{(EtO)}_3\text{Si}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{S}-\text{CH}_2\text{CH}_2\text{CH}_2-\text{O}-\left[\text{CH}_2\text{CH}_2\text{O}\right]_4$$

2.1b

Molecular weight: 486.74 g/mol (C₂₁H₄₆O₈SSi)

¹³C NMR (101 MHz, CDCl₃): δ 72.1 (S-CH₂CH₂CH₂-O), 70.8 (5 CH₂), 70.7 (CH₂), 70.4 (CH₂), 70.0 (CH₂), 59.2 (CH₃O-), 58.6 (2 CH₃CH₂-O-Si), 58.5 (CH₃CH₂-O-Si), 35.4 (Si-CH₂CH₂CH₂-S), 29.9 (S-CH₂CH₂CH₂-O), 28.8 (S-CH₂CH₂CH₂-O), 23.3 (Si-CH₂CH₂CH₂-S), 18.6 (CH₃CH₂-O-Si), 18.5 (2 CH₃CH₂-O-Si), 10.1 (Si-CH₂CH₂CH₂-S).

2.8.4 Synthesis of ligands neutral hydrophobic **2.2a** and **2.2b**

C=CCOCC(C)C
2.8a

Molecular weight: 170.30 g/mol (C₁₁H₂₂O)

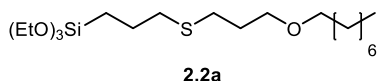
TLC: R_f 0.7 (20:1 PE:Et₂O, CAM)

53

(dt, $J = 5.6, 1.4$ Hz, 2H, CHH=CHCH₂-O), 3.41 (t, $J = 6.7$ Hz, 2H, O-CH₂CH₂), 1.56 (q, $J = 6.7$ Hz, 2H, O-CH₂CH₂), 1.39 – 1.20 (m, 10H, 5x CH₂), 0.93 – 0.77 (m, 3H, CH₃).

GC-MS: Rt= 7.788 min; m/z (Relative abundance %) 39 (16), 41 (100), 42 (14), 43 (70), 55 (29), 56 (21), 57 (86), 58 (62), 69 (26), 70 (17), 71 (66), 82 (6), 83 (14), 84 (11), 97 (2), 112 (6), 141 (9)

Synthesis of 4,4-diethoxy-3,12-dioxa-8-thia-4-silaooctacosane (**2.2a**):



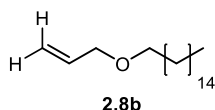
In a vial for photochemical reactions allyl ether (**2.8a**) (478 mg, 2.57 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 589 μ L, 2.57 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 651, 26 mg, 0.103 mmol) were added and then mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (65 mg, yellowish viscous oil) was obtained.

Molecular weight: 408.71 g/mol (C₂₆H₅₀O₄SSi)

¹H NMR (400 MHz, CDCl₃): δ 3.87 – 3.76 (m, 6H, 3x CH₃CH₂O-Si), 3.48 (t, $J = 6.3$ Hz, 2H, S-CH₂CH₂CH₂-O), 3.39 (t, $J = 6.7$ Hz, 2H, O-CH₂CH₂), 2.57 (t, $J = 7.3$ Hz, 2H, S-CH₂CH₂CH₂-O), 2.53 (t, $J = 7.2$ Hz, 2H, Si-CH₂CH₂CH₂-S), 1.83 (p, $J = 6.5$ Hz, 2H, S-CH₂CH₂CH₂-O), 1.70 (p, $J = 8.0$ Hz, 2H, Si-CH₂CH₂CH₂-S), 1.55 (p, $J = 6.9$ Hz, 2H, O-CH₂CH₂), 1.34 – 1.17 (m, 19H, 5x CH₂ and 3x CH₃CH₂O-Si), 0.87 (t, $J = 6.8$ Hz, 3H, CH₃), 0.77 – 0.68 (m, 2H, Si-CH₂CH₂CH₂-S).

¹³C NMR (101 MHz, CDCl₃): δ 71.2 (O-CH₂CH₂), 69.4 (S-CH₂CH₂CH₂-O), 58.5 (3x CH₃CH₂O-Si), 35.3 (Si-CH₂CH₂CH₂-S), 32.0 (CH₂), 30.0 (S-CH₂CH₂CH₂-O), 29.9 (O-CH₂CH₂), 29.6 (CH₂), 29.4 (CH₂), 28.8 (S-CH₂CH₂CH₂-O), 26.3 (CH₂), 23.3 (Si-CH₂CH₂CH₂-S), 22.8 (CH₂), 18.4 (3x CH₃CH₂O-Si), 14.2 (CH₃), 10.0 (Si-CH₂CH₂CH₂-S).

Synthesis of 1-(allyloxy)hexadecane (**2.8b**):



NaH (60% in mineral oil, 1.650 g, 41.2 mmol) was suspended in dry THF (68.7 mL) under Ar atmosphere. The reaction was cooled to 0°C and hexadecanol (5.000 g, 20.6 mmol) was slowly added. After 10 minutes the reaction was brought to room temperature. After 1 hour at room temperature allyl bromide (2.7 mL, 30.9 mmol) was injected very slowly. As last tetrabutylammonium bromide was added (332 mg, 1.03 mmol). The reaction flask was sealed with a screw cap and then heated to 80°C. After 18 hours, the reaction was cooled to room temperature and s.s. NH₄Cl was added and the organic solvent was partially removed. The mixture was diluted with AcOEt and transferred in a separatory funnel. The organic phase was extracted 3 times with s.s. NH₄Cl, washed with brine, dried (Na₂SO₄), filtered and evaporated to dryness. The crude allylether (5.819 g, yellowish oil) was sufficiently pure to be used in the next step without further purification.

Molecular weight: 282.51 g/mol (C₁₉H₃₈O)

TLC: Rf 0.7 (20:1 PE:Et₂O, CAM)

¹H NMR (400 MHz, CDCl₃): δ 5.91 (ddt, $J = 17.2, 10.5, 5.6$ Hz, 1H, CHH=CHCH₂-O), 5.25 (dq, $J = 17.2, 1.6$ Hz, 1H, CHH^[cis]=CHCH₂-O), 5.15 (dq, $J = 10.4, 1.3$ Hz, 1H, CHH^[trans]=CHCH₂-O), 3.95 (dt, $J = 5.6,$

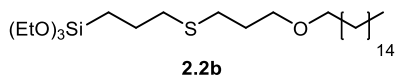
1.4 Hz, 2H, CHH=CHCH₂-O), 3.41 (t, *J* = 6.7 Hz, 2H, O-CH₂CH₂), 1.61 – 1.52 (m, 2H, O-CH₂CH₂), 1.36 – 1.19 (m, 26H, 13 CH₂), 0.87 (t, *J* = 7.1 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃): δ 135.3 (CHH=CHCH₂-O), 116.8 (CHH=CHCH₂-O), 71.9 (CHH=CHCH₂-O), 70.7 (O-CH₂CH₂), 32.1 (CH₂), 29.9 (CH₂), 29.84 (3 CH₂), 29.81 (3 CH₂), 29.76 (2 CH₂), 29.66 (CH₂), 29.5 (CH₂), 26.3 (CH₂), 22.8 (CH₂), 14.3 (CH₃).

GC-MS: Rt= 8.295 min ; m/z (Relative abundance %) : 39 (6), 41 (63), 43 (24), 44 (7), 45 (32), 55 (6), 58 (29), 59 (100), 71 (7), 73 (26), 85 (13), 87 (16), 89 (11), 103 (22)

IR: wavenumber (cm⁻¹): 2922, 2852, 1466, 1346, 1106, 994, 920, 721

Synthesis of 4,4-diethoxy-3,12-dioxa-8-thia-4-silaoctacosane (**2.2b**)



In a vial for photochemical reactions allyl ether (**2.8b**) (35 mg, 0.125 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 30 μL, 0.125 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 651, 1.0 mg, 0.00499 mmol) were added and then mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (65 mg, yellowish viscous oil) was obtained. The crude ligand was sufficiently pure to be used in the next step without further purification.

Molecular weight: 520.93 g/mol (C₂₆H₅₀O₄SSi)

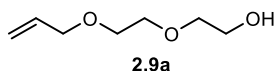
¹H NMR (400 MHz, CDCl₃): δ 3.88 – 3.76 (m, 6H, 3x CH₃CH₂-O-Si), 3.48 (t, *J* = 6.3 Hz, 2H, S-CH₂CH₂CH₂-O), 3.39 (t, *J* = 6.7 Hz, 2H, O-CH₂CH₂), 2.58 (t, *J* = 7.1 Hz, 2H, S-CH₂CH₂CH₂-O), 2.54 (t, *J* = 7.1 Hz, 2H, Si-CH₂CH₂CH₂-S), 1.84 (p, *J* = 6.4 Hz, 2H, S-CH₂CH₂CH₂-O), 1.77 – 1.63 (m, 2H, Si-CH₂CH₂CH₂-S), 1.61 – 1.49 (m, 2H, O-CH₂CH₂), 1.36 – 1.19 (m, 35H, 13x CH₂ e 3x CH₃CH₂-O-Si), 0.88 (t, *J* = 6.8 Hz, 3H, CH₃), 0.77 – 0.71 (m, 2H, Si-CH₂CH₂CH₂-S).

¹³C NMR (101 MHz, CDCl₃): δ 71.2 (O-CH₂CH₂), 69.4 (S-CH₂CH₂CH₂-O), 58.5 (3x CH₃CH₂-O-Si), 35.3 (Si-CH₂CH₂CH₂-S), 32.1 (CH₂), 30.0 (S-CH₂CH₂CH₂-O), 29.8 (CH₂), 29.8 (6 CH₂), 29.8 (2 CH₂), 29.7 (CH₂), 29.5 (CH₂), 28.8 (S-CH₂CH₂CH₂-O), 26.3 (CH₂), 23.3 (Si-CH₂CH₂CH₂-S), 22.8 (CH₂), 18.4 (3 CH₃CH₂-O-Si), 14.3 (CH₃), 10.0 (Si-CH₂CH₂CH₂-S).

IR: wavenumber (cm⁻¹): 2923, 2853, 1466, 1390, 1294, 1250, 1166, 1102, 1077, 958, 781

2.8.5 Preparation of the common intermediate **2.11a**, **2.11b** and **2.12**

Synthesis of 2-(2-(allyloxy)ethoxy)ethan-1-ol (**2.9a**)



KOH (773 mg, 13.8 mmol) was solubilized in a DMSO:H₂O mixture (4:1, 5.0 mL). Diethylene glycol (4.1 mL, 45.9 mmol) was slowly added. After 15 minutes allyl bromide (1.0 mL, 11.5 mmol) was injected dropwise. After 3 hours at rt, s.s. NH₄Cl was added, the reaction crude was diluted with ethyl acetate and transferred in a separatory funnel. The aqueous phase was extracted 8 times with ethyl acetate. The reunited organic phase was washed with brine and dried (Na₂SO₄), filtered and evaporated to dryness. The crude mono-allylether was purified by FC on silica (PE:EA 3:7) to give the product as pale-yellow oil (1.412 g, 84% yield).

Molecular weight: 146.19 g/mol (C₇H₁₄O₃)

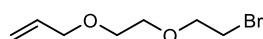
TLC: Rf 0.4 (3:7 PE:EA, KMnO₄ Basic)

¹H NMR (400 MHz, CDCl₃): δ 5.91 (ddt, *J* = 16.8, 10.4, 5.7 Hz, 1H, CHH=CHCH₂-O), 5.27 (dq, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.19 (dq, *J* = 10.4, 1.1 Hz, 1H, CHH^[trans]=CHCH₂-O), 4.02 (dt, *J* = 5.7, 1.3 Hz, 2H, CHH^[cis]=CHCH₂-O), 3.75 – 3.66 (m, 4H, 2x CH₂), 3.62 – 3.58 (m, 4H, 2x CH₂).

¹³C NMR (101 MHz, CDCl₃): δ 134.6 (CHH=CHCH₂-O), 117.5 (CHH=CHCH₂-O), 72.6 (CH₂), 72.4 (O-CHH=CHCH₂), 70.6 (CH₂), 69.6 (CH₂), 61.9 (CH₂).

GC-MS: Rt= 7.045 min; m/z (Relative abundance %) 39 (10), 41 (56), 42 (4), 43 (12), 44 (9), 45 (100), 55 (4), 57 (5), 58 (8), 59 (8), 71 (6), 73 (12), 75 (5), 89 (8)

Synthesis of 3-(2-(2-bromoethoxy)ethoxy)prop-1-ene (**2.11a**)



2.11a

Monoallyl ether (**2.9a**) (307.5 mg, 2.10 mmol) was solubilized in dry DCM (2.1 mL) under inert atmosphere. CBr₄ (767.4 mg, 2.31 mmol) was added and once fully solubilized PPh₃ (662 mg, 2.52 mmol) was slowly added. The reaction flask was covered with an aluminium foil. After 4 hours at rt, the reaction solvent was fully removed under vacuum. The crude bromide was directly purified by FC on silica (PE : EA 8:2) to give the product as pale-yellow oil (421 mg, 96% yield).

Molecular weight: 209.08 g/mol (C₇H₁₃BrO₂)

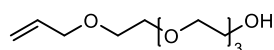
TLC: Rf 0.45 (8:2 PE: EA, KMnO₄ Basic)

¹H NMR (400 MHz, CDCl₃): δ 5.92 (ddt, *J* = 17.2, 10.4, 5.7 Hz, 1H, CHH=CHCH₂-O), 5.29 (dq, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.19 (dq, *J* = 10.4, 1.3 Hz, 1H, CHH^[trans]=CHCH₂-O), 4.04 (dt, *J* = 5.7, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.82 (t, *J* = 6.4 Hz, 2H, O-CH₂CH₂-Br), 3.71 – 3.66 (m, 2H, CH₂), 3.64 – 3.60 (m, 2H, CH₂), 3.48 (t, *J* = 6.4 Hz, 2H, O-CH₂CH₂-Br).

¹³C NMR (101 MHz, CDCl₃): δ 134.8 (CHH=CHCH₂-O), 117.4 (CHH=CHCH₂-O), 72.5 (CHH=CHCH₂-O), 71.4 (O-CH₂CH₂-Br), 70.7 (CH₂), 69.5 (CH₂), 30.4 (O-CH₂CH₂-Br).

GC-MS: Rt= 5.444 min; m/z (Relative abundance %) 39 (28), 40 (4), 41 (100), 42 (7), 43 (27), 44 (5), 45 (12), 55 (7), 56 (5), 57 (12), 71 (13), 73 (23), 85 (6), 101 (3), 107 (51), 109 (49), 151 (5), 153 (5)

Synthesis of 3,6,9,12-tetraoxapentadec-14-en-1-ol (**2.9b**)



2.9b

KOH (864 mg, 15.4 mmol) was solubilized in a DMSO:H₂O mixture (4:1, 5.6 mL). Tetraethylen glycol (6.6 mL, 38.4 mmol) was slowly added. After 15 minutes allyl bromide (1.1 mL, 12.8 mmol) was injected dropwise. After 3 hours at rt, s.s. NH₄Cl was added, the reaction crude was diluted with ethyl acetate and transferred in a separatory funnel. The aqueous phase was extracted 8 times with ethyl acetate. The reunited organic phase was washed with brine and dried (Na₂SO₄), filtered and evaporated to dryness. The crude mono-allylether was purified by FC on silica (EA to EA + 5% MeOH) to give the product as pale-yellow oil (2.309 g, 77% yield).

Molecular weight: 234.29 g/mol (C₁₁H₂₂O₅)

TLC: Rf 0.4 (EA + 0.5% MeOH, KMnO₄ Basic)

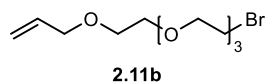
¹H NMR (400 MHz, CDCl₃): δ 5.91 (ddt, *J* = 16.4, 11.0, 5.7 Hz, 1H, CHH=CHCH₂-O), 5.31 – 5.23 (m, 1H, CHH^[cis]=CHCH₂-O), 5.21 – 5.15 (m, 1H, CHH^[trans]=CHCH₂-O), 4.02 (dt, *J* = 5.7, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.75 – 3.69 (m, 2H, CH₂), 3.67 (s, 10H, 5x CH₂), 3.63 – 3.58 (m, 4H, 2x CH₂), 2.80 (bt, *J* = 5.8 Hz, 1H, OH).

¹³C NMR (101 MHz, CDCl₃): δ 134.8 (CHH=**CH**CH₂-O), 117.4 (**CHH**=CHCH₂-O), 72.7 (CH₂), 72.4 (CHH=CH**CH**₂-O), 70.8 (CH₂), 70.74 (CH₂), 70.71 (CH₂), 70.70 (CH₂), 70.4 (CH₂), 69.5 (CH₂), 61.9 (CH₂).

GC-MS: Rt= 8.315 min; m/z (Relative abundance %): 39 (6), 41 (55), 42 (4), 43 (19), 44 (12), 45 (100), 55 (5), 56 (3), 57 (4), 58 (7), 59 (14), 71 (7), 73 (22), 84 (5), 85 (10), 87 (15), 88 (5), 89 (29), 101 (3), 103 (5), 133 (3)

IR: wavenumber (cm⁻¹): 3436, 2868, 1724, 1647, 1454, 1349, 1256, 1091, 996, 929, 884, 811, 731

Synthesis of 1-bromo-3,6,9,12-tetraoxapentadec-14-ene (**2.11b**)



Monoallyl ether (**2.9b**) (1.724 g, 7.36 mmol) was solubilized in dry DCM (7.4 mL) under inert atmosphere. CBr₄ (2.685 g, 8.10 mmol) was added and once fully solubilized PPh₃ (2.317 g, 8.83 mmol) was slowly added. The reaction flask was covered with an aluminium foil. After 4 hours at rt, the reaction solvent was fully removed under vacuum. The crude bromide was directly purified by FC on silica (PE : EA 1:1) to give the product as pale-yellow oil (2.143 g, 98% yield).

Molecular weight: 297.19 g/mol (C₁₁H₂₁BrO₄)

TLC: R_f 0.4 (1:1 PE: EA, KMnO₄ Basic)

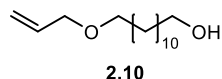
¹H NMR (400 MHz, CDCl₃): δ 5.92 (ddt, *J* = 16.1, 11.2, 5.7 Hz, 1H, CHH=**CH**CH₂-O), 5.28 (dd, *J* = 17.2, 1.5 Hz, 1H, **CHH**^[cis]=CHCH₂-O), 5.19 (dt, *J* = 10.4, 1.2 Hz, 1H, **CHH**^[trans]=CHCH₂-O), 4.03 (d, *J* = 5.7 Hz, 2H, CHH=CH**CH**₂-O), 3.82 (t, *J* = 6.3 Hz, 2H, O-**CH**₂CH₂-Br), 3.68 (s, 10H, 5x CH₂), 3.65 – 3.57 (m, 2H, CH₂), 3.48 (t, *J* = 6.3 Hz, 2H, O-CH₂**CH**₂-Br).

¹³C NMR (101 MHz, CDCl₃): δ 134.8 (CHH=**CH**CH₂-O), 117.3 (**CHH**=CHCH₂-O), 72.4 (CHH=CH**CH**₂-O), 71.3 (O-**CH**₂CH₂-Br), 70.8 (CH₂), 70.74 (2x CH₂), 70.70 (CH₂), 70.6 (CH₂), 69.5 (CH₂), 30.5 (O-CH₂**CH**₂-Br).

GC-MS: Rt= 8.855 min; m/z (Relative abundance %): 39 (12), 41 (100), 42 (7), 43 (37), 44 (8), 45 (36), 55 (11), 56 (7), 57 (7), 58 (6), 59 (19), 71 (18), 72 (3), 73 (54), 83 (4), 84 (11), 85 (19), 86 (3), 87 (21), 88 (3), 89 (9), 101 (5), 103 (8), 105 (3), 107 (64), 109 (62), 115 (6), 117 (5), 133 (5), 150 (5), 151 (15), 152 (6), 153 (14)

IR: wavenumber (cm⁻¹): 2866, 1647, 1453, 1423, 1350, 1278, 1249, 1094, 1040, 994, 927, 880, 664

Synthesis of 12-(allyloxy)dodecan-1-ol (**2.10**)



NaH (60% in mineral oil, 238 mg, 5.94 mmol) was suspended in dry THF (16.5 mL) under Ar atmosphere. Later, 1,12-dodecanediol (3.000 g, 14.9 mmol) was slowly added in portions. After 1 hour at room temperature allyl bromide (0.43 mL, 4.95 mmol) was injected very slowly and lastly tetrabutylammonium bromide was added (80 mg, 0.247 mmol). The reaction flask was sealed with a screw cap and then heated to 80°C. After 18 hours, the reaction was cooled to room temperature, s.s. NH₄Cl was added and the organic solvent was partially removed. The mixture was diluted with AcOEt and transferred in a separatory funnel. The aqueous phase was extracted 3 times with ethyl acetate, the reunited organic phases were washed with brine, dried (Na₂SO₄), filtered and evaporated to dryness. The crude mono-allyl ether was purified by FC on silica (PE:EA 8:2) to give the product as pale-yellow oil (1.200 g, 82% yield).

Molecular weight: 242.40 g/mol (C₁₅H₃₀O₂)

TLC: R_f 0.3 (8:2 PE:EA, CAM)

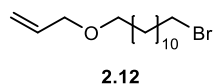
¹H NMR (400 MHz, CDCl₃): δ 5.92 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H, CHH=CHCH₂-O), 5.27 (dq, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.17 (dq, *J* = 10.4, 1.3 Hz, 1H, CHH^[trans]=CHCH₂-O), 3.97 (dt, *J* = 5.6, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.64 (bt, *J* = 6.5 Hz, 2H, CH₂CH₂-OH), 3.42 (t, *J* = 6.7 Hz, 2H, O-CH₂CH₂), 1.63 – 1.52 (m, 4H, O-CH₂CH₂ e CH₂CH₂-OH), 1.38 – 1.24 (m, 17H, 8x CH₂ and OH).

¹³C NMR (101 MHz, CDCl₃): δ 135.2 (CHH=CHCH₂-O), 116.9 (CHH=CHCH₂-O), 71.9 (CHH=CHCH₂-O), 70.7 (O-CH₂CH₂), 63.2 (CH₂CH₂-O), 32.9 (O-CH₂CH₂), 29.9 (CH₂CH₂-O), 29.7 (4x CH₂), 29.6 (CH₂), 29.6 (CH₂), 26.3 (CH₂), 25.9 (CH₂).

GC-MS: Rt= 9,260 min; m/z (Relative abundance %): 39 (12), 40 (2), 41 (96), 42 (13), 43 (40), 44 (3), 53 (3), 54 (9), 55 (100), 56 (13), 57 (31), 58 (46), 59 (4), 66 (3), 67 (29), 68 (20), 69 (77), 70 (8), 71 (21), 79 (3), 80 (2), 81 (28), 82 (34), 83 (48), 84 (5), 85 (8), 95 (27), 96 (18), 97 (32), 98 (4), 99 (2), 109 (16), 110 (8), 111 (10), 121 (2), 123 (5), 124 (3), 213 (3)

IR: wavenumber (cm⁻¹): 3360, 2923, 2853, 1647, 1464, 1347, 1099, 1057, 995, 920, 799, 721

Synthesis of 1-(allyloxy)-12-bromododecane (**2.12**)



Monoallyl ether (**2.10**) (1.812 g, 7.48 mmol) was solubilized in dry DCM (7.5 mL) under inert atmosphere. CBr₄ (3.471 g, 10.5 mmol) was added and once fully solubilized PPh₃ (2.941 g, 11.2 mmol) was slowly added. The reaction flask was covered with an aluminium foil. After 4 hours at rt, the reaction solvent was fully removed under vacuum. The crude bromide was directly purified by FC on silica (PE:Et₂O 30:1) to give the product as pale-yellow oil (2.129 g, 93% yield).

Molecular weight: 305.30 g/mol (C₁₅H₂₉BrO)

TLC: R_f 0.3 (30:1 PE:Et₂O CAM)

¹H NMR (400 MHz, CDCl₃): δ 5.91 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H, CHH=CHCH₂-O), 5.26 (dq, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.16 (dq, *J* = 10.4, 1.3 Hz, 1H, CHH^[trans]=CHCH₂-O), 3.95 (dt, *J* = 5.6, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.44 – 3.36 (m, 4H, O-CH₂CH₂ e CH₂CH₂CH₂-Br), 1.84 (p, *J* = 6.9 Hz, 2H, CH₂CH₂CH₂-Br), 1.56 (q, *J* = 7.6 Hz, 2H, O-CH₂CH₂), 1.45 – 1.36 (m, 2H, CH₂CH₂CH₂-Br), 1.35 – 1.21 (m, 14H, 7 CH₂).

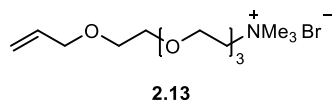
¹³C NMR (101 MHz, CDCl₃): δ 135.2 (CHH=CHCH₂-O), 116.9 (CHH=CHCH₂-O), 71.9 (CHH=CHCH₂-O), 70.7 (O-CH₂CH₂), 34.3 (CH₂CH₂CH₂-Br), 33.0 (CH₂CH₂CH₂-Br), 29.9 (O-CH₂CH₂), 29.70 (CH₂), 29.67 (CH₂), 29.65 (CH₂), 29.63 (CH₂), 29.56 (CH₂), 28.9 (CH₂), 28.3 (CH₂CH₂CH₂-Br), 26.3 (CH₂).

GC-MS: Rt= 9,695 min; m/z (Relative abundance %): 39 (13), 41 (100), 42 (12), 43 (57), 53 (3), 54 (5), 55 (70), 56 (12), 57 (60), 58 (83), 59 (5), 67 (12), 68 (9), 69 (63), 70 (9), 71 (41), 81 (10), 82 (11), 83 (41), 84 (5), 85 (17), 95 (9), 96 (5), 97 (39), 98 (4), 99 (4), 109 (9), 111 (16), 123 (5), 125 (5), 135 (5), 137 (6), 148 (8), 149 (4), 150 (7), 151 (3), 162 (4), 164 (4), 305 (1) [M]⁺

IR: wavenumber (cm⁻¹): 2922, 2853, 1460, 1377, 1261, 1105, 994, 920, 722, 651

2.8.6 Preparation of ligand hydrophilic cationic **2.3**

Synthesis of N,N,N-trimethyl-3,6,9,12-tetraoxapentadec-14-en-1-aminium bromide (**2.13**)



Bromide (**2.11b**) (2.030 g, 6.831 mmol) was solubilized in CH₃OH (68.3 mL). Then, trimethyl amine (28% in water, 16 mL, 68.3 mmol) was added. The reaction flask was sealed with a screw cap and then heated to 40°C. After 3 days at 40°C, the reaction was cooled to room temperature. Trimethylamine and methanol were eliminated using a rotavapor. Water was removed by freeze drying. The crude quaternary ammonium salt (2.434 g, yellowish very viscous oil) was sufficiently pure to be used in the next step without further purification.

Molecular weight: 356.30 g/mol (C₁₄H₃₀BrNO₄)

TLC: R_f 0.04 (DCM:MeOH 8:2, CAM, KMnO₄ Basic)

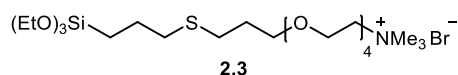
¹H NMR (400 MHz, CDCl₃): δ 5.88 (ddt, *J* = 17.2, 10.4, 5.6 Hz, 1H, CHH=CHCH₂-O), 5.26 (dq, *J* = 17.2, 1.5 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.17 (dd, *J* = 10.4, 1.6 Hz, 1H, CHH^[trans]=CHCH₂-O), 3.99 (dt, *J* = 5.7, 1.3 Hz, 2H, CHH=CHCH₂-O), 3.95 (s, 4H, 2x CH₂), 3.68 – 3.63 (m, 2H, CH₂), 3.62 – 3.55 (m, 10H, 5x CH₂), 3.45 (s, 9H, 3x CH₃).

¹³C NMR (101 MHz, CDCl₃): δ 134.7 (CHH=CHCH₂-O), 117.5 (CHH=CHCH₂-O), 72.3 (CHH=CHCH₂-O), 70.7 (2x CH₂), 70.6 (CH₂), 70.4 (CH₂), 70.2 (CH₂), 69.6 (CH₂), 65.7 (CH₂), 65.3 (CH₂), 54.8 (3x CH₃).

HPLC-ESI-MS: Rt= 8.07 min, exact mass expected: 276 m/z (C₁₄H₃₀NO₄), observed 276 m/z (M)⁺

IR: wavenumber (cm⁻¹): 3420, 3006, 2869, 1645, 1478, 1350, 1248, 1100, 997, 955, 926, 876, 841, 746, 660

Synthesis of 4,4-diethoxy-N,N,N-trimethyl-3,12,15,18,21-pentaoxa-8-thia-4-silatricosan-23-aminium bromide (**2.3**)



In a vial for photochemical reactions allyl ether (**2.13**) (710 mg, 1.99 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 481 μL, 1.99 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmap or Irgacure 651, 20 mg, 0.0797 mmol) were added and then solubilized and mixed in dry chloroform (1.3 mL) under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (1.183 g, yellowish viscous oil) was obtained. The crude ligand was sufficiently pure to be used in the next step without further purification.

Molecular weight: 594.72 g/mol (C₂₃H₅₂BrNO₇SSi)

¹H NMR (400 MHz, CDCl₃): δ 4.01 – 3.96 (m, 4H, 2x CH₂), 3.85 – 3.77 (m, 2H, CH₃CH₂OSi), 3.76 – 3.66 (m, 6H, 2x CH₃CH₂OSi and CH₂), 3.65 – 3.53 (m, 12H, 5x CH₂ and S-CH₂CH₂CH₂-O), 3.53 – 3.42 (m, 9H, 3x -NCH₃), 2.56 (t, *J* = 7.2 Hz, 2H, S-CH₂CH₂CH₂-O), 2.52 (t, *J* = 7.3 Hz, 2H, Si-CH₂CH₂CH₂-S), 1.84 (p, *J* = 6.7 Hz, 2H, S-CH₂CH₂CH₂-O), 1.69 (p, *J* = 8.5 Hz, 2H, Si-CH₂CH₂CH₂-S), 1.28 – 1.18 (m, 9H, 3 CH₃CH₂OSi), 0.76 – 0.70 (m, 2H, Si-CH₂CH₂CH₂-S).

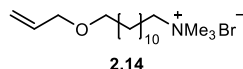
¹³C NMR (101 MHz, CD₃OD): δ 73.7 (S-CH₂CH₂CH₂-O), 71.5 (2x CH₂), 71.3 (2x CH₂), 66.9 (CH₂), 65.9 (2x CH₂), 62.2 (CH₂), 58.3 (3x CH₃CH₂OSi), 54.8 (N-(CH₃)₃), 36.0 (Si-CH₂CH₂CH₂-S), 31.0 (S-

CH₂CH₂CH₂-O), 29.4 (S-CH₂CH₂CH₂-O), 24.4 (Si-CH₂CH₂CH₂-S), 18.4 (3x CH₃CH₂OSi), 11.6 (Si-CH₂CH₂CH₂-S).

IR: wavenumber (cm⁻¹): 3369, 2919, 1719, 1477, 1350, 1280, 1247, 1097, 1068, 955, 876, 749, 715, 690, 664

2.8.7 Preparation of ligand hydrophobic cationic **2.4**

Synthesis of 12-(allyloxy)-N,N,N-trimethyldodecan-1-aminium bromide, (**2.14**)



Bromide (**2.12**) (2.113 g, 6.921 mmol) was solubilized in CH₃OH (69 mL). Then, trimethyl amine (28% in water, 16 mL, 69.2 mmol) was added. The reaction flask was sealed with a screw cap and then heated to 40°C. After 3 days at 40°C, the reaction was cooled to room temperature. Trimethylamine and methanol were eliminated using a rotavapor. Water was removed by freeze drying. The crude quaternary ammonium salt (2.522 g, uncoloured amorphous solid) was sufficiently pure to be used in the next step without further purification.

Molecular weight: 364.41 g/mol (C₁₈H₃₈BrNO)

TLC: R_f 0.04 (DCM:MeOH 8:2, CAM, KMnO₄ Basic)

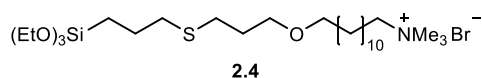
¹H NMR (400 MHz, CDCl₃): δ 5.92 (ddt, *J* = 17.1, 10.4, 5.5 Hz, 1H, CHH=CHCH₂-O), 5.27 (dq, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.17 (dq, *J* = 10.4, 1.5 Hz, 1H, CHH^[trans]=CHCH₂-O), 3.97 (dt, *J* = 5.6, 1.3 Hz, 2H, CHH=CHCH₂-O), 3.62 – 3.52 (m, 2H, CH₂CH₂-N), 3.48 (s, 9H, 3x CH₃), 3.42 (t, *J* = 6.7 Hz, 2H, O-CH₂CH₂), 1.82 – 1.68 (m, 2H, CH₂CH₂-N), 1.58 (p, *J* = 6.7 Hz, 2H, O-CH₂CH₂), 1.43 – 1.22 (m, 16H, 8x CH₂).

¹³C NMR (101 MHz, CDCl₃): δ 135.2 (CHH=CHCH₂-O), 116.9 (CHH=CHCH₂-O), 71.9 (CHH=CHCH₂-O), 70.6 (O-CH₂CH₂), 67.2 (CH₂CH₂-N), 53.5 (3x -NCH₃), 29.9 (O-CH₂CH₂), 29.6 (CH₂), 29.59 (2x CH₂), 29.53 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 26.3 (CH₂), 26.3 (CH₂), 23.3 (CH₂CH₂-N).

HPLC-ESI-MS: Rt= 17.45 min, exact mass expected: 284 m/z (C₁₈H₃₈NO), observed 284 m/z (M)⁺

IR: wavenumber (cm⁻¹): 3019, 2919, 2850, 2790, 1648, 1482, 1435, 1404, 1364, 1302, 1243, 1140, 1114, 1083, 1030, 987, 962, 936, 913, 770, 721, 616

Synthesis of 4,4-diethoxy-N,N,N-trimethyl-3,12-dioxa-8-thia-4-silatetracosan-24-aminium bromide (**2.4**)



In a vial for photochemical reactions allyl ether (**2.14**) (91 mg, 0.249 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 60 μL, 0.249 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpap or Irgacure 651, 3 mg, 0.00995 mmol) were added and then solubilized and mixed in dry chloroform (0.17 mL) under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (150 mg, yellowish viscous oil) was obtained. The crude ligand was sufficiently pure to be used in the next step without further purification.

Molecular weight: 602.83 g/mol (C₂₇H₆₀BrNO₄SSi)

¹H NMR (400 MHz, CDCl₃): δ 3.87 – 3.76 (m, 4H, 2x CH₃CH₂OSi), 3.72 (q, *J* = 7.0 Hz, 2H, CH₃CH₂OSi), 3.60 – 3.52 (m, 2H, -CH₂CH₂-N), 3.47 (d, *J* = 5.0 Hz, 11H, S-CH₂CH₂CH₂-O and -N(CH₃)₃), 3.39 (t, *J* =

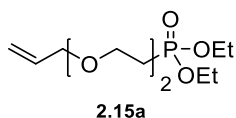
6.7 Hz, 2H, O-CH₂CH₂-), 2.57 (t, *J* = 7.3 Hz, 2H, S-CH₂CH₂CH₂-O), 2.53 (t, *J* = 7.3 Hz, 2H, Si-CH₂CH₂CH₂-S), 1.83 (p, *J* = 6.4 Hz, 2H, S-CH₂CH₂CH₂-O), 1.78 – 1.62 (m, 4H, Si-CH₂CH₂CH₂-S and CH₂), 1.59 – 1.50 (m, 2H, CH₂), 1.43 – 1.12 (m, 25H, 8x CH₂ and (CH₃CH₂O)₃Si), 0.77 – 0.69 (m, 2H, Si-CH₂CH₂CH₂-S).

¹³C NMR (101 MHz, DMSO-*D*₆): δ 70.0 (O-CH₂CH₂-), 68.4 (S-CH₂CH₂CH₂-O), 65.2 (-CH₂CH₂-N), 56.0 (3x CH₃CH₂OSi), 52.1 (-N(CH₃)₃), 33.9 (Si-CH₂CH₂CH₂-S), 29.5 (CH₂), 29.2 (O-CH₂CH₂-), 29.01 (2x CH₂), 28.94 (2x CH₂), 28.86 (CH₂), 28.83 (CH₂), 28.55 (CH₂), 28.51 (CH₂), 27.8 (S-CH₂CH₂CH₂-O), 25.8 (-CH₂CH₂-N), 22.0 (CH₂), 18.5 (3x CH₃CH₂OSi), 10.7 (Si-CH₂CH₂CH₂-S).

IR: wavenumber (cm⁻¹): 3343, 2924, 2852, 1718, 1481, 1277, 1108, 1056, 964, 911, 752, 713, 647

2.8.8 Studies for the preparation of anionic ligands

Synthesis of diethyl (2-(2-(allyloxy)ethoxy)ethyl)phosphonate (**2.15a**)



Triethyl phosphite was reacted with metallic Na in inert atmosphere for 4 hours in a reaction flask to remove traces of water. When bubbling stops, triethyl phosphite is distilled under reduced pressure to remove any impurity and stored under Ar.

Bromide (**2.11a**) (421 mg, 2.01 mmol) was solubilized in the freshly distilled triethyl phosphite (1.3 mL, 8.05 mmol) under inert atmosphere. The reaction flask was heated to reflux (roughly 160°C). After 18 hours at reflux, the reaction solvent was fully removed under vacuum. The crude diethyl phosphonate was directly purified by FC on silica (EA + 1% MeOH) and diethyl ethyl phosphonate was fully removed using high vacuum with a gentle heating for 12 h. The product was obtained pure (442 mg, 82% yield) as a pale yellow oil.

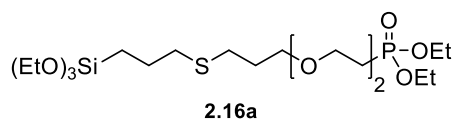
Molecular weight: 266.27 g/mol (C₁₁H₂₃O₅P)

TLC: R_f 0.42 (EA + 1% MeOH, CAM, KMnO₄ Basic)

¹H NMR (400 MHz, CDCl₃): δ 5.91 (ddt, *J* = 17.2, 10.4, 5.7 Hz, 1H, CHH=CHCH₂-O), 5.28 (dt, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.19 (dq, *J* = 10.4, 1.2 Hz, 1H, CHH^[trans]=CHCH₂-O), 4.16 – 4.05 (m, 2H, P-(O-CH₂CH₃)₂), 4.02 (dt, *J* = 5.7, 1.3 Hz, 2H, CHH=CHCH₂-O), 3.74 (dt, *J* = 10.7, 7.6 Hz, 2H, O-CH₂CH₂-P), 3.64 – 3.57 (m, 4H, 2x CH₂), 2.14 (dt, *J* = 19.1, 7.6 Hz, 2H, O-CH₂CH₂-P), 1.32 (t, *J* = 7.1 Hz, 6H, P-(O-CH₂CH₃)₂).

¹³C NMR (101 MHz, CDCl₃): δ 134.8 (CHH=CHCH₂-O), 117.4 (CHH=CHCH₂-O), 72.4 (CHH=CHCH₂-O), 70.4 (CH₂), 69.4 (CH₂), 65.3 (O-CH₂CH₂-P), 61.8 (P-O-CH₂CH₃), 61.7 (P-O-CH₂CH₃), 27.8-26.4 (d, *J* = 139.1 Hz, O-CH₂CH₂-P), 16.6 (P-O-CH₂CH₃), 16.5 (P-O-CH₂CH₃).

Synthesis of diethyl (4,4-diethoxy-3,12,15-trioxa-8-thia-4-silaheptadecan-17-yl)phosphonate (**2.16a**)



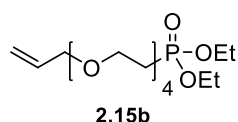
In a vial for photochemical reactions allyl ether (**2.15a**) (189 mg, 0.711 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 172 μ L, 0.711 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 651, 9 mg, 0.0355 mmol) were added and then mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (359 mg, yellowish viscous oil) was obtained.

Molecular weight: 504.69 g/mol ($C_{20}H_{45}O_8PSSi$)

^1H NMR (400 MHz, $CDCl_3$): δ 4.18 – 4.03 (m, 4H, P-(O- CH_2CH_3)₂), 3.82 (q, J = 7.0 Hz, 2H, CH_3CH_2 -O-Si), 3.77 – 3.68 (m, 6H, (CH_3CH_2 -O)₂-Si e O- CH_2CH_2 -P), 3.64 – 3.52 (m, 6H, 2x CH_2 and S- $CH_2CH_2CH_2$ -O), 2.57 (t, J = 7.2 Hz, 2H, S- $CH_2CH_2CH_2$ -O), 2.53 (t, J = 7.4 Hz, 2H, Si- $CH_2CH_2CH_2$ -S), 2.14 (dt, J = 18.7, 7.7 Hz, 2H, O- CH_2CH_2 -P), 1.86 (p, J = 6.6 Hz, 2H, S- $CH_2CH_2CH_2$ -O), 1.70 (p, J = 7.6 Hz, 2H, Si- $CH_2CH_2CH_2$ -S), 1.32 (t, J = 7.1 Hz, 6H, P-(O- CH_2CH_3)₂), 1.27 – 1.19 (m, 9H, (CH_3CH_2 -O)₃-Si), 0.77 – 0.69 (m, 2H, Si- $CH_2CH_2CH_2$ -S).

^{13}C NMR (101 MHz, $CDCl_3$): 70.3 (CH_2), 70.2 (CH_2), 70.0 (S- $CH_2CH_2CH_2$ -O), 65.3 (O- CH_2CH_2 -P), 61.8 (P-O- CH_2CH_3), 61.7 (P-O- CH_2CH_3), 58.6 (CH_3CH_2 -O-Si), 58.5 (CH_3CH_2 -O-Si), 58.4 (CH_3CH_2 -O-Si), 35.3 (Si- $CH_2CH_2CH_2$ -S), 29.7 (S- $CH_2CH_2CH_2$ -O), 28.7 (S- $CH_2CH_2CH_2$ -O), 27.08 (d, J = 139.0 Hz, O- CH_2CH_2 -P), 23.3 (Si- $CH_2CH_2CH_2$ -S), 18.6 ((CH_3CH_2 -O)₂-Si), 18.4 (CH_3CH_2 -O-Si), 16.6 (P-O- CH_2CH_3), 16.5 (P-O- CH_2CH_3), 10.0 (Si- $CH_2CH_2CH_2$ -S).

Synthesis of diethyl (3,6,9,12-tetraoxapentadec-14-en-1-yl)phosphonate (**2.15b**)



Triethyl phosphite was reacted with metallic Na in inert atmosphere for 4 hours in a reaction flask to remove traces of water. When bubbling stops, triethyl phosphite is distilled under reduced pressure to remove any impurity and stored under Ar.

Bromide (**2.11b**) (609 mg, 2.05 mmol) was solubilized in the freshly distilled triethyl phosphite (1.4 mL, 8.20 mmol) under inert atmosphere. The reaction flask was heated to reflux (roughly 160°C). After 18 hours at reflux, the reaction solvent was fully removed under vacuum. The crude diethyl phosphonate was directly purified by FC on silica (DCM+2% MeOH) and diethyl ethyl phosphonate was fully removed using high vacuum with a gentle heating for 12 h. The product was obtained pure (616 mg, 85% yield) as a pale-yellow oil.

Molecular weight: 354.38 g/mol ($C_{15}H_{31}O_7P$)

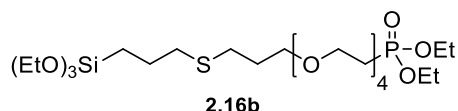
TLC: Rf 0.32 (DCM + 2%MeOH, CAM, $KMnO_4$ Basic)

^1H NMR (400 MHz, $CDCl_3$): δ 5.92 (ddt, J = 16.1, 11.2, 5.8 Hz, 1H, $CHH=CHCH_2$ -O), 5.28 (dd, J = 17.2, 1.6 Hz, 1H, $CHH^{[cis]}=CHCH_2$ -O), 5.18 (dd, J = 10.4, 1.1 Hz, 1H, $CHH^{[trans]}=CHCH_2$ -O), 4.16 – 4.04 (m, 4H, P-(O- CH_2CH_3)₂), 4.03 (dd, J = 5.7, 1.4 Hz, 2H, $CHH^{[cis]}=CHCH_2$ -O), 3.72 (dt, J = 10.6, 7.7 Hz, 2H,

O-CH₂CH₂-P), 3.68 – 3.63 (m, 8H, 4x CH₂), 3.63 – 3.58 (m, 4H, 2x CH₂), 2.13 (dt, *J* = 18.8, 7.9 Hz, 2H, O-CH₂CH₂-P), 1.32 (t, *J* = 7.1 Hz, 6H, P-(O-CH₂CH₃)₂).

¹³C NMR (101 MHz, CDCl₃): δ 134.9 (CHH=CHCH₂-O), 117.3 (CHH=CHCH₂-O), 72.4 (CHH=CHCH₂), 70.8 (3x CH₂), 70.6 (CH₂), 70.3 (CH₂), 69.5 (CH₂), 65.3 (O-CH₂CH₂-P), 61.8 (P-O-CH₂CH₃), 61.7 (P-O-CH₂CH₃), 27.09 (d, *J* = 139.1 Hz, O-CH₂CH₂-P), 16.6 (P-O-CH₂CH₃), 16.5 (P-O-CH₂CH₃).

Synthesis of diethyl (4,4-diethoxy-3,12,15,18,21-pentaoxa-8-thia-4-silatricosan-23-yl)phosphonate (**2.16b**):



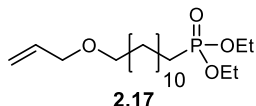
In a vial for photochemical reactions allyl ether (**2.15b**) (384 mg, 1.08 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 262 μL, 1.08 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 11 mg, 0.0433mmol) were added and then mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap, and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (642 mg, yellowish viscous oil) was obtained. The crude ligand was sufficiently pure to be used in the next step without further purification.

Molecular weight: 592.80 g/mol (C₂₄H₅₃O₁₀PSSi)

¹H NMR (400 MHz, CDCl₃): δ 4.15 – 4.05 (m, 4H, P-(O-CH₂CH₃)₂), 3.82 (q, *J* = 7.0 Hz, 4H, (CH₃CH₂-O)₂-Si), 3.77 – 3.68 (m, 4H, CH₃CH₂-O-Si e O-CH₂CH₂-P), 3.68 – 3.57 (m, 12H, 6x CH₂), 3.54 (t, *J* = 6.3 Hz, 2H, S-CH₂CH₂CH₂-O), 2.57 (t, *J* = 7.3 Hz, 2H, S-CH₂CH₂CH₂-O), 2.53 (t, *J* = 7.3 Hz, 2H, Si-CH₂CH₂CH₂-S), 2.13 (dt, *J* = 18.8, 7.8 Hz, 2H, O-CH₂CH₂-P), 1.85 (p, *J* = 6.7 Hz, 2H, S-CH₂CH₂CH₂-O), 1.70 (p, *J* = 7.7 Hz, 2H, Si-CH₂CH₂CH₂-S), 1.32 (t, *J* = 7.1 Hz, 6H, P-(O-CH₂CH₃)₂), 1.28 – 1.20 (m, 9H, (CH₃CH₂-O)₃-Si), 0.78 – 0.70 (m, 2H, Si-CH₂CH₂CH₂-S).

¹³C NMR (101 MHz, CDCl₃): 70.7 (3 CH₂), 70.6 (CH₂), 70.3 (2 CH₂), 70.0 (S-CH₂CH₂CH₂-O), 65.2 (O-CH₂CH₂-P), 61.8 (P-O-CH₂CH₃), 61.7 (P-O-CH₂CH₃), 58.5 ((CH₃CH₂-O)₃-Si), 35.2 (Si-CH₂CH₂CH₂-S), 29.8 (S-CH₂CH₂CH₂-O), 28.7 (S-CH₂CH₂CH₂-O), 27.07 (d, *J* = 139.0 Hz, O-CH₂CH₂-P), 23.3 (Si-CH₂CH₂CH₂-S), 18.6 (CH₃CH₂-O-Si), 18.4 ((CH₃CH₂-O)₂-Si), 16.6 (P-O-CH₂CH₃), 16.5 (P-O-CH₂CH₃), 10.0 (Si-CH₂CH₂CH₂-S).

Synthesis of diethyl (12-(allyloxy)dodecyl)phosphonate (**2.17**)



NaH (60% w/w in oil, 82 mg, 2.05 mmol) was suspended in dry DMF (0.5 mL) under inert atmosphere, Ar. The reaction flask was cooled to 0°C, and freshly distilled diethyl phosphonate (0.237 mL, 1.84 mmol) was slowly added. When the intense bubbling stops the reaction flask is warmed to room temperature. After 1 h at room temperature, the reaction flask is cooled to 0°C and bromide (**2.12**) (313 mg, 1.02 mmol) solubilized in DMF (0.5 mL) is slowly added to the white suspension. After 15 minutes, the reaction flask is warmed to room temperature. After 16 h at room temperature s.s. NH₄Cl is slowly added to stop the reaction. The crude is diluted with EA and transferred in a separatory funnel. The aqueous layer is extracted three times using EA, the reunited organic layer are washed with brine, six times with 5% w/w LiCl aqueous solution, dried with

Na₂SO₄ and the solvent removed in negative pressure. The reaction crude was purified by FC on silica (PE:EA 2:8) delivering the product (259 mg, 70% yield) as a pale yellow oil.

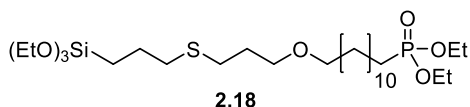
Molecular weight: 362.49 g/mol (C₁₉H₃₉O₄P)

TLC: R_f 0.28 (2:8 PE:EA, CAM, KMnO₄ Basic)

¹H NMR (400 MHz, CDCl₃): δ 5.92 (ddt, *J* = 17.1, 9.9, 5.5 Hz, 1H, CHH=CHCH₂-O), 5.27 (dq, *J* = 17.2, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.17 (dq, *J* = 10.4, 1.3 Hz, 1H, CHH^[trans]=CHCH₂-O), 4.17 – 4.02 (m, 4H, P-(O-CH₂CH₃)₂), 3.97 (dt, *J* = 5.6, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.42 (t, *J* = 6.7 Hz, 2H, O-CH₂CH₂), 1.80 – 1.66 (m, 2H, CH₂CH₂-P), 1.65 – 1.53 (m, 4H, CH₂CH₂-P e O-CH₂CH₂), 1.41 – 1.23 (m, 22H, 8x CH₂ e P-(O-CH₂CH₃)₂).

¹³C NMR (101 MHz, CDCl₃): δ 135.2 (CHH=CHCH₂-O), 116.9 (CHH=CHCH₂-O), 71.9 (CHH=CHCH₂-O), 70.7 (O-CH₂CH₂), 61.5 (P-O-CH₂CH₃), 61.5 (P-O-CH₂CH₃), 30.76 (d, *J* = 17.1 Hz CH₂CH₂CH₂-P), 29.9 (O-CH₂CH₂), 29.7 (3x CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.2 (CH₂), 25.80 (d, *J* = 140.2 Hz, CH₂CH₂CH₂-P), 26.3 (CH₂), 22.52 (d, *J* = 5.2 Hz, CH₂CH₂CH₂-P), 16.6 (P-O-CH₂CH₃), 16.6 (P-O-CH₂CH₃).

Synthesis of diethyl (4,4-diethoxy-3,12-dioxa-8-thia-4-silatetracosan-24-yl)phosphonate (**2.18**)



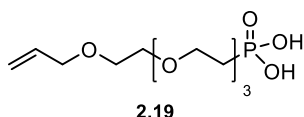
In a vial for photochemical reactions allyl ether (**2.17**) (326 mg, 0.900 mmol), 3-mercaptopropyltriethoxysilane (MPTES, 217 μL, 0.900 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 651, 9 mg, 0.0360 mmol) were added and then mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap, and the vial was crimped. The vial was placed in the photochemical plate and stirred for 24 h shining 365 nm light on it. After 24 h the ligand (541 mg, yellowish viscous oil) was obtained. The crude ligand was sufficiently pure to be used in the next step without further purification.

Molecular weight: 600.91 g/mol (C₂₈H₆₁O₇PSSi)

¹H NMR (400 MHz, CDCl₃): 4.17 – 4.01 (m, 4H, P-(O-CH₂CH₃)₂), 3.82 (q, *J* = 7.0 Hz, 4H, (CH₃CH₂-O)₂-Si), 3.72 (q, *J* = 7.0 Hz, 2H, CH₃CH₂-O-Si), 3.48 (t, *J* = 6.3 Hz, 2H, S-CH₂CH₂CH₂-O), 3.40 (t, *J* = 6.7 Hz, 2H, OCH₂CH₂), 2.58 (t, *J* = 7.3 Hz, 2H, S-CH₂CH₂CH₂-O), 2.54 (t, *J* = 7.3 Hz, 2H, Si-CH₂CH₂CH₂-S), 1.84 (p, *J* = 6.5 Hz, 2H, S-CH₂CH₂CH₂-O), 1.78 – 1.65 (m, 4H, CH₂CH₂-P e Si-CH₂CH₂CH₂-S), 1.65 – 1.50 (m, 4H, CH₂CH₂-P e O-CH₂CH₂), 1.41 – 1.18 (m, 31H, 8x CH₂ e P-(O-CH₂CH₃)₂ and (CH₃CH₂-O)₃-Si), 0.77 – 0.71 (m, 2H, Si-CH₂CH₂CH₂-S).

¹³C NMR (101 MHz, CDCl₃): δ 71.2 (O-CH₂CH₂), 69.4 (S-CH₂CH₂CH₂-O), 61.5 (P-O-CH₂CH₃), 61.5 (P-O-CH₂CH₃), 58.6 (CH₃CH₂-O-Si), 58.5 ((CH₃CH₂-O)₂-Si), 35.3 (Si-CH₂CH₂CH₂-S), 30.77 (d, *J* = 17.2 Hz, CH₂CH₂CH₂-P), 30.0 (S-CH₂CH₂CH₂-O), 29.9 (O-CH₂CH₂), 29.7 (2x CH₂), 29.6 (CH₂), 29.5 (CH₂), 29.2 (CH₂), 28.8 (CH₂), 26.3 (CH₂), 25.97 (d, *J* = 139.9 Hz, CH₂CH₂CH₂-P), 23.3 (Si-CH₂CH₂CH₂-S), 22.53 (d, *J* = 5.1 Hz, CH₂CH₂CH₂-P), 18.6 (CH₃CH₂-O-Si), 18.4 ((CH₃CH₂-O)₂-Si), 16.6 (P-O-CH₂CH₃), 16.6 (P-O-CH₂CH₃), 10.0 (Si-CH₂CH₂CH₂-S).

Synthesis of (3,6,9,12-tetraoxapentadec-14-en-1-yl)phosphonic acid (**2.19**):



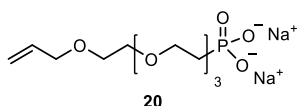
In a reaction flask diethyl phosphonate (**2.15b**) (51 mg, 0.145 mmol) is solubilized in dry DCM (0.1 mL) under inert atmosphere, Ar. Later, TMS-Br (77 μ L, 0.580 mmol) is slowly added to the reaction. After the addition, the crude turns pale orange. After 5 h at room temperature, the solvent is completely removed in negative pressure and methanol is added (1 mL). The reaction crude is heated to 40°C for 5 hours, cooled to room temperature and the solvent is removed in high vacuum. The product (45 mg) is recovered as a pale orange oil. The crude is used directly in the next transformation without purifications.

Molecular weight: 298.27 g/mol (C₁₁H₂₃O₇P)

TLC: R_f 0.45 (7:3:3 *n*-butanol:H₂O:AcOH, CAM, KMnO₄ Basic)

¹H NMR (400 MHz, CD₃OD): δ 5.92 (ddt, J = 17.3, 10.5, 5.5 Hz, 1H, CHH=CHCH₂-O), 5.28 (dq, J = 17.3, 1.7 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.16 (dq, J = 10.4, 1.3 Hz, 1H, CHH^[trans]=CHCH₂-O), 4.02 (dt, J = 5.6, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.74 (dt, J = 11.1, 7.6 Hz, 2H, O-CH₂CH₂-P), 3.68 – 3.57 (m, 12H, 6x CH₂), 2.07 (dt, J = 18.7, 7.6 Hz, 2H, O-CH₂CH₂-P)

Synthesis of sodium (3,6,9,12-tetraoxapentadec-14-en-1-yl)phosphonate (**2.20**)



In a reaction flask the phosphonic acid (**2.19**) (43 mg, 0.145 mmols) was reacted with 0.1M NaOH aqueous solution (3 mL, 0.290 mmol) for 20 minutes under constant stirring. The solution containing the phosphonate di-sodium salt is purified through inverse phase chromatography using water (A) and methanol (B) as solvents (from 100% A to 100% B). The organic solvent was distilled in negative pressure and water was removed through freeze drying. The di-sodium salt (**2.20**), a colourless amorphous solid, was obtained as a pure compound (41 mg, quantitative yield in two steps).

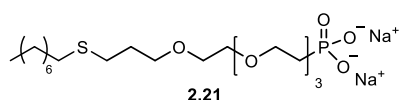
Molecular weight: 342.24 g/mol (C₁₁H₂₁Na₂O₇P)

TLC: R_f 0.45 (7:3:3 *n*-butanol:H₂O:AcOH, CAM, KMnO₄ Basic)

¹H NMR (400 MHz, CD₃OD): δ 5.94 (ddt, J = 17.2, 10.4, 5.7 Hz, 1H, CHH=CHCH₂-O), 5.29 (dq, J = 17.3, 1.6 Hz, 1H, CHH^[cis]=CHCH₂-O), 5.19 (dq, J = 10.4, 1.2 Hz, 1H, CHH^[trans]=CHCH₂-O), 4.06 (dt, J = 5.7, 1.4 Hz, 2H, CHH=CHCH₂-O), 3.76 (dt, J = 10.7, 7.5 Hz, 2H, O-CH₂CH₂-P), 3.68 – 3.58 (m, 12H, 6x CH₂), 1.85 (dt, J = 17.1, 7.5 Hz, 2H, O-CH₂CH₂-P).

¹³C NMR (101 MHz, CD₃OD): 136.1 (CHH=CHCH₂-O), 117.7 (CHH=CHCH₂-O), 73.1 (CHH=CHCH₂-O), 71.3 (CH₂), 71.2 (CH₂), 71.1 (CH₂), 71.0 (CH₂), 70.5 (CH₂), 70.0 (CH₂), 69.5 (O-CH₂CH₂-P), 31.31 (d, J = 128.3 Hz, O-CH₂CH₂-P).

Synthesis of sodium (3,6,9,12-tetraoxa-16-thiatetracosyl)phosphonate (**2.21**)



In a vial for photochemical reactions allyl ether (**2.20**) (23 mg, 0.0660 mmol), octanethiol (11 μ L, 0.0660 mmol) and 2,2-dimethoxy-2-phenylacetophenone (dmpa or Irgacure 651, 1 mg, 0.00264 mmol) were added and then solubilized in dry DCM (44 μ L). The crude was mixed under Ar atmosphere. Ar was constantly fluxed through a silicon septum for 10 minutes. After 10 minutes the septum was replaced by the cap and the vial was crimped. The vial was placed in the photochemical plate and stirred for 6 h, shining 365 nm light on it. After 6 h the thiol-ene product was collected, the solvent removed in negative pressure and solubilized in 1 mL of deionized water. The crude was purified through inverse phase chromatography using water (A) and methanol (B) as solvents (from 100% A to 100% B). The organic solvent was distilled in negative pressure and water was removed through freeze drying. The product, a colourless amorphous solid, was obtained pure (32 mg, quantitative yield)

Molecular weight: 488.53 g/mol ($C_{19}H_{39}Na_2O_7Ps$)

TLC: Rf 0.58 (7:3:3 *n*-butanol:H₂O:AcOH, CAM, KMnO₄ Basic)

¹H NMR (400 MHz, CD₃OD): δ 3.80 – 3.70 (m, 2H, O-CH₂CH₂-P), 3.67 – 3.54 (m, 14H, 6x CH₂ glycol and S-CH₂CH₂CH₂-O), 2.58 (t, *J* = 7.2 Hz, 2H, S-CH₂CH₂CH₂-O), 2.51 (t, *J* = 7.2 Hz, 2H, CH₂CH₂-S), 1.92 (dt, *J* = 17.8, 7.8 Hz, 2H, O-CH₂CH₂-P), 1.83 (p, *J* = 6.4 Hz, 2H, S-CH₂CH₂CH₂-O), 1.57 (p, *J* = 7.2 Hz, 2H, CH₂CH₂-S), 1.45 – 1.24 (m, 10H, 5x CH₂ thiol), 0.90 (t, *J* = 6.9 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CD₃OD): 71.4 (CH₂), 71.4 (CH₂), 71.3 (CH₂), 71.3 (CH₂), 71.1 (CH₂), 70.7 (S-CH₂CH₂CH₂-O and CH₂), 68.6 (O-CH₂CH₂-P), 33.0 (CH₂), 32.9 (CH₂CH₂-S), 30.8 (CH₂CH₂-S and S-CH₂CH₂CH₂-O), 30.67 (d, *J* = 130.9 Hz, O-CH₂CH₂-P), 30.4 (CH₂), 30.35 (CH₂), 30.2 (CH₂), 29.9 (CH₂), 29.4 (S-CH₂CH₂CH₂-O), 23.7 (CH₂), 14.4 (CH₃).

HPLC-ESI-MS: Rt= 13.065 min, negative ionization exact mass expected: 443 m/z ($C_{19}H_{40}O_7Ps$), observed 443 m/z (M)⁻ and positive ionization exact mass expected: 444 m/z ($C_{19}H_{41}O_7Ps$), observed 445 m/z (M+H)⁺

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Chapter 3

VISIBLE LIGHT INDUCED PD CATALYSED ISOCYANIDE INSERTION INTO INACTIVE IODIDES

The project that will be described in the next pages started in 2019 from an idea of Professor Lambruschini, where I had the honour to take part, sharing ideas, transposing them into practice and helping in the final development of the work. Here will be reported a brief explanation about what has been done before my contribution and in a separated paragraph all my work. Finally, all the work done led to this publication: Messina, A.; Monticelli, F.; Miroglio, T.; Gagliardi, A.; Viviani, I.; Banfi, L.; Riva, R.; Moni, L.; Basso, A.; Lambruschini, C. Photoexcited Palladium Complex-Catalyzed Isocyanide Insertion into Inactivated Alkyl Iodides. *Molecules* 2025, 30 (12), 2584. <https://doi.org/10.3390/molecules30122584>.

3.1 Pd catalysed Cross Coupling reactions

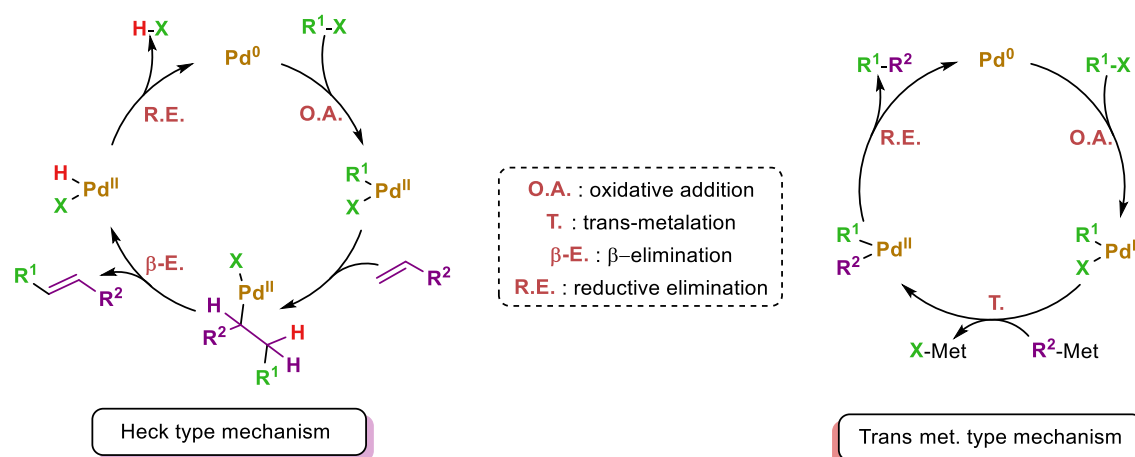
Cross-coupling reactions have been a breakthrough in organic chemistry and for this reason in 2010 Richard Heck, Ei-ichi Negishi e Akira Suzuki received the Nobel prize for chemistry¹. Cross-coupling reactions are reactions that enable the formation of carbon-carbon (or carbon-heteroatom) bonds without requiring the classical chemistry of nucleophiles and electrophiles. Generally, mild conditions are required and the compatibility with other functional groups is very high, therefore, enabling shorter and easier synthetic strategies. Extended studies on the use of orthogonal protecting groups are not required. In specific cases cross-coupling reactions have been used for domino and cascade processes. Due to the use of transition metals for the catalysis, cross-coupling reactions do not satisfy all the “Green Chemistry” parameters, nonetheless they are one of the most useful tools in the hands of an organic chemist.

The three most famous types of reaction mechanisms are: heck type mechanism, transmetalation type mechanism and insertion type mechanism.

The *Heck type mechanism* (Scheme 3.1-left) took its name from the homonymous reaction firstly published in 1968², which from the beginning had a great success, enabling the vinylation or arylation of olefines in mild conditions. It was used for the creation of systems with extended conjugation of π electrons. The reactive partners are alkenes (or alkynes) and aryl (or vinyl) halides (in alternative triflates) in presence of a palladium complex as the catalyst³.

The *transmetalation-type mechanism* (Scheme 3.1-right) is common to many cross-coupling reactions, and the most well-known and widely used are: the Suzuki coupling and the Sonogashira coupling. The Suzuki coupling is probably the most famous and extensively applied cross-coupling reaction. It was first reported by Akira Suzuki in 1979⁴ and involves two reacting partners: an organoboron compound (such as a boronic acid or boronate ester) and an aryl (or alkenyl) halide

(or triflate, tosylate, etc.). The popularity of the Suzuki coupling arises, in part, from the use of boron-containing organic compounds, which are stable and exhibit low toxicity. Moreover, it is a high yield reaction, without suffering from issues of regioselectivity or stereoselectivity. All those great advantages made this reaction particularly valuable for drug synthesis. The Sonogashira coupling is slightly more complex than the Suzuki coupling, as it requires the in-situ formation of an organocuprate intermediate obtained from an alkyne. This reaction proceeds efficiently when alkynes are coupled with aryl (alkenyl or alkynyl) halides in the presence of an external base, typically triethylamine (Et_3N), and a Cu(I) salt. The Sonogashira coupling enables the synthesis of arylalkynes, enynes, enediynes, and conjugated alkynes, that are high-value compounds important for the preparation of pharmaceutically active molecules⁵ and organic materials for electronic applications. Notably, in both the reaction mechanisms reported in *Scheme 3.1* each oxidation and reduction of the palladium complex is a process that involves the exchange of 2 electrons.



Scheme 3.1: General mechanism of a cross-coupling reaction with Heck type mechanism (left) and trans metalation mechanism

3.1.1 Isocyanide chemistry and the palladium catalysed isocyanide Insertion

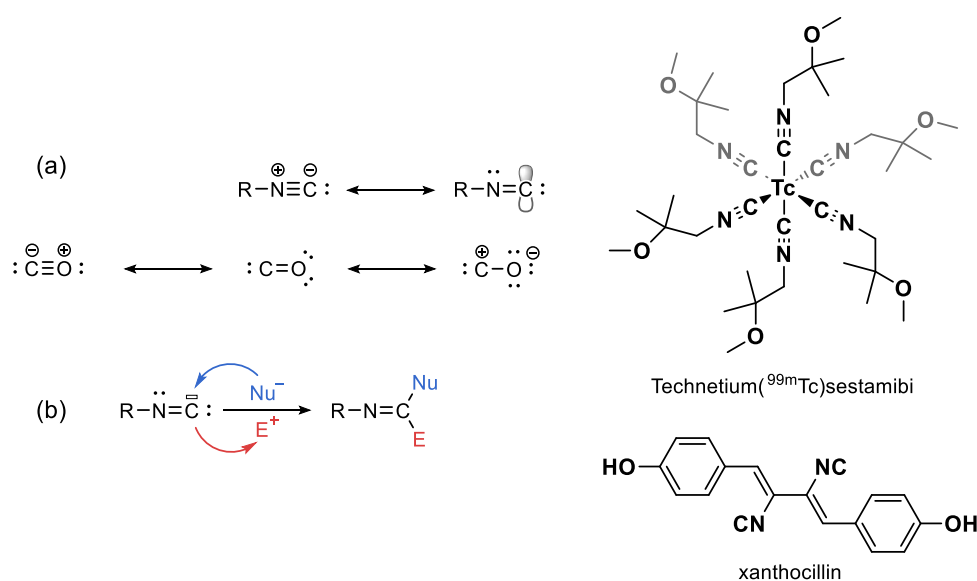
Insertion reactions are defined as reactions in which an agent (for example: isocyanide or carbon monoxide) is inserted in a σ or π bond⁶. The reaction always requires the presence of a catalyst that could be a Lewis or a Brønsted acid or a transition metal. In this paragraph, it will be discussed only the isocyanides insertion promoted by transition metal complexes, which follows a classical cross-coupling mechanism, normally called *imidoylative reactions*.

Isocyanides are very peculiar organic compounds, since they are the only stable functional group with a formally divalent carbon, that can easily undergo oxidation (C^{II} to C^{IV}).

The R-NC group possesses chameleonic electronic properties, it can be used as nucleophile and electrophile, even in the same reaction mechanism (e.g. Ugi and Passerini multicomponent reactions), and its reactivity can be modulated by changing the reaction conditions. Thus, this functional group can be employed in reactions with electrophiles, nucleophiles, oxidants and

reductants and for this reason isocyanides are used to increase the molecular complexity when properly reacted^{7,8}.

An isocyanide can be described as a resonance structure between a zwitterion form and a divalent form (*Scheme 3.2-a*). The former helps to understand the linear geometry, while the latter shows the carbene-like reactivity. The carbene-like form is used to explain the tendency of isocyanide to undergo the simultaneous reaction with a nucleophile and an electrophile at its carbon atom, the so called α -addition (*Scheme 3.2-b*). Isocyanides share this peculiar characteristic only with carbenes and carbon monoxide, while other organic molecules usually react on two different centres⁹.



Scheme 3.2: (a) resonance forms of isocyanide and its most representative Lewis structure; (b) α -addition to an isocyanide; structure of Technetium(^{99m}Tc)sestamibi and xanthocillin

The presence of a lone pair on the terminal carbon atom accounts for its strong metal coordinative properties, which in the past allowed the preparation of a variety of coordination complexes. An example is Technetium(^{99m}Tc)sestamibi, a metal complex commonly used in nuclear medicine imaging¹⁰ (*Scheme 3.2*).

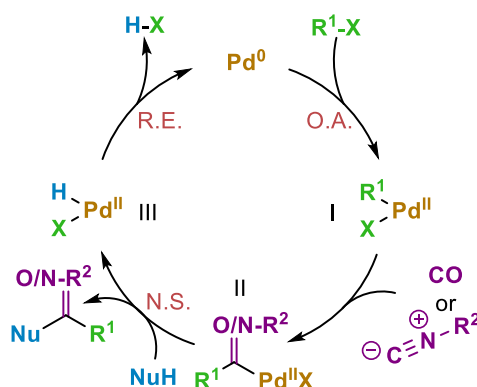
It is reported that the interaction between the metal centre and the isocyanides can be highly dependent on the nature of the isocyanide. In aromatic isocyanide the extended conjugation allows for a better delocalization of the backdonated electrons, thus making aromatic isocyanides better π acceptors, which leads to more stable complexes. For this property, they are defined as carbon monoxide-like ligands. Instead, the aliphatic ones are strong σ -donors to the metal and terrible π acceptors, causing the formation of unstable and reactive complexes, thus being defined as cyanide anion-like ligands¹¹. The coordination with the metal has changed the electron density on the isocyanide carbon atom, which is now prone to insertion reactions via nucleophilic attack, like alcohols, amines, and thiols. This type of isocyanides ligands is called non-innocent¹².

Lastly, it is worth mentioning that, in spite of their peculiar reactivity, isocyanides are also occurring as natural products from marine organisms. Interestingly, most of them possess

remarkable fungicidal, antitumoral, insecticide and, most importantly, antibiotic properties (*Scheme 3.2-xanthocillin*). Those substances were recognized among the less subject to the pharmaco-resistance phenomena¹³.

A compound that has similar properties to isocyanides is carbon monoxide, that is widely applied in metal catalysed insertion reactions. Thanks to their similarities isocyanides and carbon monoxide share the same mechanism in the palladium catalysed insertion reaction, like it is shown in *Scheme 3.3*.

The mechanism starts with the oxidative addition (O.A.) of the catalyst to the starting material (R^1-X , X: good leaving group) giving the intermediate **I**, a Pd^{II} complex. Subsequently, the insertion of CO or $CN-R^2$ takes place, delivering intermediate **II**, a new organometal compound, in which Pd^{II} is covalently bound to the carbonyl (or iminic) carbon. This carbon-metal bond is weak, and palladium is easily replaced by another nucleophile through a nucleophilic substitution reaction (S_N). In this stage, intermediate **III** and the final product are formed; the latter is released from the mechanism and can be recovered and purified. Finally, the initial catalyst is restored after a reductive elimination reaction (R.E.) with the elimination of hydrogen halide (X: halide) and the reduction of palladium from Pd^{II} to Pd^0 . The formation of the acid requires always the presence of a base not nucleophilic, in the reaction crude.



Scheme 3.3: General mechanism of an isocyanide/CO insertion into an aryl (or alkenyl) halide

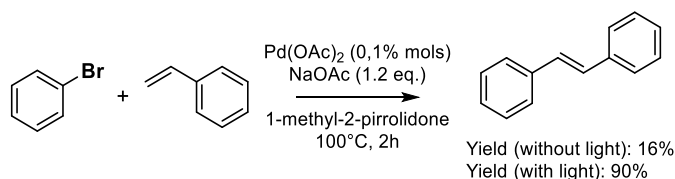
Compared to carbon monoxide, isocyanides are not gasses and less toxic, reducing the hazards and the complexity of the experimental setup. Moreover, isocyanides let the possibility of further functionalization, in fact, if $Nu \neq H_2O$ (otherwise an amide is yielded), the imine that is formed as the product of the insertion reaction is a substrate with a high intrinsic value. The $C=N$ bond can be easily attacked by nucleophiles to obtain asymmetric amines, hydrogenated to obtain simpler amines or hydrolysed to deliver ketones in quantitative yields. Moreover, imine finds use both in multicomponent reactions (like the Ugi tetra-components reaction) and in 4+2 and 3+2 cycloaddition reactions and it can also behave like a strong electron acceptor in SET (Single Electron Transfer) reactions activated by light and transition metal complexes^{14,15}.

3.2 Photocatalytic Pd Cross Coupling reactions

Visible light-induced reactions represent a rapidly expanding area of modern chemistry, and they are considered as one of the promising key techniques. As the name suggests this type of chemistry uses the energy of visible light, typically wavelengths between 400 and 700 nm, to drive chemical transformations. In contrast to traditional photochemistry, which often relies on high-energy ultraviolet (UV) light, visible light offers a milder, safer, and more selective alternative. These reactions often proceed at ambient temperature, harsh reagents are not necessary, and visible light is abundant, inexpensive, and environmentally friendly, making this technique aligned to the principles of green chemistry.

Recently, it was discovered that some of the classical cross-coupling reactions, if performed in the presence of light, are faster and more efficient with respect to the traditional way, because the presence of an electromagnetic radiation boosts the oxidative addition rate, which is the rate limiting step in cross-coupling reactions.

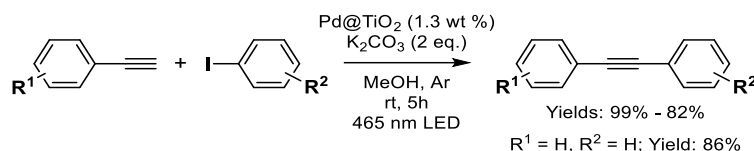
In 2010, Köhler *et al.*¹⁶ reported the first light-induced Heck reaction. In their study, aryl halides were coupled with styrene in the presence of $\text{Pd}(\text{OAc})_2$ and a base (Scheme 3.4). Under standard conditions, the desired product was obtained in only 16% yield; however, upon irradiation of the reaction mixture with a Xe-vapour lamp, the yield increased dramatically to 90%. Since Pd(II) species are not catalysts for cross-coupling reactions and must be reduced to Pd(0) to undergo oxidative addition, the authors proposed that light facilitates and accelerates this reduction through a ligand-to-metal charge-transfer (LMCT) process. DFT calculations combined with the absorption spectrums of the catalyst complex, confirmed the proposed charge transfer. The metal complex exhibits a strong absorption band at 400 nm, which was computationally assigned to excitation of electrons from the HOMO, which has a ligand character, to the LUMO, which shows a metal character. This LMCT event enriches the metal centre with electron density, promoting its reduction to catalytically active Pd(0). Notably, no radical pathways are involved in the mechanism.



Scheme 3.4: photoactivated palladium catalysed Heck coupling reaction between aryl bromide and styrene

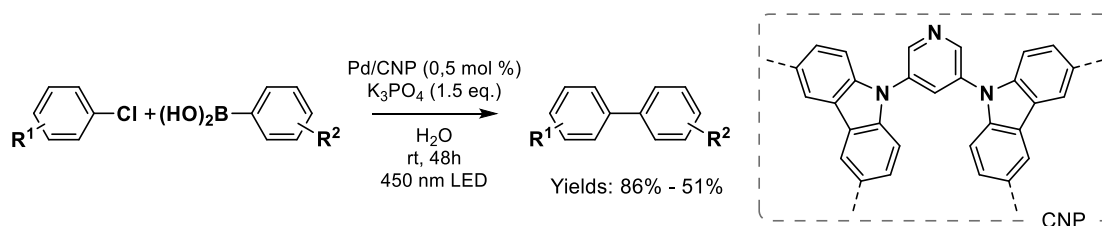
Scaiano research group found that the traditional Sonogashira reaction between aryl alkynes and iodobenzene derivatives can be catalysed by air and moisture tolerant Pd nanoparticles supported on titanium dioxide ($\text{Pd}@\text{TiO}_2$) without requiring the presence of copper as co-catalyst. The researchers tested different reaction conditions, and the best result was obtained by irradiating the reaction mixture with 465 nm LED light in 5h, obtaining the desired product from unfunctionalized starting materials in 86% yield (Scheme 3.5). The necessity of direct excitation of the catalyst was proved by action spectrum. Action spectroscopy is a technique used to study how molecules or ions respond when they absorb light. Instead of directly measuring light absorption,

it measures the resulting action caused by the absorbed light. Action spectroscopy generally shows higher sensitivity respect to absorption spectroscopy. Researcher demonstrated that the catalyst efficiency drastically decreases after two catalytic cycles due to the poisoning by hydrogenated arylalkynes side products. However, Pd NPs can be easily reactivated through a straightforward reductive photochemical approach following any loss of activity. Unfortunately, only aryl iodine can participate, aryl bromide and aryl chloride are not sufficiently reactive¹⁷.



Scheme 3.5: photoactivated palladium catalysed Sonogashira coupling reaction

Close to the work proposed by Scaiano, the research group of Professor Lang decided to explore the use of supported palladium nanoparticles for the heterogeneous catalysis of Suzuki-Miyaura coupling in water. The researchers chose to react aryl chlorides with different boronic acids using palladium nanoparticles supported on conjugated nanoporous polycarbazole (Pd/CNP) obtaining products from good to satisfying yields (Scheme 3.6). The researchers found a broad compatibility with different functional groups and the catalyst can be recycled without a significant reduction in its catalytic activity. A deep investigation of the reaction mechanism unveiled that light is essential to increase the reaction rate. When irradiated, CNP excited electrons can be easily transferred to the palladium⁽⁰⁾ increasing its electron density, thus making the oxidative addition faster. At the same time the CNP, with less electrons in the ground state, subtracts e⁻ from the boronic acid causing the formation of electronic holes in the substrate, making the transmetalation reaction easier¹⁸.

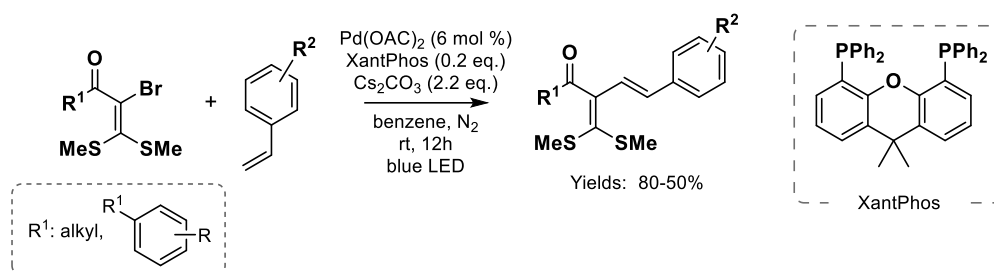


Scheme 3.6: photoactivated palladium catalysed Suzuki-Miyaura coupling reaction

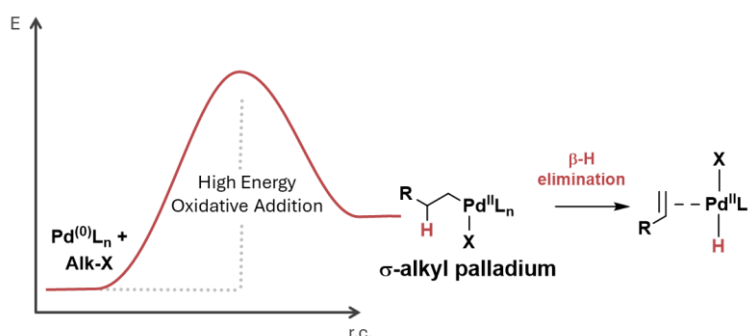
In the three examples reported, light helps to favour efficiency and reactivity, but the mechanism remains the classic one and each oxidation, reduction and transformation follows the typical two electron transfer chemistry. However, light can also favour single electron transfer reactions, causing the formation of radicals in solution, thus causing changes in the reaction mechanism. The SET pathway is an emerging field in palladium catalysis.

Yu and co-workers¹⁹ in 2021 reported a visible-light-induced, palladium-catalyzed Heck reaction between S,S-functionalized vinyl bromides and styrenes. They first optimized the reaction conditions, demonstrating that blue-light irradiation was essential for reactivity. An on-off experiment excluded the possibility of a radical chain process: conversion of the starting material

occurred only during periods of irradiation. Nevertheless, the radical nature of the mechanism was supported by trapping experiments, in which the initial vinyl radical was intercepted by TEMPO or BHT (butylated hydroxytoluene). Under these mild conditions, a variety of diene products were obtained in moderate to excellent yields (Scheme 3.7). These dienes could then be transformed into highly functionalized trisubstituted furan derivatives. Based on the collected experimental evidence, the authors proposed a mechanism initiated by a single-electron-transfer oxidative addition (SET OA) from the photoexcited catalyst to the vinyl bromide, generating a hybrid Pd(I)/vinyl-radical species. The mechanism then proceeds via radical addition to styrene, followed by β -hydrogen elimination and a final reductive elimination to yield the product and restore the catalyst.



Scheme 3.7: photoactivated palladium catalysed Heck coupling reaction through a radical mechanism



Scheme 3.8: Representation of the problematics correlated to the reaction between an alkyl electrophile (Alk-X) and a palladium complex ($\text{Pd}^{(0)}\text{L}_n$).

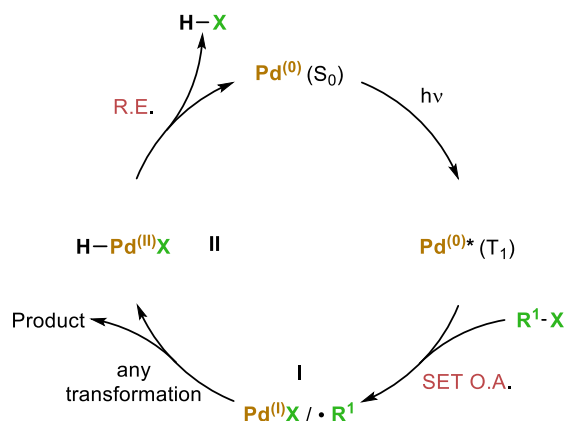
Palladium cross-coupling reactions possess a big disadvantage, although being a strong and reliable tool in the hand of an organic chemist as reported. In the electrophile the carbon bound to the leaving group ($\text{R}^1\text{-X}$) must be hybridized sp^2 or sp . Instead, if a sp^3 hybridized carbon is used in lieu of a sp^2 or sp hybridized one, the reaction does not work properly, and the answer is correlated to the chemical nature of the electrophile²⁰:

- High activation energy barrier is found in the initial oxidative addition, due to the electron rich nature of the σ -bond between $\text{C}(\text{sp}^3)$ and X. Therefore, the first step of the mechanism is very slow and unlikely to happen (Scheme 3.8).
- If the oxidative addition somehow succeeds, the first obtained intermediate is unstable and very reactive. In the σ -alkyl palladium complex formed is absent the stability given by the π -electrons backdonation from the organic compound to the empty d orbitals of the $\text{Pd}^{(\text{II})}$ core. Thus, the first reaction that is likely to happen is a rapid and irreversible β -elimination reaction

with the formation of a terminal alkene and $\text{X-Pd}^{\text{III}}\text{-H}$ complex that gives a rapid reductive elimination (Scheme 3.8).

Recently, it was discovered that the extension of palladium cross-coupling reactions to alkyl-halides can be achieved using photons²¹.

Overall, the mechanism presents some analogies to the “classical” one, but also significative differences like SET reaction step and the formation of free radicals. In detail, once the palladium complex absorbs light, it is excited to a singlet state (S_1) and rapidly undergoes intersystem crossing to a triplet state (T_1). In this excited state, the $\text{Pd}(0)$ complex performs a single-electron transfer oxidative addition (SET O.A.). In practice, the excited $\text{Pd}(0)^*$ species performs a homolytic cleavage of the $\text{R}_1\text{-X}$ bond, generating a hybrid radical $\text{Pd}(\text{I})\text{X}$ complex and an $\text{R}_1\cdot$ radical (intermediate I). This intermediate can follow different pathways depending on the type of reaction, ultimately evolving into the final intermediate (II), a $\text{Pd}(\text{II})$ complex, which can give a final reductive elimination (R.E.) to restore the catalyst (Scheme 3.9) and deliver the final product.



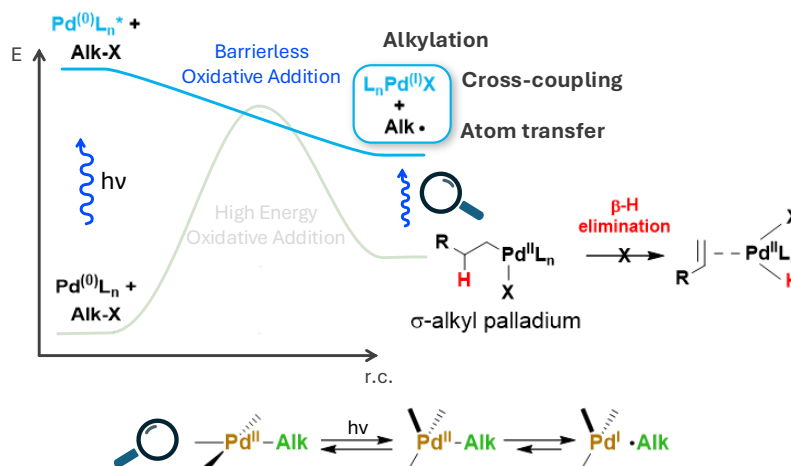
Scheme 3.9: general catalytic cycle for a light activated cross-coupling reaction with a single electron transfer oxidative addition

The new SET chemistry approach described, extends the cross-coupling reactions worlds towards inactivated alkyl electrophiles. The theoretical explanation behind the reasons of this unusual reactivity is complicated and still needs to be fully understood, although a lot of studies are now ongoing. The most accredited reasons are reported here.

When the palladium complex is excited in the triplet state ($\text{Pd}(0)^*(\text{T}_1)$), it acquires enough energy to overcome any activation barrier. Practically, it can carry out a barrierless oxidative addition²², meaning that it becomes fast and easy to perform. Moreover, once the radical interaction between $\text{R}^1\cdot$ and $\text{Pd}(\text{I})\text{X}$ is established, the β -elimination reaction is totally suppressed²³, because $\text{R}^1\cdot$ is not directly bound to the palladium complex and, thus, it is not acting as a ligand, instead, as an independent species (Scheme 3.10 top).

Another theory that explains this special reactivity is based on the study of the first intermediate formed after the two-electron oxidative addition. It was discovered that, although it is difficult to form with alkyl electrophiles, the σ -alkyl palladium intermediate can absorb light and becomes photoexcited. Upon photoexcitation, the $\text{R-Pd}(\text{II})\text{-X}$ complex changes its geometry from square

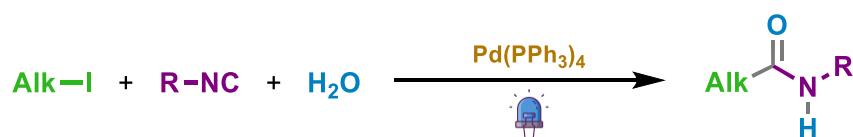
planar to tetrahedral (*Scheme 3.10 bottom*)²⁴. This geometric transformation is a direct consequence of the involvement of higher-energy orbitals during excitation. In this new geometry, the Pd–R¹ bond is weakened and undergoes homolytic cleavage, forming intermediate I (*Scheme 3.9 and Scheme 3.10*)²⁵. The simultaneous presence of the metal centre in its triplet state and light irradiation further accelerates the bond cleavage.



Scheme 3.10: graphical explanation of the two possible mechanisms for the formation of the hybrid radical intermediate Pd^(II) R• (with R = Alk, alkyl)

3.3 Project's previews results

The single-electron transfer (SET) approach applied to cross-coupling reactions has recently emerged as an important trend in palladium-catalysed chemistry. Since its first presentation in the literature, numerous research groups have adopted this strategy, leading to the development and optimisation of several reactions. However, the multicomponent isocyanide insertion under these conditions remained unprecedented in the literature. For this reason, an initial attempt to access the desired product was made following the general reaction scheme shown in *Scheme 3.11*. In the first experiment, a secondary alkyl iodide and tert-butyl isocyanide were employed in a 1:1.1 ratio, together with Et₃N as base, Pd(PPh₃)₄ as the metal catalyst, and a light source. This preliminary test demonstrated that the desired amide could be formed, albeit in low yield. Several parameters were not optimal, for example, the reaction was irradiated with a white lamp rather than LEDs; the small amount of water present in the solvents and ambient air was used as a component for the reaction, instead of using a stoichiometric quantity; and no co-catalysts were included. These observations motivated a deeper exploration of the multicomponent transformation.

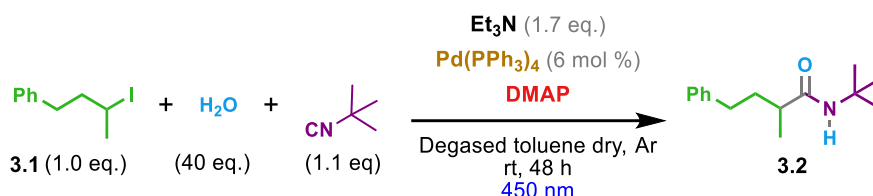


Scheme 3.11: general reagent used in the multicomponent palladium catalysed isocyanide insertion reaction

The initial optimisation studies and preliminary scope exploration were carried out by Master's and Bachelor's students. A brief overview of their work and the experimental results obtained prior to my contribution is provided below.

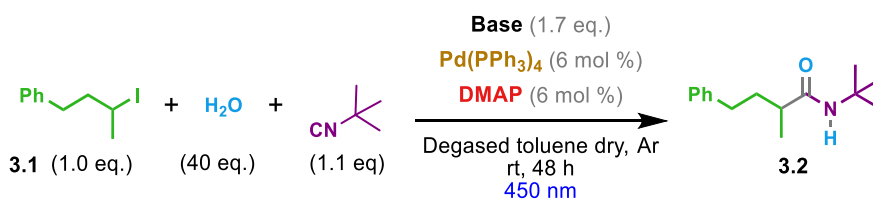
Initially, the appropriate iodide and isocyanide reagents for the reaction were selected. As the iodide, 3-iodobutylbenzene was chosen because it is easy to visualize in TLC, can be detected using an HPLC-DAD (DAD: diode array detector) and lacks other functional groups that could interfere with the reaction. As the isocyanide, *tert*-butyl isocyanide was selected since it is readily available, its excess can be removed by distillation under reduced pressure, and it produces only a single signal in the ¹H-NMR spectrum. The first breakthrough in optimizing the reaction was the discovery that the addition of a catalytic amount of DMAP (4-(dimethylamino)pyridine) significantly increased the yield (Table 3.1).

Table 3.1: 4-(dimethylamino)pyridine screening



Entry	DMAP	Yield (HPLC)	Iodide conversion	Toluene Conc.
1	No	2 %	29 %	0.1 M
2	6 mol%	72 %	91 %	0.1 M
3	30 mol%	63 %	99 %	0.3 M
4	Pyridine instead of DMAP	10 %	41 %	0.3 M

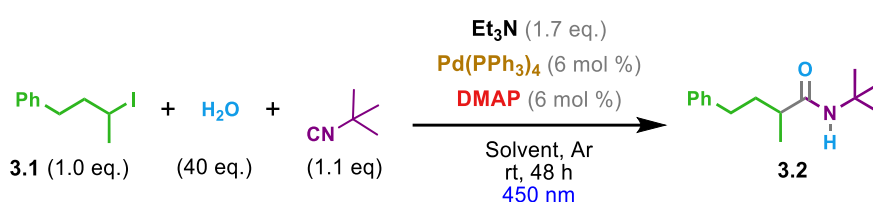
Table 3.2: Bases screening (DIPEA: *N,N*-diisopropylethylamine)



Entry	Base	Yield (HPLC)	Iodide conversion
1	Et₃N	72%	91 %
2	DIPEA	52%	94 %
3	Pyridine	25%	63 %
4	KOAc	18%	83 %
5	K ₃ PO ₄	11%	54 %
6	K ₂ CO ₃	15%	62 %
7	K ₂ HPO ₄	48%	92 %
8	Cs ₂ CO ₃	10%	60 %

The second reaction component studied was the base. Both organic and inorganic bases were screened, as shown in *Table 3.2*. The organic bases provided higher conversions and yields, indicating better efficiency under the experimental conditions. A possible explanation can be related to the biphasic nature of the reaction mixture (toluene and water are immiscible). The reaction likely takes place at the interface, and since most of the mechanism occurs in toluene, a water-soluble base loses its effectiveness. Consequently, bases that are partially soluble in both phases are more efficient than those distributed only in the aqueous or organic phase. Among the organic bases tested, triethylamine (Et₃N) proved to be the best option, affording a 72% amide yield and 91% iodide conversion.

Table 3.3: solvents screening (DCM: dichloromethane, DMSO: dimethyl sulfoxide, DMF: dimethylformamide).



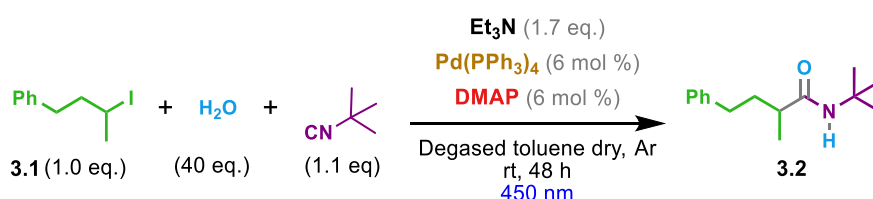
Entry	Solvent	Yield (HPLC)	Yield (isolated)	Iodide Conversion
1	Toluene (0.3 M)	72 %	72 %	93 %
2	DCM (0.3 M)	48 %	57 %	82 %
3	Et ₂ O (0.3 M)	28 %	24 %	83 %
4	MeCN (0.3 M)	61 %	59 %	-
5	DMSO (0.3 M)	58%	-	-
6	DMF (0.3 M)	50%	-	-
7	Dioxane (0.3 M)	-	-	-
8	Toluene (0.05 M)	-	0 %	-
9	Toluene (0.1 M)	-	63 %	-
10	Toluene (0.6 M)	-	64 %	-
11	Toluene (1 M)	-	30 %	-

Parallel to the bases screening, different types of solvents and reaction mixture concentrations were tested. As reported in *Table 3.3*, apolar solvent (toluene), moderately polar solvents (diethyl ether, DCM, dioxane and acetonitrile) and polar aprotic solvents (DMSO and DMF) were used. Water miscible solvents, like DMSO, DMF and dioxane, were evaluated thinking that a monophasic reaction mixture would improve the iodide conversion and the final yield. This theory was proven to be wrong by the experimental results, probably, water, since interacts immediately with delicate and reactive compounds could promote side reactions or degradations of the starting materials. Dioxane participated in the reaction proving to be not inert in those conditions, leading to zero iodide conversion (entry 7). In the end, the best solvents found were toluene and acetonitrile (entry 4), because a clean iodide conversion into the final product was promoted, parallel to the reduction

of the quantity of side-compounds formed during the transformation. Toluene was furthermore studied by changing the reaction concentration. Generally, the reaction resulted to be more efficient when the reaction mixture was less diluted (entry 1, 9 and 10), however, when a too high concentration was chosen the yield dropped to 30% (entry 11).

Another very relevant parameter that was considered in the optimization of the reaction was the ligand for the palladium. Each ligand can have a different impact on the chemical properties of the active complex, in terms of reactivity, absorption (*molar attenuation coefficient* ϵ and λ_{max}), energy of the excited state and redox potential at the excited state. Moreover, the processes of ligand to metal charge transfer or metal to ligand charge transfer can be differently favoured, affecting the outcome of the photocatalytic transformation (this is valid for thermal reactions too). The organic phosphines tested were dppf (1,1'-Bis(diphenylphosphino)ferrocene), dppe (1,2-Bis(diphenylphosphino)ethane), dppb (1,4-Bis(diphenylphosphino)butane), XanthPhos (4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene) and BippyPhos (5-(Di-*tert*-butylphosphino)-1', 3', 5'-triphenyl-1'*H*-[1,4']bipyrazole), however, the commercial palladium tetra-triphenylphosphine outperformed all the other palladium complexes. Experimentally was proven that a higher amount of catalyst is not beneficial for the reaction, while a lower amount reduces the overall conversion of the iodide and consequently the yield.

Table 3.4: essential components screening



Entry	Variations from standard conditions	Yield (HPLC)	Iodide Conversion
1	none	72 %	93 %
2	dark	0 %	64 %
3	350 nm	28 %	22 %
4	Green (528 nm)	14 %	57 %
5	Without Et ₃ N	10 %	48 %
6	Without H ₂ O	11 %	67 %
7	Without Pd(PPh ₃) ₄	0 %	32 %
8	Without stirring	5 %	60 %
9	Without DMAP	2 %	29 %

Finally, the relevance of each reaction component was evaluated to gain a better understanding of its specific role (Table 3.4). This final screening once again confirmed the crucial role of DMAP, highlighting its importance for the correct outcome of the chemical transformation (entry 9). The omission of Et₃N led to a decrease in both iodide conversion and overall yield (entry 5). In fact, in all classical palladium-catalysed reactions, the base is responsible for neutralizing the hydrogen

halide formed during the reductive elimination step that closes the catalytic cycle. The absence of $\text{Pd}(\text{PPh}_3)_4$ (entry 7) and water (entry 6) revealed two important aspects: the catalyst is the only component in the mixture capable of absorbing light and initiating the SET oxidative addition, while water serves as the sole source of oxygen, which is essential for the formation of the final amide. Moreover, it was found that the amount of water must be carefully controlled; if it is either increased or decreased, the reaction does not proceed as expected. In conclusion, an extended study of the operative wavelength was performed, demonstrating that the reaction proceeds only under continuous irradiation at 450 nm (the complete study is not reported here).

Finally, it was possible to define the best conditions to perform the isocyanide insertion reaction (Scheme 3.12).

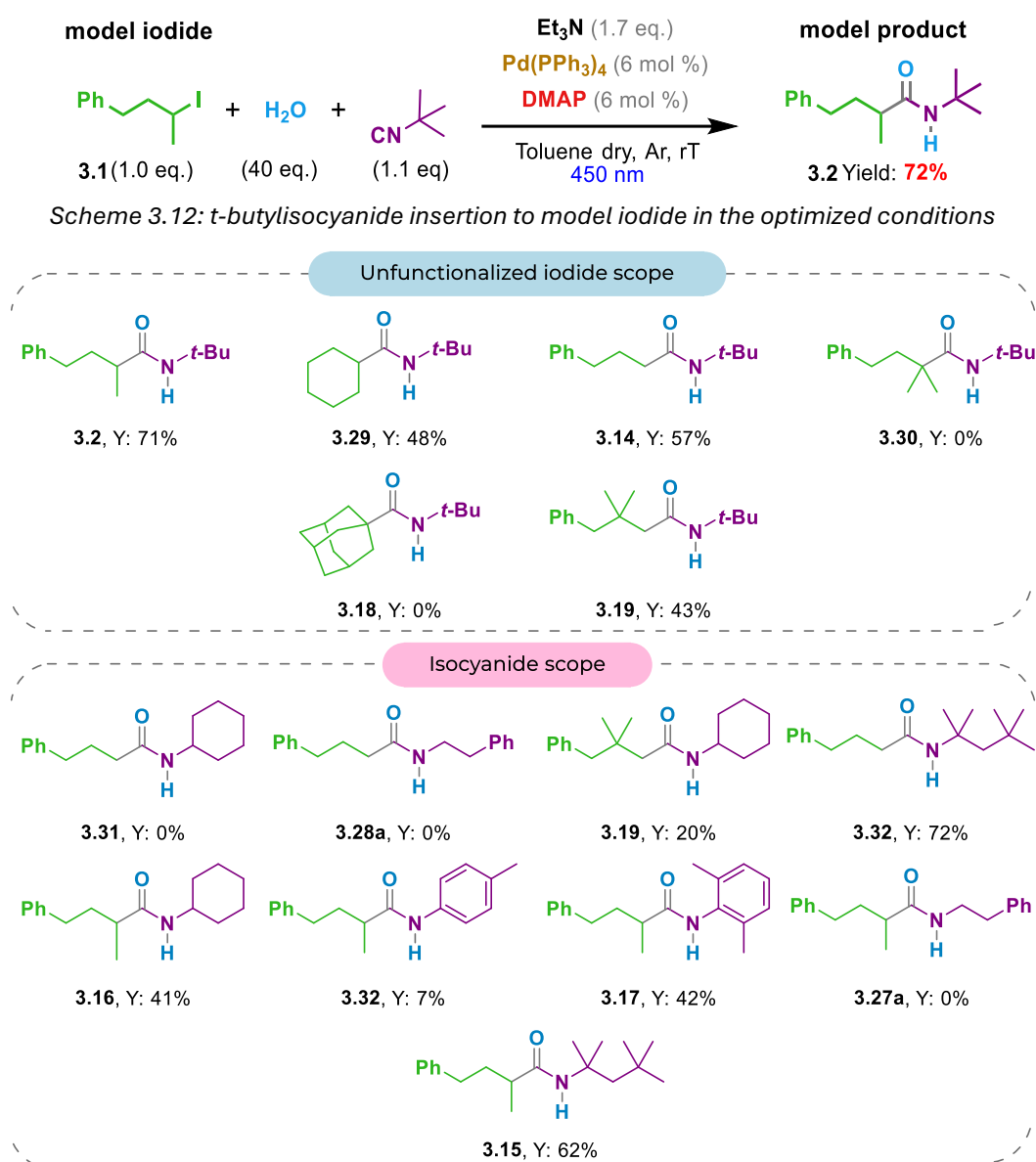


Figure 3.1: first scope of the reaction using the optimized conditions

After defining the best conditions for the insertion reaction, a library of different amide was made. Initially, the role of steric hindrance in the reaction mechanism was investigated. To this end, primary, secondary, tertiary, and sterically hindered primary iodides were tested. The steric demand of various commercial isocyanides was also considered; therefore, *t*-butyl isocyanide, *c*-hexyl isocyanide, 1,1,3,3-tetramethyl isocyanide, 1-isocyano-4-methylbenzene, 2,6-dimethylphenyl isocyanide and (2-isocyanoethyl)benzene were selected for the study (*Figure 3.1*).

The experimental results highlighted a significant influence of steric hindrance of the reaction partners, with the most effective combination being secondary iodides and tertiary isocyanides. The combination of secondary iodides and secondary isocyanides delivered good results. Instead, a higher steric hindrance of the iodide led to a total suppression of the reactivity. Notably, working in the opposite direction by reducing the steric hindrance (e.g. using primary iodides) brought again to a reduction of the isolated yields. Further studies on the latter case are presented in section 3.5.

3.4 New optimization of the isocyanide insertion reaction

After completing the first scope evaluating the steric requirements of the reaction, I started to test the compatibility and orthogonality of the insertion reaction towards different functional groups. Therefore, a new library of amides was prepared, combining functionalized secondary iodides to tertiary and secondary isocyanides (*t*-butyl isocyanide and *c*-hexyl isocyanide). Only those combinations of reagents and unfunctionalized isocyanides were tested, because they are the most reliable and efficient partners, as it was demonstrated in first reaction scope.

It was found that with a specific secondary iodide bearing a simple thiophene ring, the reaction performed worse than expected. Part of my PhD was used to deeply investigate this specific isocyanide insertion, and in *Table 3.5* are reported the 18 experiments used to clarify this reaction, that finally lead to the definition of new condition to perform the photoactivated reaction.

When the conditions reported in *Scheme 3.12* were applied to iodide **3.1**, the desired product was isolated in 12 % yield, and only a very low conversion of the starting iodide was observed (*Table 3.5, entry 1*). It was assumed that the sulphur atom present in the thiophene ring could poison the catalyst by strongly coordinating the metal centre, thus reducing the available quantity of free catalyst and consequently the conversion and the final yield. To improve the conversion, the reaction time was extended to 72 hours, which led to a slight improvement (*entry 2*): the conversion of the starting iodide increased, although it remained incomplete, and the yield approximately doubled. A further combination of longer reaction time (72 h) with the addition of fresh Pd(PPh₃)₄, DMAP (4-(dimethylamino)pyridine), and isocyanide after 48 hours afforded the product in 62 % yield (*entry 3*). Analysis of the crude reaction mixture by GC-MS and ¹H NMR revealed full conversion of the starting iodide, mainly to the desired product. The experiment was repeated twice under the same conditions to confirm reproducibility, and identical results were obtained (*entry 4*). After confirming the robustness of the conditions, the reaction time was further increased to 96

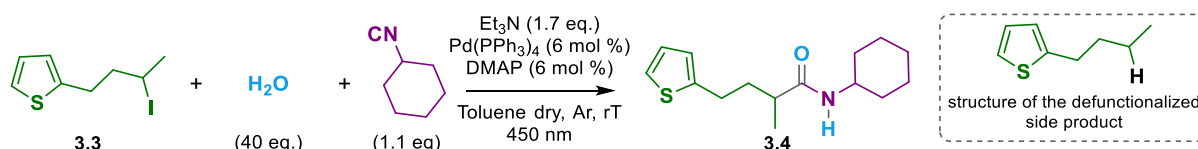
hours (24 hours longer than in *entries* 3 and 4) to test whether the yield could be improved. However, GC-MS and ^1H NMR analyses of the crude mixture revealed a significant amount of defunctionalized iodide (scheme of *Table* 3.5), the main side product of the reaction, and the desired amide was isolated in 59 % yield. The byproduct of the reaction could form when the alkyl radical, formed after the SET oxidative addition, abstracts a hydrogen radical from water or solvent, instead of combining to the isocyanide. Alternatively, it is the byproduct of amide degradation. Thus, prolonged irradiation was found to be detrimental to the reaction outcome.

To better understand which reagent was responsible for the sharp increase in yield, a series of additional experiments were performed. Fresh $\text{Pd}(\text{PPh}_3)_4$ and isocyanide were added after 48 hours (*entries* 6 and 7), leading to comparable results but still inconclusive evidence. A similar test was conducted by increasing the amount of isocyanide from the start of the reaction (2.2 eq instead of 1.1 eq), while maintaining irradiation for 48 hours (*entry* 8). Under these conditions, the desired amide was obtained in 58 % yield, almost matching the highest recorded yield (62 %, *entry* 3). It is worth noting that in all three entries, no unreacted iodide was detected after the reaction, and only trace amounts of the defunctionalized alkane side product were observed. So far, the best conditions are those reported in *entry* 3; however, they require a relatively high catalyst loading (increasing costs), an excess of isocyanide, and a prolonged reaction time of three days.

Aiming at decreasing the reaction time and the amount of catalyst a new parameter was added: the temperature. The operative temperature employed in *entry* 1-8 ranged from 15°C to 18°C due to the photochemical setup (circulating tap water to cool down the reaction vial), so it was decided to modify the setup by adding a thermostat. 25°C (*entry* 9) and 35°C (*entry* 10) were tested and the best yield was obtained at 25 °C even if it was not as good as in *entry* 3. In both entries the full conversion of the iodide was reached, but from the analysis of the crude was detected 10% at 25 °C and 13% at 35°C in moles of side product, that reduced the yield. Those two results are impressive if compared to *entry* 1, because the temperature alone had a big impact in enhancing the rate of the reaction, and normally this variable is believed to be negligible in pure photochemical reactions. Therefore, it shows how the mechanism of the reaction is very complex and delicate. To avoid the formation of the side product, two other experiments were carried out, in which a larger excess of isocyanide from the beginning was used (*entry* 11 and 12). The use of a higher excess of this reagent favors a rapid and smother capture of the alkyl radical, reducing its half-life in solution and avoiding hydrogen abstraction reactions. In both cases the product was isolated in a 62% yield with only traces of alkyl side product and full conversion of iodide **3.3**. Willing to increase even more the yield, it was attempted the combination of more catalyst and DMAP (*entry* 15) and 25 °C, however the product was isolated in 18% yield. Instead, performing the reaction with less catalyst and DMAP (*entry* 17) led to the recovery of the product in 45% yield. The reproduction of the conditions found in *entry* 3 controlling the temperature to 25 °C (*entry* 13) led to a 10 % reduction of the amide yield. The addition of fresh PPh_3 after 48 hours (*entry* 14) and *c*-HexNC after 24 hours (*entry* 16) led to not fully satisfying results (respectively 50% and 54% yield).

It is worth noting that the reaction mixture is transparent, biphasic and light yellow and over time darkens (dark orange after 48 h) (*Figure 3.2-a*). During the addition of fresh reagents after 48 hours, if palladium and DMAP were added in the reaction mixture, nothing happened, but after the injection of isocyanide the crude reaction mixture color reverted to the initial one (*Figure 3.2-b*). The experimental evidence can be explained considering that isocyanide has the possibility to make a ligand exchange equilibrium with PPh_3 in the coordination sphere of Pd. Perego *et al.*²⁶ demonstrated the ligand exchange in the coordination sphere of palladium by theoretical calculations, $^1\text{H-NMR}$, $^{31}\text{P-NMR}$ and isolating the relative intermediates. Initially, the researchers saw the exchange between dibenzylideneacetone and *t*-BuNC, and then the same phenomenon between bidentate phosphine ligands and the same isocyanide. Inspired by this fact, a reaction with $\text{Pd}(\text{OAc})_2$ as catalyst was carried out (*entry 18*) and the desired product was isolated in 45% yield. Thus, it was demonstrated that isocyanide has a role in the coordination of palladium since $\text{Pd}(\text{OAc})_2$ is inactive in this type of reaction.

Table 3.5: second optimization (in brackets the starting equivalents)



Entry	Reaction time	Yield	Variations from the original conditions
1	48 h	12 %	-
2	72 h	28 %	-
3	72 h	62 %	Addition of: DMAP 0.06 eq. / $\text{Pd}(\text{PPh}_3)_4$ 0.06 eq / c-HexNC 1.1 eq after 48 h
4	72 h	62 %	Addition of: DMAP 0.06 eq. / $\text{Pd}(\text{PPh}_3)_4$ 0.06 eq / c-HexNC 1.1 eq after 48 h
5	96 h	59 %	Addition of: DMAP 0.06 eq. / $\text{Pd}(\text{PPh}_3)_4$ 0.06 eq / c-HexNC 1.1 eq after 48 h
6	72 h	60 %	Addition of: $\text{Pd}(\text{PPh}_3)_4$ 0.06 eq after 48 h
7	72 h	50 %	Addition of: c-HexNC 1.1 eq after 48 h
8	48 h	58 %	The reaction starts with 2.2 eq of c-HexNC
9	48 h	45 %	Controlled temperature (25°C)

10	48 h	43 %	Controlled temperature (35°C)
11	48 h	62 %	25°C and 2.2 eq of c-HexNC From the beginning
12	48 h	62 %	35°C and 2.2 eq of c-HexNC From the beginning
13	72 h	52 %	25°C + addition of: DMAP 0.06 eq. / Pd(PPh₃)₄ 0.06 eq / c-HexNC 1.1 eq after 48 h
14	72 h (48 + 24)	50 %	25°C + addition of: PPh₃ 0.3 eq after 48 h
15	48 h	18 %	25°C DMAP 0.12 eq. / Pd(PPh₃)₄ 0.12 eq From the beginning
16	48 h	54 %	25°C + addition of: c-HexNC 1.1 eq after 24 h
17	72 h	45 %	25°C DMAP 0.06 eq. / Pd(PPh₃)₄ 0.03 eq From the beginning
18	48 h	45 %	25°C and 2.2 eq of c-HexNC using Pd(OAc)₂ instead of Pd(PPh₃)₄

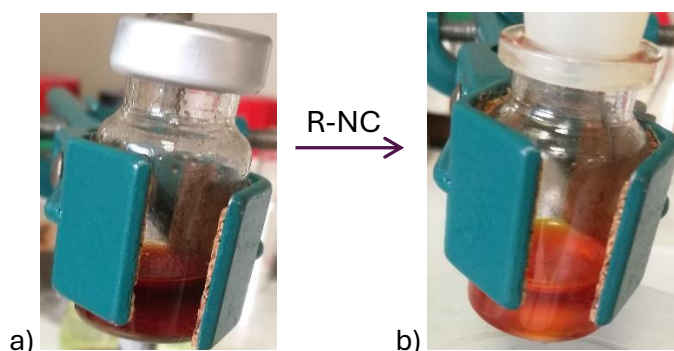


Figure 3.2: photos of the reaction crude: a) after 48 hours of reaction; b) after 48 hours of reaction and the addition of fresh isocyanide

At the end of the optimization study, three reaction conditions (*entries* 3, 11, and 12) were identified as capable of affording the desired amide **3.4** with the highest yields. Among these, the conditions from *entry* 11 (25 °C, 2.2 eq of *c*-hexNC, Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 eq), blue light, 48 h, Ar) were selected as the best for the isocyanide insertion. This choice was based on the use of lower amounts of palladium and DMAP, as well as a shorter irradiation time, which reduces both reagent and operational costs (and prolongs the lifespan of the LED lamps). Furthermore, these conditions result in fewer side products compared to *entry* 12, simplifying and accelerating the purification process.

Once the new conditions for the isocyanide insertion were selected, they were tested reproducing some of the amides initially prepared in the unfunctionalized iodides scope. This

choice was made to allow a direct comparison between the isolated yields obtained under both sets of conditions. Once finished, the same work was made with functionalized iodides.

3.5 Reaction scope with new conditions

Figure 3.3 reports the structures of the synthesized amides, comparing for each compound the yields obtained under the first and second optimized conditions. The experimental results highlight that the new conditions (2nd cnd) are generally beneficial, leading to an overall increase in isolated yields. This demonstrates that the result obtained for the synthesis of compound **3.4** was not an isolated case. Compounds **3.14**, **3.17**, and **3.18** were successfully obtained under the new conditions, with compound **3.18** being particularly noteworthy, as it could not be isolated under the first reaction conditions. Even for compound **3.2**, the product of the first optimization, the new conditions allowed for a slight increase in yield. For other compounds, such as **3.16** and **3.19**, the change in conditions appeared to have no influence. In contrast, the lower yield observed for compound **3.15** is believed to be related to the batch of isocyanide used for the insertion, which likely underwent degradation. Therefore, it is not possible to assess whether the new conditions would have been beneficial in this case. However, we believe that any improvement would have been negligible.

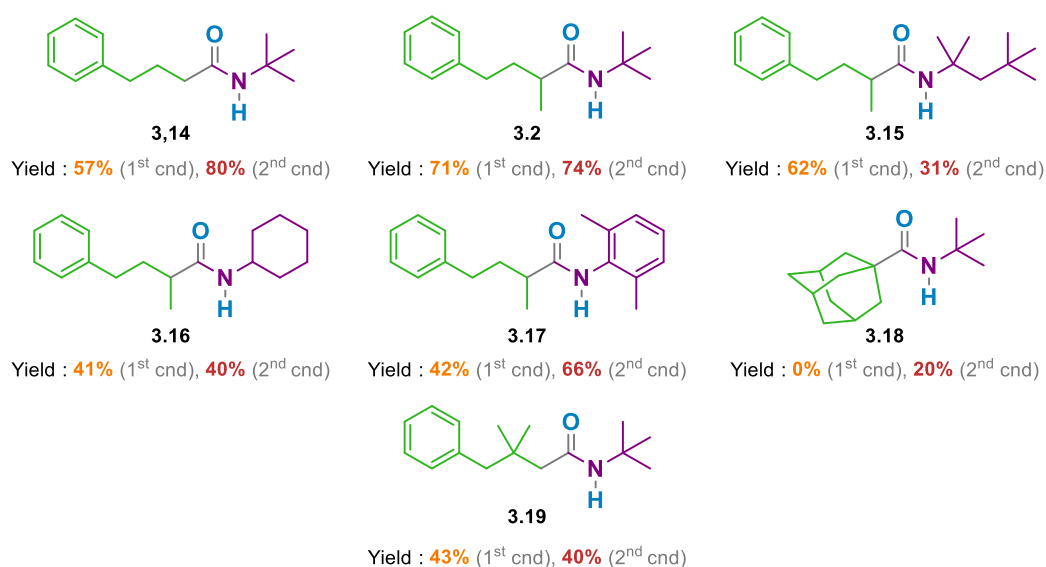


Figure 3.3: unfunctionalized iodides scope (1st cnd: iodide (1.0 eq), isocyanide (1.1 eq), Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 mmol), blue light, rt, 48 h, Ar, degassed anhydrous toluene (0.3 M); 2nd cnd: iodide (1.0 eq), isocyanide (2.2 eq), Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 mmol), blue light, rt, 48 h, Ar, degassed anhydrous toluene (0.3 M)). Reaction performed on a 100 mg scale

It is worth mentioning that the conditions identified during the second optimization (2nd cnd) were beneficial for the entire scope of the reaction, thereby rejecting the initial hypothesis of a possible poisoning of the catalyst due to the presence of sulphur in the thiophene ring.

Parallel to the steric hinderance study, experiments to evaluate the tolerance towards different function groups were conducted, and in Figure 3.4 are reported the experimental results, where the yields obtained from the two different conditions employed are compared.

As anticipated, only the combination of secondary iodides with secondary and tertiary isocyanides were preferred, because they are more versatile and also the highest yielding reactive partners employed in the unfunctionalized iodides scope.

The experimental results showed that the light-activated isocyanide insertion reaction is compatible with various functional groups, excluding nucleophiles. For example, the presence of a free hydroxy group completely suppresses the reaction, and product **3.20** could not be isolated under either the first or the second optimized conditions. However, when the same functional group is protected as a silyl ether (iodide **3.11**) or a benzoate (iodide **3.13**), the corresponding amide products can be isolated in high yields (71% for **3.21** and 72% for **3.22**, respectively).

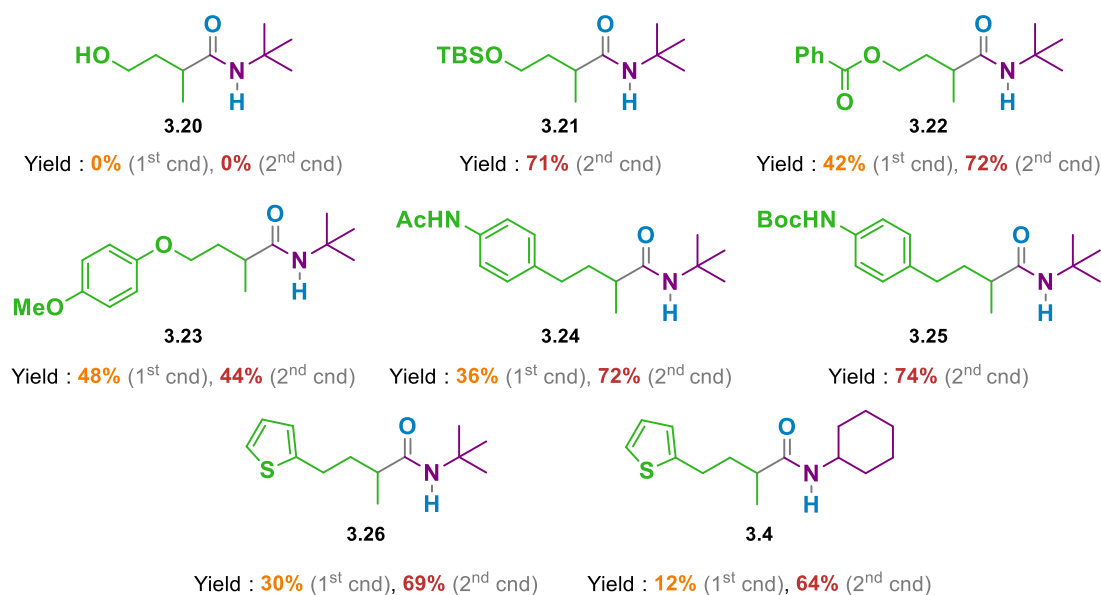


Figure 3.4: functionalized iodides scope (1st cnd: iodide (1.0 eq), isocyanide (1.1 eq), Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 mmol), blue light, rt, 48 h, Ar, degassed anhydrous toluene (0.3 M); 2nd cnd: iodide (1.0 eq), isocyanide (2.2 eq), Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 mmol), blue light, rt, 48 h, Ar, degassed anhydrous toluene (0.3 M)). Reaction performed on a 100 mg scale

It is worth mentioning that the conditions identified during the second optimization (2nd cnd) led to a significant increase in the yields of almost all amides. Moreover, many crude reaction mixtures contained fewer impurities, which facilitated purification. Amide **3.23** was the only product whose yield did not improve; this is likely due to the corresponding iodide undergoing an alternative pathway after the SET oxidative addition step, resulting in the formation of side products. This supposition is consistent with the GC–MS and ¹H NMR analyses of the crude mixture, which revealed several unidentified by-products that complicated the final chromatographic purification.

The two studies performed highlighted the same feature that initially was discovered: the most versatile and high yielding combination is secondary iodide and *t*-butyl isocyanide. Interestingly, the new conditions helped to understand the reason for the absence of product formation when the steric hinderance of both partners is reduced (i.e. primary iodide and primary isocyanide like phenethyl isocyanide). The reaction for the synthesis of the two amides **3.27a** and **3.28a** (reported in Figure 3.5) were reproduced with the second optimization conditions, the products of double

isocyanide insertion were isolated (**3.27b** and **3.28b** respectively), although in low yield. Notably, the formation of the desired products was not observed. Our hypothesis is that the double insertion products are more favoured when a higher excess of isocyanide is used.

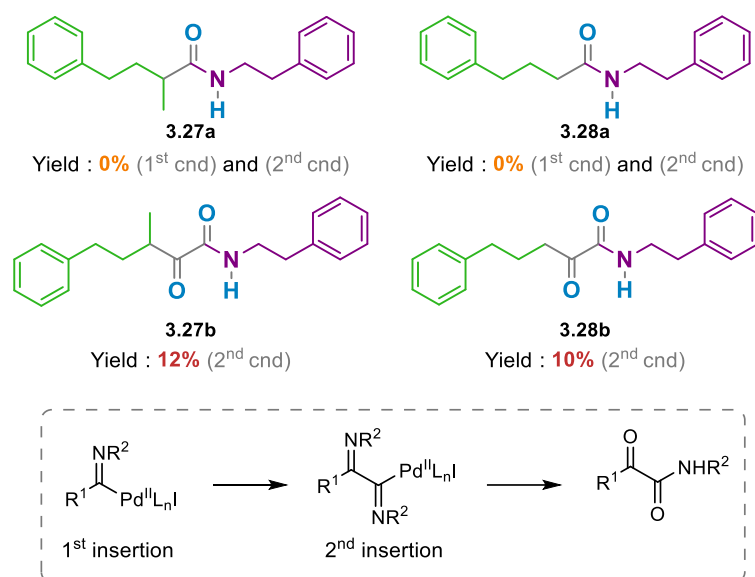


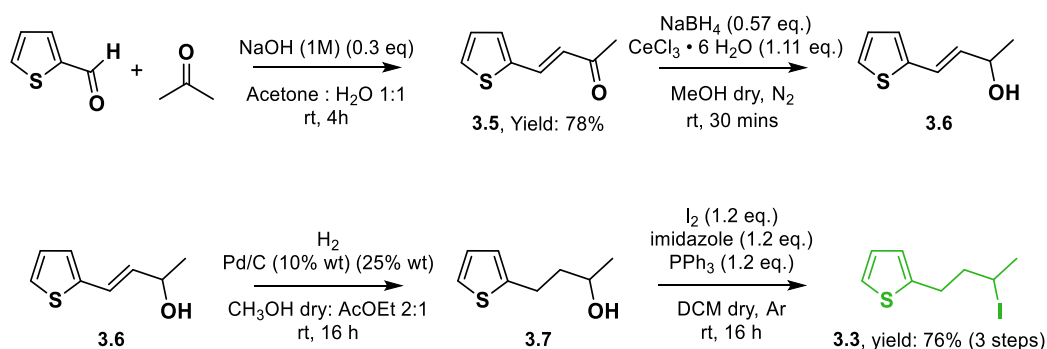
Figure 3.5: structures of the double insertion products (bottom) and expected products (top). (1st cnd: iodide (1.0 eq), isocyanide (1.1 eq), Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 mmol), blue light, rt, 48 h, Ar, degassed anhydrous toluene (0.3 M); 2nd cnd: iodide (1.0 eq), isocyanide (2.2 eq), Pd(PPh₃)₄ (6 mol%), DMAP (6 mol%), Et₃N (1.7 eq), H₂O (40 mmol), blue light, rt, 48 h, Ar, degassed anhydrous toluene (0.3 M)). Reaction performed on a 100 mg scale

3.6 Preparation of the iodides

All the iodides used for the preparation of the library of amidic products are not commercially available and they needed to be synthesized. In the next paragraph are reported only the iodides synthesized by me, while all the other were prepared by Master's and Bachelor's students.

3.6.1 Preparation of 2-(3-iodobutyl)thiophene (**3.3**)

The first iodide that was synthesized was 2-(3-iodobutyl)thiophene **3.3**.



Scheme 3.13: multistep synthesis of iodide **3.3** from 2-thiophenecarboxaldehyde.

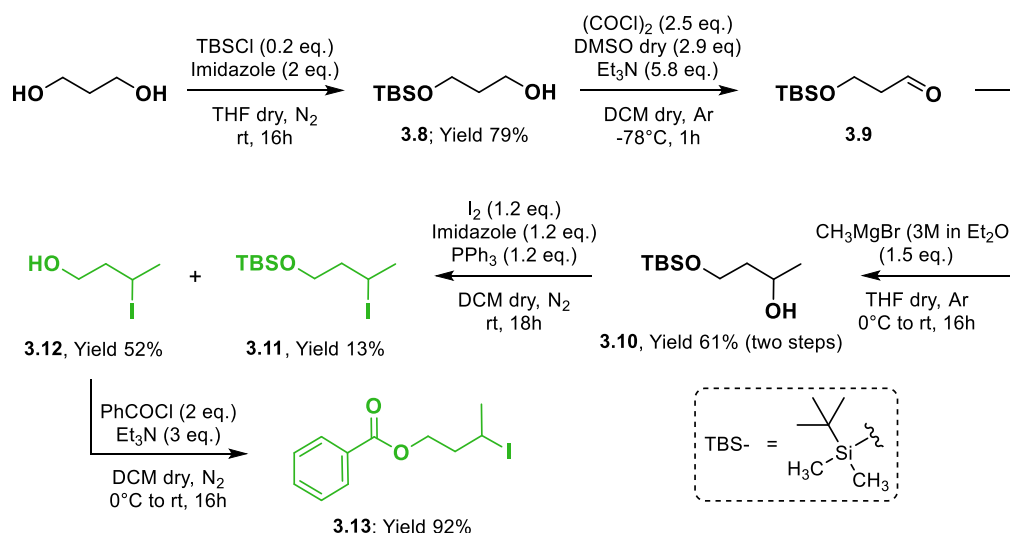
The entire straightforward preparation of the iodide is reported in Scheme 3.13. The synthesis started with an aldol condensation between acetone and 2-thiophenecarboxaldehyde. The former compound act as the nucleophile, after the *in-situ* formation of the enolate anion as a

consequence of the acid-base reaction with sodium hydroxide, instead the latter compound acts as the electrophile. The preparation of compound **3.5** is reported in literature but following those conditions the product was recovered in 63% yield and many poly-aldol addition products were found in the reaction crude. When the reaction mixture was reacted for only 4h, the conjugated ketone **3.5** was isolated in a satisfying 78% yield and the byproducts were drastically reduced.

The Luche reduction²⁷ was performed as the following reaction. Ketone **3.5** was reduced in presence of stoichiometric NaBH₄ and catalytic CeCl₃ and no issues were encountered, leading smoothly to the desired allylic alcohol **3.6** with no impurities. The crude reaction mixture was then directly subjected to hydrogenation in the presence of H₂ and catalytic palladium on carbon (Pd/C), yielding quantitatively the saturated product **3.7**. The hydrogenation of the C–C double bond was performed to remove a functional group that could interfere with the subsequent isocyanide insertion, as interactions between carbon–carbon π bonds and palladium are well documented²⁸. After filtration of the supported catalyst, the saturated alcohol was directly converted into iodide **3.3** without purification. The final transformation was an Appel reaction, carried out in the presence of triphenylphosphine, imidazole, and iodine. The product was obtained after chromatographic purification in a remarkable 76% overall yield over three steps.

3.6.2 Preparation of a series of iodides deriving from 1,3-propanediol

In this paragraph is reported the synthesis of iodides **3.11**, **3.12** and **3.13** starting from commercial 1,3-propanediol.



Scheme 3.14: multistep synthesis of iodides **3.11**, **3.12** and **3.13** from 1,3-propanediol

As the first reaction, one of the two hydroxy group of 1,3-propanediol was protected with TBSCl (*tert*-butyldimethylsilyl chloride), affording the mono protected alcohol **3.8** in 79% yield after silica column chromatography. The mono-protection of symmetric diols are challenging reactions, because statistically a complicated mixture of both double protected and mono protected alcohol could be obtained. Thus, the reaction was performed using the protecting agent as the limiting reagent and the commercial diol in large excess, like what was done in Scheme 2.3 (mono-

allylation reaction). In this case *t*-butyldimethylsilyl was chosen as protecting group for its reliability and chemical stability under oxidation conditions and in the presence of strong nucleophiles. The alcohol **3.8** was oxidized to aldehyde **3.9**, under the Swern oxidation conditions, and the freshly prepared compound was immediately subjected to a nucleophilic addition reaction using CH_3MgBr as nucleophile. The chiral secondary alcohol **3.10** was isolated after chromatographic purification in 61% yield over two steps. On intermediate **3.10** the iodination under Appel condition was carried out, to convert the secondary hydroxy group into an iodine in presence of triphenylphosphine, imidazole and iodine. Normally, the Appel iodination is a mild reaction which condition are not capable of affording the silane deprotection, however after chromatographic purification iodides **3.11** and **3.12** were collected in 13% and 52% yield respectively, despite the full conversion of the starting material. It is still unclear how and why this happened. Unexpectedly, in a single reaction two suitable iodides for the final reaction scope were obtained. The third one was prepared subjecting the deprotected alcohol **3.12** to an esterification reaction with benzoyl chloride, which yielded the desired iodide **3.13** in 92% yield after a silica column chromatography.

3.7 Study of the mechanism

Parallel to the optimization of the reaction conditions, the mechanism of the photoactivated isocyanide insertion was studied. To gain more knowledge, it was decided to perform two experiments: a radical trapping test and an on/off experiment. In the introduction (paragraph 3.2) was reported how palladium complexes in the excited state can carry out single electron transfer oxidative addition, and the radical trapping experiment was ideated to prove this. This experiment was performed before my contribution to the project. 2.0 eq of TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxyl) were added to the reaction mixture and the outcome of the reaction was analysed by GC-MS. TEMPO is a radical trapping reagent, which blocks any radical that the reaction forms by binding covalently to it, and from the chromatograph a substance was identified which absolute mass corresponded to the secondary alkyl radical bound to TEMPO.

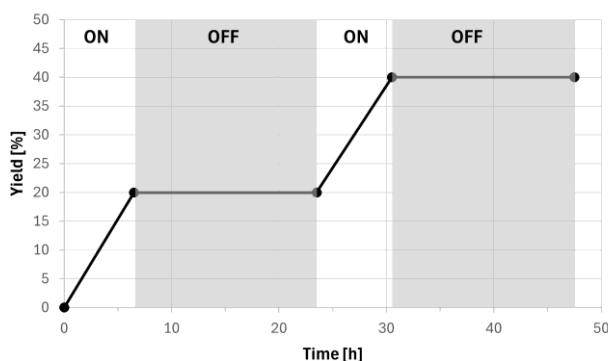


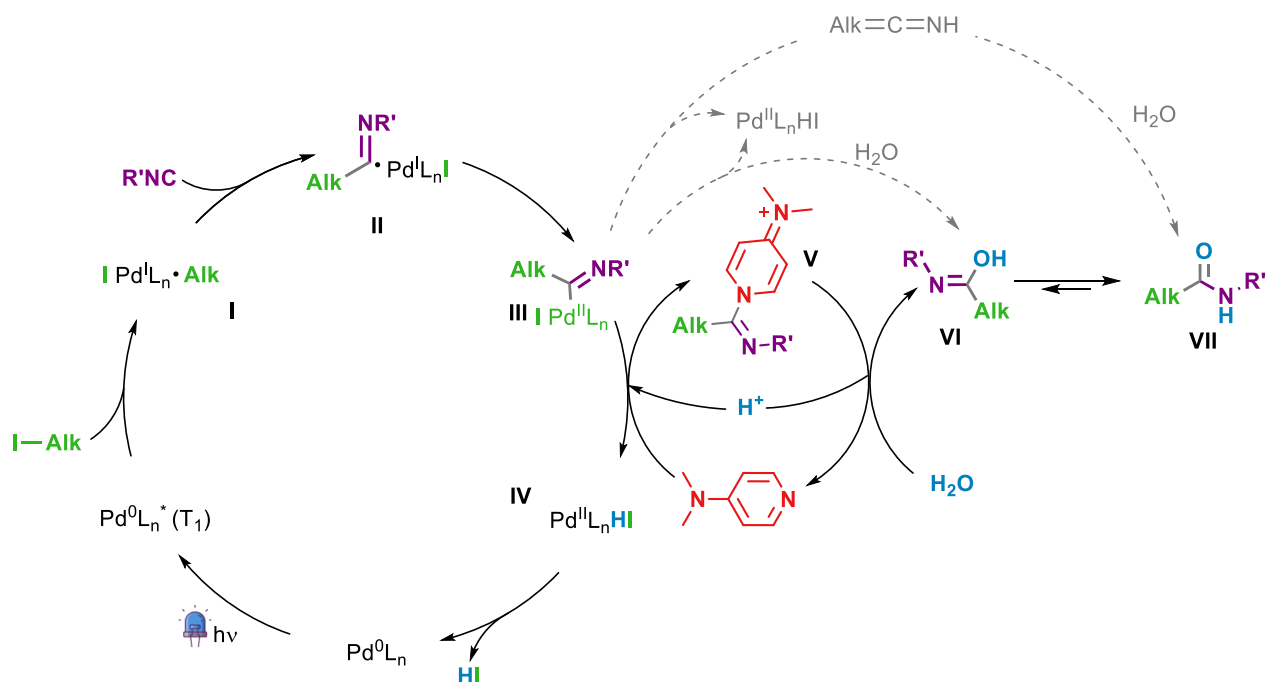
Figure 3.6: On/off experiment. The yields of the amide **3.2** were determined by ^1H NMR using 4-iodoanisole as internal standard

The on/off irradiation experiment was designed to exclude the involvement of a radical chain mechanism. The reaction between iodide **3.1** and *t*-BuNC was carried out under the conditions

established in the initial optimization (iodide 1.0 equiv, isocyanide 1.1 equiv, $\text{Pd}(\text{PPh}_3)_4$ 6 mol%, DMAP 6 mol%, Et_3N 1.7 equiv, H_2O 40 mmol, blue LED light, rt, 48 h, Ar atmosphere, degassed anhydrous toluene, 0.3 M). As illustrated in *Figure 3.6*, the product yield increased only during periods of irradiation, whereas no further conversion was observed when the reaction was kept in the dark. This behaviour supports a photocatalytic radical cycle and confirms that the product remains stable under the reaction conditions.

To perform the on/off experiment, four identical reactions were set up in separate vials. All vials were irradiated for 7 h, after which 4-iodoanisole (1 eq. internal standard) was added to one vial before workup. The crude mixture was analysed by ^1H NMR, and the yield was determined by integrating the signals corresponding to the product and the internal standard. The remaining three vials were kept in the dark for an additional 17 h. After this period, one vial was worked up and analysed by ^1H NMR, while the remaining two vials were subjected to another irradiation–dark cycle.

After the collection of all data regarding: the experimental reaction yields, which component has a stronger influence in reaction outcome, the radical trapping test and an on/off experiment, the reaction mechanism was proposed and is reported in *Scheme 3.15*.



Scheme 3.15: ideated mechanism of the light activated palladium catalysed isocyanide insertion reaction; (in grey are reported alternative paths)

Briefly, the reaction mechanism starts with the photoexcitation of $\text{Pd}(\text{PPh}_3)_4$ to $\text{Pd}(0)^*$ (T_1), in the excited state it can interact with the alkyl iodide generating the hybrid alkyl-Pd(I) di-radical interaction (I) through a smooth SET OA. Intermediate I reacts with the isocyanide delivering an imidoyl radical (II) which is immediately captured by the $\text{Pd}(\text{II})$ specie giving an imidoyl-Pd(III) (III). During the optimization of the reaction, it was highlighted the importance of DMAP, and, it is believed that the nitrogen of DMAP attacks the intermediate III forming the imidoyl-DMAP

intermediate **V**. This supposition is based on the DMAP ability to accelerate the acyl substitution reaction rate. Intermediate **V** is highly activated, and water can perform a rapid nucleophilic substitution with the concomitant formation of the tautomer of the amide **VI** and release of DMAP, which can be employed in catalytic amounts. In this reaction mechanism, DMAP is believed to have a dual role, it can work as a nucleophile and as a phase transfer catalyst, because the imido-DMAP intermediate is cationic and more soluble in water where the reaction can continue. While the product is transferred in the aqueous layer, in solution the H-Pd(II)-I species **IV** undergoes a reductive elimination, releasing HI (quenched by Et₃N) and regenerating the Pd(0) catalyst. Parallel to the steps here described can happen other secondary reactions that could lead to the product formation (*Scheme 3.15 in grey*). The simple addition of water, instead of DMAP, can be excluded as previously reported (*entry 1 Table 3.1*). The ketenimine side-mechanism is reported in a study from imido-Pd²⁹, although, in that case, the β -H elimination occurs in a benzylic position and the extended conjugation of the ketenimine might be the driving force. The ¹H-NMR spectrum of an experiment conducted in absence of DMAP and water and worked-up in presence of 1M solution HCl proved an iodide:product ratio of 9 to 1, suggesting that the ketenimine is not playing a crucial role under our conditions. Nonetheless all the secondary mechanisms reported could be possible, they are less relevant for the formation of the product, since the absence of DMAP leads to dramatic decrease in the isolated yield of the product.

3.8 Conclusions

In this part of the thesis, one of the three research projects followed during the PhD was described. The initial experimental investigations, conducted to determine the optimal conditions for the reaction, were reviewed and improved. A new parameter was introduced, temperature, which was demonstrated to have a big impact on the outcome of the reaction. Moreover, during the second optimization of the reaction it was shown, with surprise, that the transformation can be performed even without the use of phosphine-based ligands for palladium. This highlighted how isocyanides can substitute phosphines in the coordination sphere of the catalyst, however a higher excess of that precious component is essential. The reaction scope was explored, leading to the synthesis of a diverse range of amides in good to high yields. The experimental results highlighted a broad compatibility to a variety of functional groups and the significant influence of steric hindrance of the reaction partners, with the most effective combination being secondary iodides and tertiary isocyanides. A higher steric hindrance of the iodide leads to a total suppression of the reactivity. Instead, a low steric hindrance of both partners causes the formation of the double isocyanide insertion products, although in a low yield, and the presence of the desired amides was not observed.

Finally, the reaction mechanism was investigated. Specific experiments were carried out: on-off experiment and radical trapping experiment. Based on the results collected from those experiments and the reaction optimization, a mechanism, in a true multicomponent fashion, was

hypothesized and discussed. The radical nature of the mechanism was demonstrated as well as the relevance of DMAP for the correct formation of the product.

3.9 Experimental procedures

3.9.1 General aspects

Solvents and reagents

Commercial solvents and reagents were used directly without any other purification, unless differently indicated. In every reaction performed under inert atmosphere, it was used commercial dry solvents taking every possible precaution (syringe and Luer-lock®).

Routine procedure

Every piece of glassware was dried and stored in oven at 100°C. TLC analyses were carried out on pre-coated Merck silica gel 60 F254 plates and viewed at UV (254 nm) and developed with Hanessian stain (dipping into a solution of 21 g $(\text{NH}_4)_4\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ and 1g $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$ in 31 mL of H_2SO_4 and 469 mL of H_2O and heating, indicated for simplicity with CAM), KMnO_4 Basic (dipping into a solution of 1.5 g of KMnO_4 , 10 g of K_2CO_3 , 1 mL of 10% aq. NaOH in 200 mL of deionized water, and heating gently) and 20 % H_2SO_4 in ethanol (spraying with a solution of 20 mL of 96% sulphuric acid solution in 80 mL of EtOH and heating). R_f were measured after an elution of 7–9 cm. The chromatographic purification on silica was performed in flash modality (under compressed air pressure) using silica gel Ge Duran SI 60 Å (230–400 mesh). All products were characterized by ^1H , ^{13}C , IR, GC-MS and melting point when possible.

Nuclear magnetic resonance spectroscopic analysis (NMR)

^1H and ^{13}C NMR spectra were recorded on a JEOL 400 (at 400 MHz and 101 MHz respectively). NMR spectra were recorded using residual solvent and TMS as the internal standard ^1H NMR: TMS = 0.00 and ^{13}C NMR: CDCl_3 = 77.16. Data for ^1H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity, coupling constants (Hz) and integration. Data for ^{13}C NMR spectra are reported in terms of chemical shift (δ ppm). Interpretation of spectra has been made also with the aid of COSY, HSQC and HMBC experiments. The following abbreviations are used to indicate the multiplicity in NMR spectra: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and their combinations; the b in front of the multiplicity stands for a broad signal. For some compounds the extra peaks are due to residual undefined impurities (<5%) due to difficulties in the purification. All the NMR spectrums were analysed using the software MestReNova (Mestrelab Research® v. 14.2).

Infrared absorption spectrums (IR)

Infrared spectra were recorded as solid, oil or amorphous solids samples with the ATR (attenuated total reflectance) method using the spectrometer FT Perkin Elmer Spectrum 65. Data for IR absorption peaks are reported as decreasing wavenumber (cm^{-1}). All the spectrums were

elaborated using the software Spectrum (PerkinElmer® v.10.03). Each absorption spectrum was processed with a 10% smooth algorithm.

Gas chromatography Mass spectrum coupled (GC-MS) analysis

GC-MS analyses were carried out on Shimadzu GC-MS-QP2010 SE with an AOC-20i Plus auto-injector mounting an Avantor Hichrom HI-5 MS column (internal diameter 0.25 mm, film thickness 0.25 mm, length 30 m). A Mass Analyzer Metal quadrupole mass filter with pre-rods was used and the ionization was obtained through Electronic Impact operating with an ionization potential of 70 eV. He was used as gas carrier keeping a flux through the column of 1.0 mL/min. The split ratio used is 1:10. Samples were prepared upon dilution of a mother solution of an estimated 1000 ppm concentration to 100 ppm in ethyl acetate. The amount of sample injected is 1 µL.

Two different methods were applied for the analysis:

- Method A: solvent delay 2.5 min, mass range 35–400, injector temperature 250 °C, detector temperature 250 °C, MS temperature around 200 °C. Starting temperature 100 °C, starting time 3 min, temperature gradient 25 °C/min, final temperature 300 °C.
- Method B: solvent delay 2.5 min, mass range 35–400, injector temperature 250 °C, detector temperature 250 °C, MS temperature around 200 °C. Starting temperature 70 °C, starting time 3 min, temperature gradient 25 °C/min, final temperature 300 °C.

All the chromatograph and MS fragmentation patterns were analysed using the software GCMS LabSolution Shimadzu (Version 4.52) Shimadzu.

Melting point analysis (m.p.)

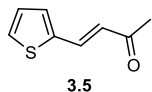
The melting point of every solid product was determined with the instrument Buchi 565. The thermal program used is 1°C/min

Photochemical Apparatus

Batch reactions were conducted in a dedicated apparatus consisting of an aluminum cooling plate (LWH 160x100x25 mm) with 6 OSRAM® Oslon SSL 80 LDCQ7P (nominal 450 nm, royal blue) LEDs mounted in series powered by a MeanWell® LPC-20-700 constant current power supply (700 mA) and a water-cooled aluminum vessel holder. The vessel holder (LWH 170x110x38 mm) holds the sealed vials 10 mm over the LEDs in a fixed position while cooling both the LED plate and the vial, keeping the temperature of the latter below 20°C. Both the LED plate and the water-cooled vessel were custom built, while the vials were purchased from Wicom International Co. (WIC43005, 5 mL crimp top vial 38.5x22.0 mm; WIC44510 20 mm crimp caps with 3.0 mm PTFE septum). For reactions performed under controlled temperature the vessel holder was connected to a Lab. Companion RW-0525G. The temperature used was 25°C and 35°C.

3.9.2 Preparation of 2-(3-iodobutyl)thiophene (**3.5**)

Synthesis of (*E*)-4-(thiophen-2-yl)but-3-en-2-one (**3.5**)



Thiophene-2-carbaldehyde (1.38 mL, 15.16 mmol) was solubilized in acetone/H₂O (1:1, 19 mL), then 1 M NaOH (5.0 mL, 5.00 mmol) was slowly added. After 4 h at rt, 1 M HCl was added until pH ~6: The mixture was extracted with AcOEt, washed with brine, dried (Na₂SO₄), filtrated and evaporated to dryness. The crude product was purified by FC on silica (PE/Et₂O 8:2) to give the product as a bright-yellow oil (594 mg, 78% yield).

MW: 152,21 (C₈H₈OS)

TLC: R_f 0.40 (ETP/Et₂O 8:2, UV, CAM)

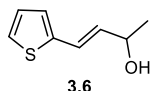
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.64 (d, *J* = 15.9 Hz, 1H, **CH=CHCO**), 7.43 – 7.39 (m, 1H, **CH_{arom}**), 7.31 – 7.29 (m, 1H, **CH_{arom}**), 7.08 (dd, *J* = 5.1, 3.6 Hz, 1H, S-CH**CH_{arom}**), 6.54 (d, *J* = 15.9 Hz, 1H, **CH=CHCO**), 2.35 (s, 3H, **CH₃**).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 197.9 (**C=O**), 139.9 (**CHC_{quat arom}-CH**), 135.9 (**CH=CHCO**), 131.7 (**CH_{arom}**), 129.1 (**CH_{arom}**), 128.4 (S-CH**CH_{arom}**), 126.0 (**CH=CHCO**), 27.8 (**CH₃**).

GC-MS: Rt= 6.815 min (Method A); m/z (Relative Intensity %): 38 (6), 39 (27), 43 (33), 45 (20), 50 (7), 51 (10), 58 (7), 63 (10), 65 (41), 69 (15), 108 (7), 109 (74), 110 (6), 137 (100), 138 (9), 139 (5), 151 (9), 152 (51) [M]⁺, 153 (5)

IR wavenumber (cm⁻¹): 3103, 3006, 1684, 1661, 1591, 1514, 1422, 1356, 1305, 1270, 1251, 1225, 1198, 1167, 1096, 1079, 1045, 1000, 961, 904, 854, 816, 702, 632

Synthesis of (*E*)-4-(thiophen-2-yl)but-3-en-2-ol (**3.6**)



Ketone **3.5** (1.616 g, 10.6 mmol) was solubilized in dry methanol (53 mL) under N₂ atmosphere. CeCl₃·6 H₂O (4.40 g, 11.8 mmol) was added, then the reaction flask was cooled to 0 °C and NaBH₄ (229 mg, 6.05 mmol) was added portion-wise. The reaction mixture was warmed to rt. The bright-yellow mixture turned pale pink over 30 min. The solvent was removed by rotavapor, and the residue was extracted with H₂O and Et₂O. The organic phases were washed with brine, dried (Na₂SO₄), filtrated and evaporated to dryness. The crude α,β-unsaturated alcohol (1.531 g, pale yellow oil) was sufficiently pure to be used in the next step without further purification.

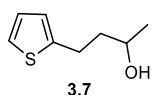
MW: 154,23 (C₈H₁₀OS)

TLC: R_f 0.35 (ETP/Et₂O 7:3, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.20 – 7.12 (m, 1H, **CH_{arom}**), 6.99 – 6.92 (m, 2H, 2x **CH_{arom}**), 6.71 (d, *J* = 15.7 Hz, 1H, **CH=CHCHOH**), 6.11 (dd, *J* = 15.7, 6.3 Hz, 1H, **CH=CHCHOH**), 4.53 – 4.37 (m, 1H, **CH=CHCHOH**), 1.55 (bd, *J* = 3.9 Hz, 1H, **OH**), 1.36 (d, *J* = 6.4 Hz, 3H, **CH₃**).

GC-MS: Rt= 6.525 min (Method A); m/z (Relative Intensity %): 38 (4), 39 (28), 41 (6), 43 (48), 44 (3), 45 (37), 50 (8), 51 (14), 52 (4), 53 (10), 55 (55), 57 (4), 58 (10), 59 (5), 62 (4), 63 (10), 65 (20), 66 (7), 67 (12), 69 (14), 70 (6), 71 (5), 74 (3), 77 (24), 78 (8), 79 (3), 82 (3), 84 (26), 85 (8), 91 (21), 95 (4), 97 (100), 98 (7), 99 (4), 103 (4), 108 (4), 109 (11), 110 (9), 111 (85), 112 (7), 113 (4), 121 (6), 134 (5), 135 (27), 136 (15), 137 (8), 139 (12), 154 (35) [M]⁺, 155 (3)

Synthesis of 4-(thiophen-2-yl)butan-2-ol (**3.7**)



The crude α,β -unsaturated alcohol **3.6** (1.531 g, 9.93 mmol) was solubilized in dry MeOH/AcOEt (2:1, 99 mL) under N₂ atmosphere, then 10 % wt. Pd/C (0.383 g, 25% wt. respect to crude alcohol) was added. The inert atmosphere was replaced by H₂, and after 18 h the mixture was filtered through celite using AcOEt for washing and evaporated to dryness. The crude alcohol (1.551 g, pale yellow oil) was sufficiently pure to be used in the next step without further purification.

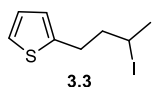
MW: 156,24 (C₈H₁₂OS)

TLC: R_f 0.33 (ETP/Et₂O 7:3, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.12 (dd, J = 5.1, 1.2 Hz, 1H, S-CH_{arom}CHCHC-), 6.92 (dd, J = 5.1, 3.4 Hz, 1H, S-CHCH_{arom}CHC-), 6.83 – 6.80 (m, 1H, S-CHCHCH_{arom}C-), 3.87 (dt, J = 11.1, 6.1 Hz, 1H, CH₂CH₂CHOH), 3.04 – 2.86 (m, 2H, -CH₂CH₂CHOH), 1.88 – 1.78 (m, 2H, -CH₂CH₂CHOH), 1.41 (s, 1H, OH), 1.24 (d, J = 6.2 Hz, 3H, CH₃).

GC-MS: Rt= 6.050 min (Method A); m/z (Relative Intensity %): 39 (18), 41 (5), 43 (15), 45 (52), 50 (3), 51 (6), 53 (15), 57 (4), 58 (6), 59 (3), 63 (3), 65 (5), 66 (3), 67 (5), 69 (6), 71 (5), 77 (10), 78 (4), 79 (6), 84 (13), 85 (8), 97 (100), 98 (41), 99 (7), 105 (3), 109 (3), 110 (5), 111 (11), 123 (79), 124 (7), 125 (4), 137 (6), 138 (20), 139 (2), 156 (17) [M]⁺

Synthesis of 2-(3-iodobutyl)thiophene (**3.3**)



PPh₃ (2.935 g, 11.2 mmol) and imidazole (762 mg, 11.2 mmol) were solubilized in DCM (3.7 mL) under N₂ atmosphere. After 10 min, iodine (110 mg, 11.2 mmol) was slowly added. Alcohol **3.7** (1.456 g, 9.32 mmol) was then added by syringe as a solution in anhydrous DCM (19 mL). After stirring for 16 h at rt, Et₂O was added, and the mixture was filtered to remove the precipitated imidazolium salts and triphenylphosphine oxide. The mother liquor was evaporated to dryness and the crude product was purified by silica FC (ETP/DCM 9:1) to deliver the product as a pale-yellow oil (1.884 g, 76 % yield three steps)

MW: 265,96 (C₈H₁₁IS)

TLC: R_f 0.6 (ETP/DCM 9:1, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.14 (dd, J = 5.1, 1.2 Hz, 1H, S-CH_{arom}CHCHC-), 6.93 (dd, J = 5.1, 3.4 Hz, 1H, S-CHCH_{arom}CHC-), 6.84 (dd, J = 3.4, 0.9 Hz, 1H, S-CHCHCH_{arom}C-), 4.14 (dq, J = 9.4, 6.8, 4.4 Hz, 1H, CH₂CH₂CHI), 3.06 (ddd, J = 14.2, 8.3, 5.2 Hz, 1H, CHHCH₂CHI), 2.94 (dt, J = 15.3, 7.8 Hz, 1H, CHHCH₂CHI), 2.24 – 2.13 (m, 1H, CH₂CHHCHI), 1.99 – 1.86 (m, 4H, CH₂CHHCHI and CH₃).

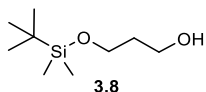
¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 143.3 (S-CHCHCHC_{quat, arom}-), 127.0 (S-CHCH_{arom}CHC-), 124.9 (S-CHCHCH_{arom}C-), 123.5 (S-CH_{arom}CHCHC-), 44.5 (CH₂CH₂CHI), 30.3 (CH₂CH₂CHI), 29.1 (CH₂CH₂CHI), 29.1 (CH₃).

GC-MS: Rt= 7.350 min (Method A); m/z (Relative Intensity): 39 (8), 41 (3), 45 (13), 53 (10), 69 (3), 97 (100), 98 (7), 99 (5), 123 (3), 139 (12), 266 (9) [M]⁺

IR wavenumber (cm⁻¹): 2914, 1535, 1440, 1377, 1295, 1242, 1197, 1165, 1130, 1080, 1037, 995, 902, 848, 822, 800, 768, 744, 690, 609

3.9.3 Preparation of tert-butyl(3-iodobutoxy)dimethylsilane (**3.11**), 3-iodobutan-1-ol (**3.12**) and 3-iodobutyl benzoate (**3.13**)

Synthesis of 3-((tert-butyldimethylsilyl)oxy)propan-1-ol (**3.8**)



1,3-Propanediol (4.7 mL, 65.7 mmol) was solubilized in dry THF (66 mL) under Ar atmosphere, then imidazole (8.945 g, 131.4 mmol) was slowly added. When all the reagents were solubilized, tert-butyldimethylsilyl chloride (1.980 g, 13.14 mmol) was added. After 16 h, the solvent was removed using the rotavapor and the crude mixture was diluted with water and extracted with Et₂O. The organic phases were washed with brine, dried (Na₂SO₄), filtrated and evaporated to dryness. The crude product was purified by silica FC (PE/AcOEt 8:2) delivering the product as colourless oil (1.976 g, 79% yield).

MW: 190.36 (C₉H₂₂O₂Si)

TLC: R_f 0.31 (ETP/AcOEt 8:2, UV, CAM, basic KMnO₄)

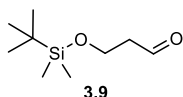
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 3.87 – 3.82 (m, 2H, SiO-CH₂CH₂CH₂-O), 3.81 – 3.77 (m, 2H, SiO-CH₂CH₂CH₂-O), 2.64 (s, 1H, OH), 1.78 (p, J = 5.6 Hz, 2H, SiO-CH₂CH₂CH₂-O), 0.90 (s, 9H, Si-C(CH₃)₃), 0.08 (s, 6H, Si(CH₃)₂)

¹³C NMR (100 MHz, CDCl₃, 27 °C) δ 63.1 (SiO-CH₂CH₂CH₂-O), 62.6 (SiO-CH₂CH₂CH₂-O), 34.4 (SiO-CH₂CH₂CH₂-O), 26.0 (C(CH₃)₃), 18.3 (C(CH₃)₃), -5.4 ppm (Si(CH₃)₂)

GC-MS: Rt= 7,570 min (Method B); m/z (Relative Intensity %): 41 (7), 43 (3), 45 (9), 47 (6), 57 (3), 59 (6), 61 (3), 73 (8), 74 (3), 75 (100), 76 (7), 77 (5), 87 (3), 91 (3), 105 (35), 106 (3), 133 (13)

IR wavenumber (cm⁻¹): 3350, 2953, 2929, 2857, 1472, 1388, 1361, 1254, 1084, 1006, 960, 939, 832, 773, 719, 661

Synthesis of 3-((tert-butyldimethylsilyl)oxy)propanal (**3.9**)



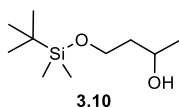
Dry DMSO (1.165 mL, 16.40 mmol) was solubilized in dry DCM (30 mL) under inert atmosphere, Ar, in a 2-neck flask. The mixture was cooled to -78 °C and after 5 min (COCl)₂ (1.43 M in dry DCM, 9.9 mL, 14.16 mmol) was added. After 10 min a solution of the alcohol **3.8** (1.0766 g, 5.66 mmol) in dry DCM (20 mL) was added dropwise. 5 mL of dry DCM were used to wash the flask containing **3.8** to transfer it quantitatively. After 15 min Et₃N (4.6 mL, 32.80 mmol) was added dropwise. After 1 h at -78 °C, a 5% aq. solution of (NH₄)H₂PO₄ was added while the reaction flask was let warm to rt. 2M HCl was added until pH 5, then the mixture was extracted with Et₂O. The organic phases were washed with brine, dried (Na₂SO₄), filtrated and evaporated to dryness. The crude aldehyde (1.066 g, yellowish oil) was sufficiently pure to be used in the next step without further purification.

MW: 188.34 (C₉H₂₀O₂Si)

TLC: R_f 0.69 (ETP/AcOEt 8:2, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27°C) δ 9.81 (s, 1H, -CHO), 3.99 (t, J = 6.0 Hz, 2H, SiO-CH₂CH₂-CHO), 2.60 (td, J = 6.2, 2.1 Hz, 2H, SiO-CH₂CH₂-CHO), 0.88 (s, 9H, C(CH₃)₃), 0.07 (s, 6H, Si(CH₃)₂).

Synthesis of 4-((*tert*-butyldimethylsilyl)oxy)butan-2-ol (**3.10**)



The crude aldehyde **3.9** (5.66 mmol) was solubilized in dry THF (28 mL) under Ar atmosphere. The flask was cooled to 0°C and a solution of CH₃MgBr (3 M in Et₂O, 2.83 mL, 8.49 mmol) was slowly added. The flask was let warm to rt and after 16 h s.s. NH₄Cl was slowly added. The mixture was extracted with Et₂O, and the organic phases were washed with brine, dried (Na₂SO₄), filtrated and evaporated to dryness. The crude product was purified by silica FC (ETP/AcOEt 9:1) delivering the product as colourless oil (787 mg, 68% yield two steps).

MW: 204.39 (C₁₀H₂₄O₂Si)

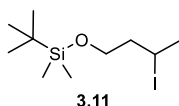
TLC: Rf 0.28 (ETP/AcOEt 9:1, UV, CAM, basic KMnO₄)

¹H NMR (400 MHz, CDCl₃, 27°C) δ 4.08 – 3.98 (m, 1H, SiO-CH₂CH₂CHCH₃), 3.93 – 3.86 (m, 1H, SiO-CH₂CH₂CHCHCH₃), 3.85 – 3.78 (m, 1H, SiO-CH₂CH₂CHCHCH₃), 3.41 (d, *J* = 2.3 Hz, 1H, OH), 1.74 – 1.60 (m, 2H, SiO-CH₂CH₂CHCH₃), 1.20 (d, *J* = 6.2 Hz, 3H, SiO-CH₂CH₂CHCH₃), 0.90 (s, 9H, SiC(CH₃)₃), 0.09 – 0.07 (m, 6H, Si(CH₃)₂).

¹³C NMR (101 MHz, CDCl₃, 27°C) δ 68.5 (SiO-CH₂CH₂CHCH₃), 63.0 (SiO-CH₂CH₂CHCH₃), 40.0 (SiO-CH₂CH₂CHCH₃), 26.0 (SiC(CH₃)₃), 23.5 (SiO-CH₂CH₂CHCH₃), 18.3 (SiC(CH₃)₃), -5.4 (SiCH₃), -5.4 (SiCH₃).

GC-MS: Rt= 7.745 min (Method A); m/z (Relative Intensity %): 39 (3), 41 (6), 43 (6), 45 (14), 47 (6), 55 (12), 57 (5), 58 (3), 59 (7), 61 (3), 73 (12), 74 (3), 75 (100), 76 (7), 77 (5), 87 (3), 89 (4), 91 (4), 101 (4), 105 (66), 106 (5), 107 (3), 129 (3), 147 (4)

Synthesis of *tert*-butyl(3-iodobutoxy)dimethylsilane (**3.11**)



PPh₃ (1.514 g, 4.64 mmol) and imidazole (316 mg, 4.64 mmol) were solubilized in DCM (3.7 mL) under N₂ atmosphere. After 10 min, iodine (1.178 g, 4.64 mmol) was slowly added. Alcohol **3.10** (0.791 g, 3.87 mmol) was then added by syringe as a solution in anhydrous DCM (2 mL). After stirring for 16 h at rt, Et₂O was added, and the mixture was filtered to remove the precipitated imidazolium salts and triphenylphosphine oxide. The mother liquor was evaporated to dryness and the crude product was purified by silica FC (ETP/DCM 8:2) to deliver *tert*-butyl(3-iodobutoxy)dimethylsilane (**3.11**) as a colourless oil (158 mg, 13 % yield) and (ETP/DCM 1:1) to deliver 3-iodobutan-1-ol (**3.12**) as a colourless oil (402 mg, 52 % yield)

MW: 314.28 (C₁₀H₂₃IOSi)

TLC: Rf 0.49 (ETP/DCM 8:2, UV, CAM, basic KMnO₄)

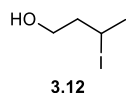
¹H NMR (400 MHz, CDCl₃, 27°C) δ 4.35 (dq, *J* = 9.5, 6.9, 4.5 Hz, 1H, SiO-CH₂CH₂CHCH₃), 3.80 – 3.71 (m, 1H, SiO-CH₂CH₂CHCH₃), 3.65 (ddd, *J* = 10.3, 8.1, 4.8 Hz, 1H, SiO-CH₂CH₂CHCH₃), 2.07 – 1.92 (m, 4H, SiO-CH₂CH₂CHCH₃ and SiO-CH₂CH₂CHCH₃), 1.85 – 1.72 (m, 1H, SiO-CH₂CH₂CHCH₃), 0.90 (s, 9H, SiC(CH₃)₃), 0.07 (d, *J* = 3.8 Hz, 6H, Si(CH₃)₂).

¹³C NMR (101 MHz, CDCl₃, 27°C) δ 62.8 (SiO-CH₂CH₂CHCH₃), 45.5 (SiO-CH₂CH₂CHCH₃), 29.3 (SiO-CH₂CH₂CHCH₃), 27.0 (SiO-CH₂CH₂CHCH₃), 26.1 (SiC(CH₃)₃), 18.4 (SiC(CH₃)₃), -5.1 (SiCH₃), -5.2 (SiCH₃).

GC-MS: Rt= 6.625 min (Method A); m/z (Relative Intensity %): 39 (21), 40 (4), 41 (50), 42 (6), 43 (23), 44 (6), 45 (34), 47 (25), 53 (5), 55 (81), 56 (10), 57 (34), 58 (28), 59 (47), 60 (7), 61 (15), 71 (5), 73 (76), 74 (8), 75 (100), 76 (7), 77 (4), 85 (9), 87 (7), 88 (12), 89 (27), 90 (3), 99 (12), 101 (22), 102 (3), 113 (3), 115 (17), 127 (3), 129 (37), 130 (5), 131 (5), 145 (5), 185 (65), 186 (5), 187 (4), 201 (6), 215 (95), 216 (8), 217 (3), 257 (72), 258 (9), 259 (3)

IR wavenumber (cm⁻¹): 2954, 2928, 2857, 1471, 1444, 1379, 1361, 1301, 1254, 1160, 1101, 1076, 1034, 1006, 991, 962, 939, 897, 831, 808, 774, 734, 662

Synthesis of 3-iodobutan-1-ol (**3.12**)



For the synthesis see section "Synthesis of *tert*-butyl(3-iodobutoxy)dimethylsilane"

MW: 200.02 (C₄H₉IO)

TLC: Rf 0.51 (ETP/DCM 1:1, UV, CAM, basic KMnO₄)

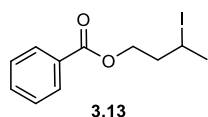
¹H NMR (400 MHz, CDCl₃, 27°C) δ 4.36 (dq, *J* = 9.7, 6.9, 4.4 Hz, 1H, O-CH₂CH₂CHCH₃), 3.83 (dt, *J* = 10.6, 5.3 Hz, 1H, O-CH₂CH₂CHCH₃), 3.74 (dq, *J* = 10.9, 5.3 Hz, 1H, O-CH₂CH₂CHCH₃), 2.09 – 1.96 (m, 4H, O-CH₂CH₂CHCH₃ + O-CH₂CH₂CHCH₃), 1.86 (dddd, *J* = 14.8, 8.1, 5.9, 4.4 Hz, 1H, O-CH₂CH₂CHCH₃), 1.52 (bs, 1H, OH).

¹³C NMR (101 MHz, CDCl₃, 27°C) δ 62.7 (O-CH₂CH₂CHCH₃), 44.9 (O-CH₂CH₂CHCH₃), 29.3 (O-CH₂CH₂CHCH₃), 26.2 (O-CH₂CH₂CHCH₃).

GC-MS: Rt= 6.520 min (Method A); m/z (Relative Intensity %): 38 (4), 39 (32), 40 (7), 41 (43), 42 (20), 43 (49), 44 (4), 45 (6), 53 (3), 55 (100), 56 (5), 57 (6), 73 (42), 127 (6), 200 (1) [M]⁺

IR wavenumber (cm⁻¹): 3317, 2915, 2882, 1444, 1417, 1377, 1300, 1253, 1218, 1149, 1123, 1091, 1047, 1022, 985, 946, 904, 846, 814, 780

Synthesis of 3-iodobutyl benzoate (**3.13**)



Alcohol **3.12** (250 mg, 1.25 mmol) was solubilized in dry DCM (13 mL) under N₂ atmosphere, then Et₃N (0.523 mL, 3.75 mmol) was added. The reaction flask was cooled to 0°C and PhCOCl (0.290 mL, 2.5 mmol) was dropwise added. After 16 h at rt, the solvent was reduced using a rotavapor and the crude mixture was extracted using Et₂O and a s.s. NH₄Cl. The organic phases were washed with brine, dried (Na₂SO₄), filtrated and evaporated to dryness. The crude product was purified by FC on silica (PE/ Et₂O 10:1) to give the product as colourless oil (350 mg, 92% yield).

MW: 304.13 (C₁₁H₁₃IO₂)

TLC: Rf 0.55 (ETP/ Et₂O 10:1, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27°C) δ 8.06 – 8.00 (m, 2H, 2x CH_{ortho,arom}), 7.61 – 7.53 (m, 1H, CH_{para,arom}), 7.45 (t, *J* = 7.7 Hz, 2H, 2x CH_{meta,arom}), 4.53 (dt, *J* = 11.2, 5.6 Hz, 1H, O-CH₂CH₂CHCH₃), 4.41 – 4.27 (m, 2H, O-CH₂CH₂CHCH₃ e O-CH₂CH₂CHCH₃), 2.27 (ddt, *J* = 14.6, 9.2, 5.4 Hz, 1H, O-CH₂CH₂CHCH₃), 2.18 – 2.08 (m, 1H, O-CH₂CH₂CHCH₃), 2.02 (d, *J* = 6.9 Hz, 3H O-CH₂CH₂CHCH₃).

¹³C NMR (101 MHz, CDCl₃, 27°C) δ 166.5 (C=O), 133.2 (CH_{para,arom}), 130.2 (C_{quat,arom}), 129.7 (2x CH_{ortho,arom}), 128.6 (2x CH_{meta,arom}), 64.8 (O-CH₂CH₂CHCH₃), 41.6 (O-CH₂CH₂CHCH₃), 29.1 (O-CH₂CH₂CHCH₃), 24.3 (O-CH₂CH₂CHCH₃).

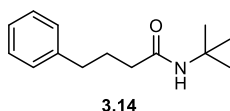
GC-MS: Rt= 9.040 min (Method A); m/z (Relative Intensity %): 39 (16), 41 (17), 42 (4), 43 (3), 50 (10), 51 (33), 53 (4), 54 (4), 55 (70), 56 (7), 76 (5), 77 (67), 78 (5), 79 (10), 105 (100), 106 (8), 123 (40), 124 (3), 177 (24), 178 (3)

IR wavenumber (cm⁻¹): 2959, 2914, 1715, 1602, 1584, 1492, 1450, 1380, 1314, 1267, 1174, 1108, 1097, 1069, 1026, 968, 846, 806, 707, 686, 675, 617

3.9.4 Amides preparation

General procedure used for isocyanide insertion reaction (2nd cnd): In a photochemical vial, the iodide (1 equiv) and DMAP (6 mol%) were added and then Ar was fluxed through a septum. Then, degassed anhydrous toluene (0.3 M), Et₃N (1.7 equiv), isocyanide (2.2 equiv), Pd(PPh₃)₄ (6 mol%), and bidistilled water (40 equiv) were added. The septum was replaced by the cap and crimped. The vial was placed in the photochemical plate and stirred for 48 h at 25 °C. The crude mixture was diluted with s.s. NH₄Cl, extracted with Et₂O, washed with brine, dried (Na₂SO₄), and evaporated to dryness. The crude product was purified by FC on silica.

Synthesis of *N*-(*tert*-butyl)-4-phenylbutanamide (**3.14**)



Following the general procedure: Primary iodide (100 mg, 0.406 mmol), DMAP (3.0 mg, 0.0244 mmol), Pd(PPh₃)₄ (28.2 mg, 0.0244 mmol), Et₃N (96 μL, 0.691 mmol), *t*-butylisocyanide (101 μL, 0.894 mmol) and bidistilled H₂O (293 μL, 16.3 mmol) in toluene dry (1.4 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (71 mg, 80% yield).

MW: 219.33 (C₁₄H₂₁NO)

TLC: R_f 0.20 (ETP/Et₂O 7:3, UV, CAM)

M.P.: 68.8 – 70.2 °C

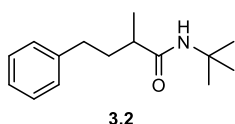
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.32 – 7.24 (m, 2H, 2x CH_{arom}), 7.22 – 7.16 (m, 3H, 2x CH_{arom} and CH_{arom, para}), 5.20 (bs, 1H, NH), 2.64 (t, *J* = 7.4 Hz, 2H, CH₂CH₂CH₂CO), 2.08 (t, *J* = 7.3 Hz, 2H, CH₂CH₂CH₂CO), 1.94 (p, *J* = 7.4 Hz, 2H, CH₂CH₂CH₂CO), 1.34 (s, 9H, C(CH₃)₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 172.1 (C=O), 141.8 (C_{quat, arom}), 128.7 (2x CH_{arom}), 128.5 (2x CH_{arom}), 126.0 (CH_{arom, para}), 51.2 (C(CH₃)₃), 36.9 (CH₂CH₂CH₂CO), 35.2 (CH₂CH₂CH₂CO), 29.0 (C(CH₃)₃), 27.2 (CH₂CH₂CH₂CO).

GC-MS: Rt= 8.705 min (Method A); m/z (Relative Intensity %): 39 (9), 41 (23), 42 (11), 43 (4), 44 (4), 51 (4), 55 (12), 56 (6), 57 (26), 58 (51), 59 (100), 60 (40), 65 (12), 72 (5), 77 (5), 78 (3), 91 (43), 92 (4), 100 (10), 104 (3), 105 (3), 115 (77), 116 (6), 117 (4), 129 (3), 147 (5), 219 (7) [M]⁺

IR wavenumber (cm⁻¹): 3308, 3070, 3030, 2966, 2931, 2864, 1665, 1639, 1546, 1497, 1453, 1421, 1390, 1360, 1281, 1227, 1158, 1133, 1081, 1033, 965, 923, 907, 843, 759, 738, 698, 667, 622

Synthesis of *N*-(*tert*-butyl)-2-methyl-4-phenylbutanamide (**3.2**)



Following the general procedure: Secondary iodide (**3.1**) (100 mg, 0.384 mmol), DMAP (2.8 mg, 0.0231 mmol), Pd(PPh₃)₄ (26.7 mg, 0.0231 mmol), Et₃N (91 μ L, 0.653 mmol), *t*-butylisocyanide (96 μ L, 0.845 mmol) and bidistilled H₂O (277 μ L, 15.36 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 8:2) delivering a pale-yellow solid (66 mg, 74% yield).

MW: 233,36 (C₁₅H₂₃NO)

TLC: Rf 0.29 (ETP/Et₂O 8:2, UV (weak), CAM (weak))

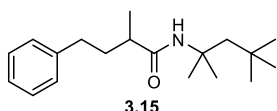
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.31 – 7.27 (m, 2H, 2x CH_{arom}), 7.24 – 7.11 (m, 3H, 3x CH_{arom}), 5.18 (s, 1H, NH), 2.66 (ddd, J = 14.3, 9.2, 5.3 Hz, 1H, CHHCH₂CH-CO), 2.61 – 2.51 (m, 1H, CHHCH₂CH-CO), 2.08 – 1.91 (m, 2H, CH₂CHHCH-CO e CH₂CH₂CH-CO), 1.71 – 1.64 (m, 1H, CH₂CHHCHCO), 1.36 (s, 9H, C(CH₃)₃), 1.12 (d, J = 6.6 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.6 (C=O), 142.0 (C_{quat}, arom), 128.6 (2x CH_{arom}), 128.5 (2x CH_{arom}), 126.0 (CH_{arom}, para), 51.2 (C(CH₃)₃), 41.6 (CH₂CH₂CHCO), 35.9 (CH₂CH₂CHCO), 33.7 (CH₂CH₂CHCO), 29.0 (C(CH₃)₃), 18.2 (CH₃).

GC-MS: Rt 8.665 min (Method A); m/z (Relative Intensity %): 39 (7), 41 (24), 42 (9), 44 (5), 51 (3), 55 (6), 56 (6), 57 (37), 58 (29), 65 (13), 69 (4), 72 (8), 73 (100), 74 (20), 77 (4), 86 (3), 91 (67), 92 (7), 100 (5), 104 (3), 114 (3), 115 (3), 129 (96), 130 (8)

IR wavenumber (cm⁻¹): 3309, 3068, 3029, 2969, 2926, 1642, 1544, 1496, 1481, 1453, 1389, 1360, 1286, 1259, 1225, 1094, 1068, 1033, 948, 928, 888, 790, 762, 742, 719, 695, 672

Synthesis of 2-methyl-4-phenyl-*N*-(2,4,4-trimethylpentan-2-yl)butanamide (**3.15**)



Following the general procedure: Secondary iodide **3.1** (100 mg, 0.384 mmol), DMAP (2.8 mg, 0.0231 mmol), Pd(PPh₃)₄ (26.7 mg, 0.0231 mmol), Et₃N (91 μ L, 0.653 mmol), 1,1,3,3-tetramethylbutylisocyanide (148 μ L, 0.845 mmol) and bidistilled H₂O (277 μ L, 15.36 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 8:2) delivering a pale-yellow solid (34 mg, 31% yield).

MW: 289,46 (C₁₉H₃₁NO)

TLC: Rf 0.27 (ETP/Et₂O 9:1, UV (weak), CAM (weak))

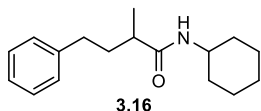
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.32 – 7.23 (m, 2H, 2x CH_{arom}), 7.22 – 7.13 (m, 3H, 3x CH_{arom}), 5.18 (s, 1H, NH), 2.71 – 2.50 (m, 2H, CH₂CH₂CHCO), 2.09 – 1.90 (m, 2H, CH₂CH₂CHCO e CH₂CHHCHCO), 1.81 (d, J = 14.9 Hz, 1H, CHH), 1.70 – 1.58 (m, 2H, CHH e CH₂CHHCHCO), 1.42 (s, 3H, CH₃), 1.39 (s, 3H, CH₃), 1.11 (d, J = 6.7 Hz, 3H, CHCH₃), 0.99 (s, 9H, C(CH₃)₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.2 (C=O), 142.1 (C_{quat}, arom), 128.6 (2x CH_{arom}), 128.5 (2x CH_{arom}), 126.0 (CH_{arom}, para), 55.3 (NHCH(CH₃)₂), 52.2 (CH₂), 41.8 (CH₂CH₂CHCO), 35.9 (CH₂CH₂CHCO), 33.7 (CH₂CH₂CHCO), 31.8 (C(CH₃)₃), 31.7 (C(CH₃)₃), 29.3 (C(CH₃)₃), 29.3 (C(CH₃)₃), 18.0 (CHCH₃).

GC-MS: Rt= 10.085 min (Method A), m/z (Relative Intensity %): 39 (4), 41 (20), 42 (7), 43 (8), 44 (3), 55 (12), 56 (5), 57 (40), 58 (100), 59 (4), 65 (9), 69 (7), 72 (8), 73 (77), 74 (9), 77 (3), 91 (88), 92 (8), 97 (15), 112 (4), 114 (14), 115 (3), 133 (8), 161 (13), 178 (9), 185 (28), 186 (3), 218 (8), 232 (5)

IR wavenumber (cm⁻¹): 3306, 3071, 2948, 2870, 1639, 1546, 1496, 1475, 1453, 1387, 1363, 1287, 1260, 1228, 1178, 1155, 1093, 1055, 1033, 956, 928, 903, 883, 861, 807, 785, 766, 742, 723, 693, 679

Synthesis of *N*-cyclohexyl-2-methyl-4-phenylbutanamide (**3.16**)



Following the general procedure: secondary iodide **3.1** (100 mg, 0.384 mmol), DMAP (2.8 mg, 0.0231 mmol), Pd(PPh₃)₄ (26.7 mg, 0.0231 mmol), Et₃N (91 μL, 0.653 mmol), *c*-hexylisocyanide (106 μL, 0.844 mmol) and bidistilled H₂O (277 μL, 15.36 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 6:4) delivering a pale-yellow solid (40 mg, 40% yield).

MW: 259,39 (C₁₇H₂₅NO)

TLC: Rf 0.28 (ETP/Et₂O 6:4, UV (weak), CAM (weak))

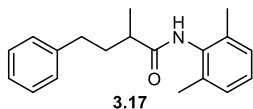
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.31 – 7.27 (m, 2H, 2x CH_{arom}), 7.22 – 7.15 (m, 3H, 3x CH_{arom}), 5.23 (bd, *J* = 7.5 Hz, 1H, NH), 3.86 – 3.74 (m, 1H, CH_{c-hex}), 2.66 (ddd, *J* = 14.7, 9.4, 5.6 Hz, 1H, CHHCH₂CHCO), 2.56 (ddd, *J* = 13.8, 8.9, 7.0 Hz, 1H, CHHCH₂CHCO), 2.15 – 2.05 (m, 1H, CH₂CH₂CHCO), 2.04 – 1.86 (m, 3H, CH₂CHHCHCO and CH_{2 c-hex}), 1.76 – 1.58 (m, 4H, CH_{2 c-hex}, CH₂CHHCHCO and CHH_{c-hex}), 1.45 – 1.30 (m, 2H, CH_{2 c-hex}), 1.22 – 1.04 (m, 6H, CHH_{c-hex}, CH₃ and CH_{2 c-hex}).

¹³C NMR (101 MHz, CHLOROFORM-*D*) δ 175.2 (C=O), 141.9 (C_{quat, arom}), 128.5 (2x CH_{arom}), 128.5 (2x CH_{arom}), 126.0 (CH_{arom, para}), 48.0 (CH_{c-hex}), 41.0 (CH₂CH₂CHCO), 35.9 (CH₂CH₂CHCO), 33.6 (CH₂CH₂CHCO), 33.5 (CH_{2 c-hex}), 33.3 (CH_{2 c-hex}), 25.6 (CHH_{c-hex}), 25.0 (2x CH_{2 c-hex}), 18.2 (CH₃).

GC-MS: Rt= 10.590 min (Method A); m/z (Relative Intensity %): 39 (6), 41 (16), 42 (3), 43 (6), 44 (5), 54 (4), 55 (21), 56 (13), 57 (4), 65 (10), 67 (6), 69 (4), 72 (4), 73 (11), 74 (100), 75 (4), 77 (3), 79 (3), 82 (5), 83 (6), 91 (70), 92 (6), 98 (5), 104 (3), 126 (11), 155 (64), 156 (7)

IR wavenumber (cm⁻¹): 3295, 3032, 2931, 2853, 2221, 1634, 1542, 1494, 1446, 1389, 1366, 1348, 1300, 1269, 1247, 1233, 1171, 1152, 1122, 1101, 1076, 1048, 1031, 973, 944, 891, 865, 846, 786, 749, 724, 697, 648

Synthesis of *N*-(2,6-dimethylphenyl)-2-methyl-4-phenylbutanamide (**3.17**)



Following the general procedure: Secondary iodide **3.1** (100 mg, 0.384 mmol), DMAP (2.8 mg, 0.0231 mmol), Pd(PPh₃)₄ (26.7 mg, 0.0231 mmol), Et₃N (91 μL, 0.653 mmol), 2,6-dimethylphenyl isocyanide (111 mg, 0.845 mmol) and bidistilled H₂O (277 μL, 15.36 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (71 mg, 66% yield).

MW: 281,40 (C₁₉H₂₃NO)

TLC: Rf 0.28 (ETP/Et₂O 7:3, UV (weak), CAM (weak))

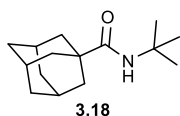
¹H NMR (400 MHz, CDCl₃, 27 °C): δ 7.33 – 7.27 (m, 2H, 2x CH_{arom} of Ph), 7.24 – 7.18 (m, 3H, CH_{arom, para} of Ph e 2x CH_{arom} of Ph), 7.12 – 7.06 (m, 3H, 2x CH_{arom, meta} of dimethylphenyl e CH_{arom, para} of dimethylphenyl), 6.68 (s, 1H, NH), 2.84 – 2.64 (m, 2H, CH₂CH₂CHCO), 2.54 – 2.40 (m, 1H, CH₂CH₂CHCO), 2.24 (s, 6H, 2x CH₃ of dimethylphenyl), 2.23 – 2.09 (m, 1H, CH₂CH₂CHCO), 1.86 – 1.73 (m, 1H, CH₂CH₂CHCO), 1.33 (d, J = 6.9 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 174.5 (C=O), 141.9 (C_{quat, arom} of Ph), 135.5 (2x C_{quat arom, orto} of dimethylphenyl), 133.9 (C_{quat, arom} of dimethylphenyl), 128.6 (2x CH_{arom} of Ph), 128.6 (2x CH_{arom} of Ph), 128.4 (2x CH_{arom, meta} of dimethylphenyl), 127.4 (CH_{arom, para} of dimethylphenyl), 126.1 (CH_{arom, para} of Ph), 41.3 (CH₂CH₂CHCO), 36.0 (CH₂CH₂CHCO), 33.9 (CH₂CH₂CHCO), 18.7 (2x CH₃ of dimethylphenyl), 18.7 (CH₃).

GC-MS: Rt = 11.415 min (Method A); m/z (Relative Intensity %): 39 (4), 41 (4), 51 (3), 55 (3), 65 (11), 77 (10), 78 (3), 79 (5), 91 (100), 92 (9), 103 (4), 104 (3), 105 (5), 106 (6), 120 (27), 121 (35), 122 (4), 133 (3), 148 (34), 149 (4), 177 (38), 178 (5), 281 (1) [M]⁺

IR wavenumber (cm⁻¹): 3226, 3027, 2967, 2926, 2850, 1669, 1648, 1602, 1526, 1496, 1474, 1453, 1430, 1367, 1265, 1234, 1206, 1162, 1122, 1090, 1052, 1033, 945, 844, 774, 765, 742, 729, 697, 648

Synthesis of (3r,5r,7r)-N-(tert-butyl)adamantane-1-carboxamide (**3.18**)



Following the general procedure: 1-iodoadamantane (100 mg, 0.381 mmol), DMAP (2.8 mg, 0.0229 mmol), Pd(PPh₃)₄ (26.5 mg, 0.0229 mmol), Et₃N (90 μL, 0.648 mmol), *t*-butyl isocyanide (95 μL, 0.839 mmol) and bidistilled H₂O (275 μL, 15.3 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 8:2) delivering a pale-yellow solid (18 mg, 20% yield).

MW: 235.37 (C₁₅H₂₅NO)

TLC: R_f 0.32 (ETP/Et₂O 7:3, UV, CAM)

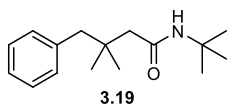
¹H NMR (400 MHz, CDCl₃, 27 °C): δ 5.37 (bs, 1H, NH), 2.03 (bs, 3H, 3x CH₂CHCH₂CCO), 1.83 – 1.78 (m, 6H, 3x CH₂CHCH₂CCO), 1.76 – 1.65 (m, 6H, 3x CH₂CHCH₂CCO), 1.33 (s, 9H, C(CH₃)₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C): δ 177.5 (C=O), 50.7 (C(CH₃)₃), 41.0 (CH₂CHCH₂CCO), 39.5 (3x CH₂CHCH₂CCO), 36.7 (3x CH₂CHCH₂CCO), 29.0 (C(CH₃)₃), 28.3 (3x CH₂CHCH₂CCO).

GC-MS: Rt = 8.980 min (Method A); m/z (Relative Intensity%): 39 (5), 41 (17), 42 (6), 53 (4), 55 (6), 56 (4), 57 (12), 58 (4), 65 (3), 67 (8), 77 (9), 79 (21), 80 (3), 81 (5), 91 (9), 92 (3), 93 (19), 94 (3), 107 (10), 135 (100), 136 (15), 163 (8), 180 (11), 193 (3), 220 (13), 235 (18) [M]⁺

IR wavenumber (cm⁻¹): 3327, 2974, 2958, 2900, 2850, 1636, 1533, 1470, 1445, 1387, 1359, 1343, 1328, 1287, 1267, 1228, 1178, 1106, 1090, 1045, 981, 944, 925, 913, 816, 746, 673, 635

Synthesis of *N*-(*tert*-butyl)-3,3-dimethyl-4-phenylbutanamide (**3.19**)



Following the general procedure: Primary iodide (100 mg, 0.365 mmol), DMAP (2.7 mg, 0.0219 mmol), Pd(PPh₃)₄ (25.3 mg, 0.0219 mmol), Et₃N (85 μL, 0.620 mmol), *t*-butyl isocyanide (90 μL, 0.804 mmol) and bidistilled H₂O (263 μL, 14.6 mmol) in toluene dry (1.2 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 8:2) delivering a pale-yellow solid (36 mg, 40% yield).

MW: 247.38 (C₁₆H₂₅NO)

TLC: Rf 0.24 (ETP/Et₂O 7:3, UV, CAM)

M.P. 72.2 – 73.7 °C

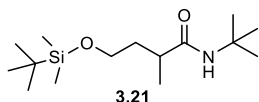
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.30 – 7.24 (m, 2H, 2x CH_{arom}), 7.23 – 7.16 (m, 3H, 2x CH_{arom} and CH_{arom, para}), 5.20 (bs, 1H, NH), 2.66 (s, 2H, Ph-CH₂), 1.94 (s, 2H, CH₂-CO), 1.35 (s, 9H, C(CH₃)₃), 1.01 (s, 6H, 2x CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 171.2 (C=O), 138.9 (C_{quat, arom}), 131.0 (2x CH_{arom}), 127.8 (2x CH_{arom}), 126.1 (CH_{arom, para}), 51.3 (C(CH₃)₃), 49.0 (CH₂-CO), 48.5 (Ph-CH₂), 34.6 (CH₂CCH₂), 28.9 (C(CH₃)₃), 27.4 (2x CH₃).

GC-MS: Rt= 8.980 min (Method A); m/z (Relative Intensity %): 39 (8), 41 (28), 42 (9), 43 (3), 44 (4), 55 (10), 56 (10), 57 (88), 58 (38), 59 (74), 60 (36), 65 (11), 72 (3), 83 (3), 91 (50), 92 (8), 100 (17), 105 (4), 115 (100), 116 (8), 117 (5), 132 (3), 133 (4), 247 (3) [M]⁺

IR wavenumber (cm⁻¹): 3311, 3064, 3030, 2966, 2925, 1637, 1607, 1543, 1492, 1452, 1389, 1354, 1329, 1317, 1290, 1265, 1225, 1182, 1145, 1114, 1073, 1031, 991, 934, 897, 791, 754, 713, 702, 685, 613

Synthesis of *N*-(*tert*-butyl)-4-((*tert*-butyldimethylsilyl)oxy)-2-methylbutanamide (**3.21**)



Following the general procedure: secondary iodide **3.11** (100 mg, 0.318 mmol), DMAP (2.3 mg, 0.0191 mmol), Pd(PPh₃)₄ (22 mg, 0.0191 mmol), Et₃N (75 μL, 0.541 mmol), *t*-butyl isocyanide (79 μL, 0.700 mmol) and bidistilled H₂O (229 μL, 12.7 mmol) in toluene dry (1.1 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow oil (65 mg, 71% yield).

MW: 287,52 (C₁₅H₃₃NO₂Si)

TLC: Rf 0.52 (ETP/Et₂O 7:3, basic KMnO₄)

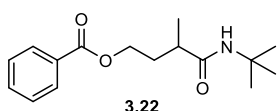
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 5.43 (bs, 1H, NH), 3.69 – 3.56 (m, 2H, O-CH₂CH₂CHCO), 2.40 – 2.26 (m, H, O-CH₂CH₂CHCO), 1.76 (ddt, *J* = 13.8, 9.3, 4.8 Hz, 1H, O-CH₂CHCHCHCO), 1.60 – 1.49 (m, 1H, O-CH₂CHCHCHCO), 1.34 (s, 9H, NH-C(CH₃)₃), 1.10 (d, *J* = 6.9 Hz, 3H, CH₃), 0.90 (s, 9H, Si-C(CH₃)₃), 0.05 (s, 6H Si(CH₃)₂).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.7 (C=O), 60.8 (O-CH₂CH₂CHCO), 51.1 (N-C(CH₃)₃), 38.0 (O-CH₂CH₂CHCO), 37.4 (O-CH₂CH₂CHCO), 29.0 (N-C(CH₃)₃), 26.1 (Si-C(CH₃)₃), 18.4 (Si-C(CH₃)₃), 17.8 (CH₃), -5.1 (Si-CH₃), -5.2 (Si-CH₃).

GC-MS: Rt= 8.035 min (Method A), m/z (Relative Intensity %): 39 (6), 41 (27), 42 (5), 43 (4), 44 (4), 45 (6), 47 (5), 55 (10), 56 (11), 57 (33), 58 (16), 59 (12), 61 (4), 72 (3), 73 (52), 74 (89), 75 (42), 76 (6), 77 (3), 84 (3), 85 (4), 88 (4), 89 (5), 99 (3), 100 (12), 101 (6), 114 (7), 115 (9), 116 (4), 129 (19), 130 (3), 156 (5), 157 (4), 174 (100), 175 (13), 176 (4), 215 (9), 216 (5), 230 (33), 231 (7)

IR wavenumber (cm⁻¹): 3300, 2961, 2930, 2858, 1643, 1552, 1462, 1389, 1361, 1256, 1227, 1089, 1024, 1005, 984, 938, 894, 834, 771, 684, 665

Synthesis of 4-(*tert*-butylamino)-3-methyl-4-oxobutyl benzoate (**3.22**)



Following the general procedure: Secondary iodide **3.13** (100 mg, 0.329 mmol), DMAP (2.4 mg, 0.0197 mmol), Pd(PPh₃)₄ (23 mg, 0.0197 mmol), Et₃N (78 μ L, 0.559 mmol), *t*-butyl isocyanide (82 μ L, 0.723 mmol) and bidistilled H₂O (237 μ L, 13.2 mmol) in toluene dry (1.2 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 6:4) delivering a pale-yellow solid (67 mg, 72% yield).

MW: 277,36 (C₁₆H₂₃NO₃)

TLC: R_f 0.27 (ETP/Et₂O 6:4, UV, CAM)

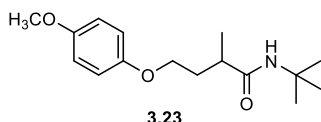
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 8.04 (dd, *J* = 8.3, 1.2 Hz, 2H, 2x CH_{arom, orto}), 7.57 (tt, *J* = 7.4, 1.3 Hz, 1H, CH_{arom, para}), 7.45 (t, *J* = 7.8 Hz, 2H, 2x CH_{arom, meta}), 5.46 (s, 1H, NH), 4.46 – 4.25 (m, 2H, O-CH₂CH₂CHCO), 2.34 – 2.21 (m, 1H, O-CH₂CH₂CHCO), 2.10 (ddt, *J* = 14.4, 8.9, 5.6 Hz, 1H, O-CH₂CH₂CHCHCO), 1.83 (ddt, *J* = 11.6, 8.0, 5.8 Hz, 1H, O-CH₂CHCHCHCO), 1.34 (s, 9H, C(CH₃)₃), 1.18 (d, *J* = 6.8 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 174.9 (C=O), 166.8 (C=ONH), 133.1 (CH_{arom, para}), 129.7 (CH_{arom, orto}), 128.5 (CH_{arom, meta}), 63.2 (C(CH₃)₃), 51.3 (O-CH₂CH₂CHCO), 38.9 (O-CH₂CH₂CHCO), 33.4 (O-CH₂CH₂CHCO), 28.9 (C(CH₃)₃), 18.3 (CH₃).

GC-MS: Rt= 9,940 min (Method A); m/z (Relative Intensity %): 39 (4), 41 (21), 42 (7), 43 (3), 44 (4), 51 (8), 55 (9), 56 (31), 57 (37), 58 (39), 72 (4), 73 (13), 74 (3), 77 (33), 78 (3), 79 (4), 99 (4), 100 (43), 101 (3), 105 (100), 106 (8), 122 (3), 123 (22), 129 (25), 140 (7), 155 (3), 178 (3), 262 (5)

IR wavenumber (cm⁻¹): 3383, 3276, 2971, 2924, 1699, 1665, 1599, 1584, 1518, 1451, 1389, 1362, 1318, 1280, 1224, 1202, 1178, 1141, 1116, 1102, 1087, 1072, 1032, 998, 978, 947, 909, 857, 812, 801, 714, 688, 675

Synthesis of *N*-(*tert*-butyl)-4-(4-methoxyphenoxy)-2-methylbutanamide (**3.23**)



Following the general procedure: secondary iodide (89.7 mg, 0.293 mmol), DMAP (2.1 mg, 0.0176 mmol), Pd(PPh₃)₄ (20.3 mg, 0.0176 mmol), Et₃N (69 μ L, 0.498 mmol), *t*-butyl isocyanide (72 μ L, 0.644 mmol) and bidistilled H₂O (211 μ L, 11.7 mmol) in toluene dry (1.1 mL). The crude was extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (36 mg, 44% yield).

MW: 279.38 (C₁₆H₂₅NO₃)

TLC: R_f 0.20 (ETP/Et₂O 7:3, UV, CAM)

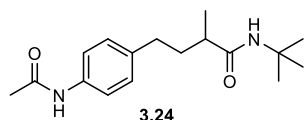
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 6.83 (s, 4H, 4x CH_{arom}), 5.37 (s, 1H, NH), 3.96 (dt, *J* = 10.1, 5.2 Hz, 1H, O-CH₂CH₂CHCO), 3.88 (td, *J* = 9.2, 4.5 Hz, 1H, O-CH₂CH₂CHCO), 3.77 (s, 3H, OCH₃), 2.50 – 2.37 (m, 1H, O-CH₂CH₂CHCO), 2.00 (ddt, *J* = 14.1, 9.4, 4.7 Hz, 1H, O-CH₂CH₂CHCHCO), 1.84 (ddt, *J* = 14.2, 8.9, 5.4 Hz, 1H, O-CH₂CHCHCHCO), 1.27 (s, 9H, C(CH₃)₃), 1.16 (d, *J* = 6.9 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.3 (C=O), 154.0 (C_{quat, arom}), 153.1 (C_{quat, arom}), 115.4 (2x CH_{arom}), 114.8 (2x CH_{arom}), 66.3 (O-CH₂CH₂CHCO), 55.8 (OCH₃), 51.1 (C(CH₃)₃), 38.5 (O-CH₂CH₂CHCO), 34.0 (O-CH₂CH₂CHCO), 28.9 (C(CH₃)₃), 18.0 (CH₃).

GC-MS: Rt= 10.125 min (Method A); m/z (Relative Intensity %): 39 (4), 41 (17), 42 (3), 44 (6), 55 (9), 56 (5), 57 (35), 58 (8), 73 (3), 77 (4), 81 (3), 83 (3), 100 (100), 101 (6), 109 (9), 124 (7), 137 (3), 156 (29), 157 (3)

IR wavenumber (cm⁻¹): 3347, 2962, 2926, 2215, 1645, 1538, 1508, 1454, 1361, 1287, 1229, 1184, 1109, 1076, 1030, 991, 970, 912, 882, 819, 727, 694, 627

Synthesis of 4-(4-acetamidophenyl)-*N*-(*tert*-butyl)-2-methylbutanamide (**3.24**)



Following the general procedure: secondary iodide (100 mg, 0.315 mmol), DMAP (2.3 mg, 0.0189 mmol), Pd(PPh₃)₄ (21.9 mg, 0.0189 mmol), Et₃N (75 μL, 0.536 mmol), *t*-butyl isocyanide (78 μL, 0.654 mmol) and bidistilled H₂O (227 μL, 12.6 mmol) in toluene dry (1.1 mL). The crude was extracted using AcOEt and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/AcOEt 2:8) delivering a pale-yellow solid (67 mg, 72% yield).

MW: 290.41 (C₁₇H₂₆N₂O₂)

TLC: Rf 0.38 (ETP/AcOEt 2:8, UV, CAM)

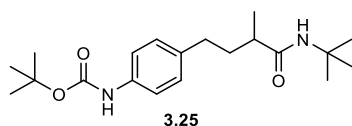
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.42 – 7.34 (m, 2H, 2x NH-CCH_{arom}), 7.19 – 7.00 (m, 3H, 2x CH_{arom}C-CH₂ and CH₃CONH), 5.16 (bs, 1H, NH), 2.65 – 2.56 (m, 1H, Ph-CHHCH₂CH-CO), 2.55 – 2.47 (m, 1H, Ph-CHHCH₂CH-CO), 2.16 (s, 3H, CH₃CONH), 2.04 – 1.87 (m, 2H, Ph-CH₂CH₂CH-CO and Ph-CH₂CHHCH-CO), 1.68 – 1.55 (m, 1H, Ph-CH₂CHHCH-CO), 1.34 (s, 9H, C(CH₃)₃), 1.10 (d, *J* = 6.6 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.5 (C=O), 168.2 (CH₃C=ONH-Ph), 138.1 (CH_{arom}C-CH₂), 135.8 (NH-CCH_{arom}), 129.0 (2x CH_{arom}C-CH₂), 120.2 (2x NH-CCH_{arom}), 51.1 (C(CH₃)₃), 41.4 (Ph-CH₂CH₂CH-CO), 35.8 (Ph-CH₂CH₂CH-CO), 33.0 (Ph-CH₂CH₂CH-CO), 29.0 (C(CH₃)₃), 24.7 (CH₃CONH), 18.2 (CH₃).

GC-MS: Rt= 11.845 min (Method A); m/z (Relative Intensity %), 39 (3), 41 (12), 42 (4), 43 (16), 44 (6), 55 (3), 56 (5), 57 (17), 58 (10), 72 (6), 73 (90), 74 (18), 77 (5), 78 (6), 79 (3), 91 (3), 100 (4), 105 (4), 106 (34), 107 (4), 114 (3), 119 (6), 120 (4), 129 (100), 130 (8), 132 (3), 146 (3), 148 (3), 162 (6), 207 (3), 290 (3) [M]⁺

IR wavenumber (cm⁻¹): 3321, 3196, 3129, 3072, 2967, 2922, 2864, 1677, 1644, 1607, 1541, 1514, 1458, 1411, 1390, 1372, 1362, 1317, 1267, 1229, 1121, 1062, 1022, 962, 914, 882, 822, 763, 720, 672, 650, 609

Synthesis of *tert*-butyl 4-(4-(*tert*-butylamino)-3-methyl-4-oxobutyl)phenyl carbamate (**3.25**)



Following the general procedure: secondary iodide (100 mg, 0.266 mmol), DMAP (2 mg, 0.0160 mmol), Pd(PPh₃)₄ (18.5 mg, 0.0160 mmol), Et₃N (63 μL, 0.453 mmol), *t*-butyl isocyanide (66 μL, 0.586 mmol) and bidistilled H₂O (192 μL, 10.7 mmol) in toluene dry (1.1 mL). The crude was

extracted using Et₂O and a saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (66 mg, 74% yield).

MW: 348,49 (C₂₀H₃₂N₂O₃)

TLC: Rf 0.31 (ETP/Et₂O 7:3, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.29 – 7.24 (m, 2H, 2x NH-CCH_{arom}), 7.09 (d, *J* = 8.1 Hz, 2H, 2x CH_{arom}C-CH₂), 6.47 (bs, 1H, OCONH), 5.18 (bs, 1H, CONH), 2.69 – 2.41 (m, 2H, CH₂CH₂CH-CO), 2.05 – 1.86 (m, 2H, CH₂CH₂CH-CO e CH₂CHHCH-CO), 1.70 – 1.55 (m, 1H, CH₂CHHCH-CO), 1.51 (s, 9H, (CH₃)₃CO), 1.35 (s, 9H, NC(CH₃)₃), 1.10 (d, *J* = 6.6 Hz, 3H, CH₃).

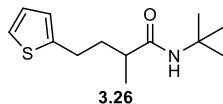
¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.6 (C=O), 153.0 (OC=ONH), 136.7 (CH_{arom}C-CH₂), 136.3 (NH-CCH_{arom}), 129.0 (2x CH_{arom}C-CH₂), 118.9 (2x NH-CCH_{arom}), 80.5 ((CH₃)₃CO), 51.2 (NC(CH₃)₃), 41.3 (CH₂CH₂CH-CO), 35.9 (CH₂CH₂CH-CO), 32.9 (CH₂CH₂CH-CO), 29.0 (NC(CH₃)₃), 28.5 ((CH₃)₃CO), 18.2 (CH₃).

GC-MS: the compound degrades during the analysis. A big and broad peak is obtained in the chromatograph together with the product without the Boc- protecting group.

Rt= 11,485 min (Method C); m/z (Relative Intensity %): 39 (13), 40 (5), 41 (25), 42 (8), 43 (8), 44 (51), 45 (5), 51 (3), 53 (3), 55 (6), 56 (11), 57 (13), 58 (9), 59 (3), 65 (3), 69 (3), 72 (5), 73 (100), 74 (17), 75 (4), 77 (7), 78 (5), 79 (4), 91 (4), 96 (4), 100 (4), 105 (3), 106 (33), 107 (5), 118 (3), 119 (11), 120 (4), 129 (52), 130 (5), 132 (3), 133 (4), 135 (8), 146 (4), 147 (8), 191 (4), 193 (9), 194 (3), 207 (39), 208 (6), 209 (5), 221 (5), 248 (10), 249 (4), 267 (3), 281 (11), 282 (4), 283 (3)

IR wavenumber (cm⁻¹): 3348, 3272, 2965, 2931, 1699, 1651, 1603, 1536, 1478, 1451, 1413, 1391, 1363, 1318, 1246, 1229, 1158, 1089, 1054, 1021, 947, 904, 833, 805, 735, 719, 665, 635, 607

Synthesis of *N*-(*tert*-butyl)-2-methyl-4-(thiophen-2-yl)butanamide (**3.26**)



Following the general procedure: secondary iodide **3.3** (100 mg, 0.376 mmol), DMAP (2.8 mg, 0.0226 mmol), Pd(PPh₃)₄ (26.4 mg, 0.0226 mmol), Et₃N (89 μL, 0.639 mmol), *t*-butyl isocyanide (93 μL, 0.827 mmol) and bidistilled H₂O (271 μL, 15.04 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (76 mg, 69% yield).

MW: 239,38 (C₁₃H₂₁NOS)

TLC: Rf 0.38 (ETP/Et₂O 7:3, UV, CAM)

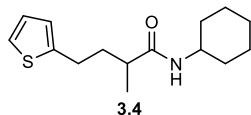
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.11 (dd, *J* = 5.1, 1.1 Hz, 1H, S-CH_{arom}CHCHC-), 6.92 (dd, *J* = 5.1, 3.4 Hz, 1H, S-CHCH_{arom}CHC-), 6.82 – 6.75 (m, 1H, S-CHCHCH_{arom}C-), 5.26 (bs, 1H, NH), 2.96 – 2.69 (m, 2H, Ar-CH₂CH₂CH-CO), 2.15 – 1.96 (m, 2H, Ar-CH₂CH₂CH-CO and Ar-CH₂CHHCH-CO), 1.70 (dtd, *J* = 13.3, 7.8, 5.0 Hz, 1H, Ar-CH₂CHHCH-CO), 1.36 (s, 9H, C(CH₃)₃), 1.12 (d, *J* = 6.6 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.4 (C=O), 144.8 (S-CHCHCHC_{arom}-), 126.9 (S-CHCH_{arom}CHC-), 124.5 (S-CHCHCH_{arom}C-), 123.2 (S-CH_{arom}CHCHC-), 51.2 (C(CH₃)₃), 41.1 (Ar-CH₂CH₂CH-CO), 36.2 (Ar-CH₂CH₂CH-CO), 29.0 (C(CH₃)₃), 27.7 (Ar-CH₂CH₂CH-CO), 18.1 (CH₃).

GC-MS: Rt= 8.720 min (Method A); m/z (Relative Intensity %): 39 (3), 41 (11), 42 (3), 44 (3), 45 (6), 53 (5), 55 (3), 56 (3), 57 (17), 58 (10), 69 (3), 72 (8), 73 (100), 74 (18), 97 (30), 98 (3), 100 (5), 110 (3), 114 (3), 129 (82), 130 (7), 239 (4) [M]⁺

IR wavenumber (cm⁻¹): 3302, 3072, 2964, 2925, 2870, 1641, 1543, 1479, 1450, 1440, 1389, 1359, 1310, 1294, 1262, 1229, 1185, 1140, 1082, 1065, 1038, 933, 890, 852, 829, 781, 762, 748, 691, 679, 670, 649, 615

Synthesis of *N*-cyclohexyl-2-methyl-4-(thiophen-2-yl)butanamide (**3.4**)



Following the general procedure: secondary iodide **3.3** (100 mg, 0.376 mmol), DMAP (2.8 mg, 0.0226 mmol), Pd(PPh₃)₄ (26.4 mg, 0.0226 mmol), Et₃N (89 μL, 0.639 mmol), *t*-butyl isocyanide (103 μL, 0.827 mmol) and bidistilled H₂O (271 μL, 15.04 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (64 mg, 64% yield).

MW: 265,42 (C₁₅H₂₃NOS)

TLC: R_f 0.28 (ETP/Et₂O 7:3, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.12 (dd, *J* = 5.1, 1.1 Hz, 1H, S-CH_{arom}CHCHC-), 6.92 (dd, *J* = 5.1, 3.4 Hz, 1H, S-CHCH_{arom}CHC-), 6.82 – 6.75 (m, 1H, S-CHCHCH_{arom}C-), 5.30 (s, 1H, NH), 3.80 (dddd, *J* = 14.9, 10.8, 8.3, 3.9 Hz, 1H, CH_{c-hex}), 2.93 – 2.73 (m, 2H, Ar-CH₂CH₂CH-CO), 2.21 – 2.10 (m, 1H, Ar-CH₂CH₂CH-CO), 2.05 (dtd, *J* = 14.4, 8.2, 5.7 Hz, 1H, Ar-CH₂CH₂CH-CO), 1.98 – 1.86 (m, 2H, CH_{2 c-hex}), 1.79 – 1.57 (m, 4H, Ar-CH₂CH₂CH-CO e CH_{2 c-hex} e CHH_{c-hex}), 1.44 – 1.30 (m, 2H, CH_{2 c-hex}), 1.23 – 1.03 (m, 6H, CH_{2 c-hex} e CHH_{c-hex} and CH₃).

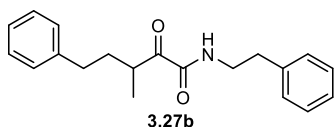
¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 175.0 (C=O), 144.8 (S-CHCHCHC_{arom}-), 126.9 (S-CHCH_{arom}CHC-), 124.5 (S-CHCHCH_{arom}C-), 123.2 (S-CH_{arom}CHCHC-), 48.1 (CH_{c-hex}), 40.6 (Ar-CH₂CH₂CH-CO), 36.1 (Ar-CH₂CH₂CH-CO), 33.5 (CH_{2 c-hex}), 33.3 (CH_{2 c-hex}), 27.7 (Ar-CH₂CH₂CH-CO), 25.7 (CH_{2 c-hex}), 25.0 (2x CH_{2 c-hex}), 18.1 (CH₃).

GC-MS: Rt= 10.620 min (Method A); *m/z* (Relative Intensity %): 39 (5), 41 (15), 42 (3), 43 (4), 44 (6), 45 (7), 53 (7), 54 (3), 55 (18), 56 (8), 57 (5), 67 (6), 69 (5), 72 (4), 73 (10), 74 (100), 75 (4), 82 (5), 83 (3), 97 (36), 98 (6), 110 (3), 126 (10), 155 (45), 156 (4), 265 (1) [M]⁺

IR wavenumber (cm⁻¹): 3282, 3099, 2931, 2853, 2221, 1636, 1544, 1440, 1387, 1372, 1346, 1303, 1271, 1245, 1234, 1225, 1181, 1151, 1099, 1075, 1030, 976, 945, 892, 854, 823, 796, 698, 614

3.9.5 α-ketoamides characterizations

Characterization of 3-methyl-2-oxo-*N*-phenethyl-5-phenylpentanamide (**3.27b**)



Following the general procedure: secondary iodide **3.1** (100 mg, 0.384 mmol), DMAP (2.8 mg, 0.0231 mmol), Pd(PPh₃)₄ (26.7 mg, 0.0231 mmol), Et₃N (91 μL, 0.653 mmol), (2-isocianoethyl)benzene (117 μL, 0.846 mmol) and bidistilled H₂O (277 μL, 15.36 mmol) in toluene dry (1.3 mL). The crude was extracted using Et₂O and saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 8:2) delivering a pale-yellow oil (14 mg, 12% yield).

MW: 309,41 (C₂₀H₂₃NO₂)

TLC: R_f 0.35 (ETP/Et₂O 8:2, UV, CAM)

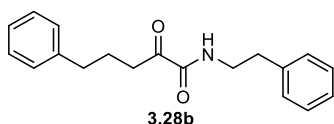
¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.34 – 7.28 (m, 2H, 2x CH_{arom}), 7.28 – 7.22 (m, 3H, 3x CH_{arom}), 7.22 – 7.14 (m, 5H, 5x CH_{arom}), 6.95 (bs, 1H, NH), 3.65 – 3.50 (m, 3H, Ar-CH₂CH₂CH-CO e N-CH₂CH₂-Ar), 2.85 (t, *J* = 7.1 Hz, 2H, N-CH₂CH₂-Ar), 2.68 – 2.52 (m, 2H, Ar-CH₂CH₂CH-CO), 2.05 (ddt, *J* = 13.3, 10.0, 6.5 Hz, 1H, Ar-CH₂CH₂CH-CO), 1.71 – 1.60 (m, 1H, Ar-CH₂CH₂CH-CO), 1.14 (d, *J* = 7.0 Hz, 3H, CH₃).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 202.0 (CH₂C=O), 159.9 (C=ONH), 141.7 (C_{quat,arom}), 138.3 (C_{quat,arom} ethylbenzene), 128.9 (2x CH_{arom}), 128.8 (2x CH_{arom}), 128.6 (2x CH_{arom}), 128.5 (2x CH_{arom}), 126.9 (CH_{arom}), 126.1 (CH_{arom}), 40.6 (N-CH₂CH₂-Ar), 38.9 (Ar-CH₂CH₂CH-CO), 35.6 (N-CH₂CH₂-Ar), 34.2 (Ar-CH₂CH₂CH-CO), 33.6 (Ar-CH₂CH₂CH-CO), 16.0 (CH₃).

GC-MS: Rt= 11.810 min (Method A); m/z (Relative Intensity %): 39 (4), 41 (3), 51 (3), 65 (12), 77 (8), 78 (3), 79 (8), 91 (100), 92 (9), 103 (5), 104 (22), 105 (34), 106 (3), 114 (6), 133 (8), 148 (3), 161 (8), 205 (10), 239 (20), 240 (3)

IR wavenumber (cm⁻¹): 3362, 3064, 3027, 2929, 2861, 1719, 1659, 1604, 1532, 1496, 1454, 1370, 1285, 1240, 1193, 1128, 1100, 1044, 1029, 977, 905, 883, 846, 815, 739, 695

Characterization of 2-oxo-*N*-phenethyl-5-phenylpentanamide (**3.28b**)



Following the general procedure: secondary iodide (100 mg, 0.406 mmol), DMAP (3.0 mg, 0.0244 mmol), Pd(PPh₃)₄ (28.2 mg, 0.0244 mmol), Et₃N (96 μL, 0.691 mmol), (2-isocynoethyl)benzene (123 μL, 0.894 mmol) and bidistilled H₂O (293 μL, 16.3 mmol) in toluene dry (1.4 mL). The crude was extracted using Et₂O and saturated solution of NH₄Cl. The crude was purified by silica column chromatography (ETP/Et₂O 7:3) delivering a pale-yellow solid (12 mg, 10% yield).

MW: 295.38 (C₁₉H₂₁NO₂)

TLC: R_f 0.32 (ETP/Et₂O 7:3, UV, CAM)

¹H NMR (400 MHz, CDCl₃, 27 °C) δ 7.34 – 7.21 (m, 5H, 5x CH_{arom}), 7.21 – 7.15 (m, 5H, 5x CH_{arom}), 6.96 (bs, 1H, NH), 3.55 (q, *J* = 7.0 Hz, 2H, N-CH₂CH₂-Ar), 2.94 (t, *J* = 7.3 Hz, 2H, Ar-CH₂CH₂CH₂-CO), 2.84 (t, *J* = 7.1 Hz, 2H, N-CH₂CH₂-Ar), 2.65 (t, *J* = 7.4 Hz, 2H, Ar-CH₂CH₂CH₂-CO), 1.93 (p, *J* = 7.5 Hz, 2H, Ar-CH₂CH₂CH₂-CO).

¹³C NMR (101 MHz, CDCl₃, 27 °C) δ 199.0 (CH₂C=O), 160.1 (C=ONH), 141.5 (C_{quat,arom}), 138.3 (C_{quat,arom}), 128.9 (2x CH_{arom}), 128.8 (2x CH_{arom}), 128.6 (2x CH_{arom}), 128.6 (2x CH_{arom}), 126.9 (CH_{arom}), 126.2 (CH_{arom}), 40.6 (N-CH₂CH₂-Ar), 36.2 (Ar-CH₂CH₂CH₂-CO), 35.6 (N-CH₂CH₂-Ar), 35.1 (Ar-CH₂CH₂CH₂-CO), 24.9 (Ar-CH₂CH₂CH₂-CO).

GC-MS: Rt= 11.805 min (Method A); m/z (Relative Intensity %): 39 (7), 41 (9), 44 (5), 51 (6), 55 (4), 63 (3), 65 (19), 73 (4), 77 (18), 78 (7), 79 (17), 89 (3), 91 (100), 92 (9), 100 (3), 103 (12), 104 (64), 105 (76), 106 (6), 115 (3), 117 (4), 119 (3), 120 (5), 129 (13), 147 (36), 148 (14), 191 (5), 207 (4), 239 (24), 240 (4), 267 (14), 268 (3), 28, 295 (2) [M]⁺

IR wavenumber (cm⁻¹): 3343, 3062, 3028, 2928, 2852, 1721, 1659, 1603, 1533, 1495, 1455, 1396, 1376, 1285, 1263, 1233, 1192, 1120, 1078, 1027, 991, 907, 853, 805, 742, 722, 693, 651

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Chapter 4

PREPARATION OF NOVEL AMPHIPHILIC DENDRIMERS FOR BIOMEDICAL APPLICATIONS THROUGH THE USE OF CLICK-CHEMISTRY

During my third year of doctoral studies, I had the opportunity and the pleasure to pursue part of my chemical investigations at the University of Malaga (UMA) and Instituto de Investigación Biomédica de Málaga y Plataforma en Nanomedicina (IBIMA-BIONAND) in Malaga, Spain, within the research group of Prof. Dr. Ezequiel Pérez-Inestrosa Villatoro.

IBIMA Plataforma BIONAND is a Health Research Institute established in July 2010 as a result of an agreement between the Andalusian Regional Ministry of Health and the University of Málaga. IBIMA is a cutting-edge and excellence centre in biomedicine accredited for health research by the Carlos III Health Institute (on January 15, 2015). Scientists from both academic and healthcare fields work together towards an efficient knowledge and technology transfer from academia to the industrial sector. This multidisciplinary workspace has the main focus of improving citizens' health and contribute to the social and economic development.

The research group of Prof. Dr. Ezequiel Pérez-Inestrosa Villatoro, called the “Biomimetic Dendrimers and Photonic Laboratory”, focusses on a broad range of topics, from organic nanomaterials to novel carbohydrate based multivalent systems. Recent recurring research themes include the synthesis of new dendrimer¹, their modification to emulate multivalent bioactive macromolecules, the preparation of solid supports functionalized with dendrimer^{2,3}, the development of fluorescent dendrimers as biological markers^{4,5} and the design of multivalent carbohydrates-based systems that mimic natural oligosaccharides involved in biological processes⁶.

4.1 What is a Dendrimer?

The dendritic architecture is one of the most diffused on our planet. Examples of these complex structures may be found in both abiotic systems (e.g., lightning patterns, snow crystals, etc.) as well as in the biological world (e.g., tree branching or roots, plant and animal vascular systems, and neurons).

Dendritic materials (or dendritic polymers) are recognized as a class of polymeric architecture. Other kind of well know and studied polymeric architecture are linear polymer, cross-linked polymer and branched polymer, to give just a few examples. When the term dendritic material is used, it is possible to indicate four subsets: random hyperbranched polymers, dendrigraft polymers, dendrons, and dendrimers. The difference among them is related to degree of structural control, starting from the least controlled to the most controlled one⁷.

- Random hyperbranched polymers (HB) are highly branched and disordered macromolecules (*Figure 4.1*). In the three-dimensional domain can be identified both globular and dendritic architectures. The molecular sizes of HB polymers can range from several nanometres to dozens of nanometres. They are characterized for having a high-density of functional groups along with high free volume. All of this gives them unique physical and chemical properties^{8,9}. In 1982¹⁰ for the first time was reported the synthesis of an hyperbranched polymer.
- Dendrigrraft polymers are highly branched macromolecules whose core consists of a linear polymer onto which the arborescent (tree-like) branches are grafted¹¹ (*Figure 4.1*). Although they resemble classic grafted polymers, their chemical and physical properties are predominantly dictated by the dendritic, highly branched regions. Among dendritic materials, dendrigrrafts are the largest, with sizes typically ranging from several tens to several hundreds of nanometres. The first dendrigrraft polymers were synthesized and reported in 1991¹².

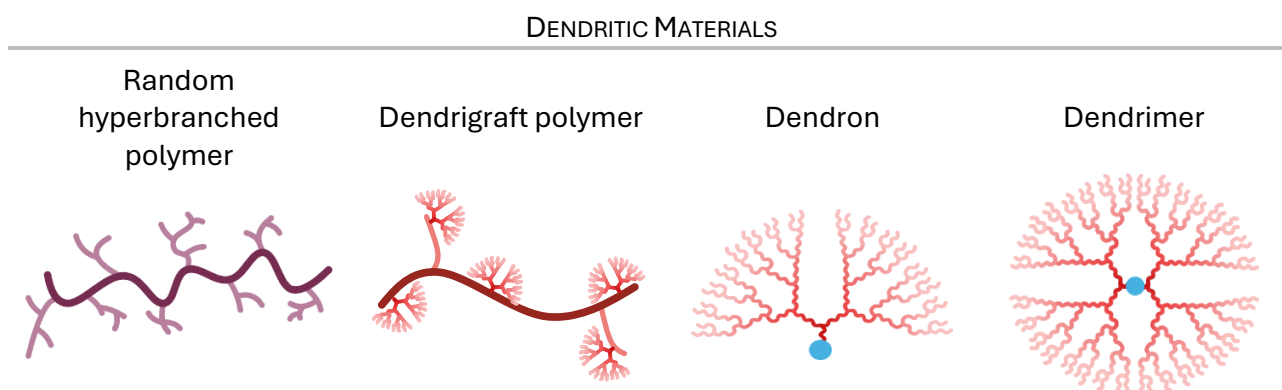


Figure 4.1: graphical representation of a random hyperbranched polymer, dendrigrraft polymer, dendron and dendrimer

A dendrimer can be described as a highly controlled, ordered, monodisperse and ramified three-dimensional macromolecule (*Figure 4.1*). It is characterized by a very high degree of superficial functionalization, that imparts the final properties to the material and makes it highly versatile. Dendrimers always consist of an inner core, a dendritic unit, that gives structure to the macromolecule, and is bound to the core, and a terminal unit on the globular surface that rules the reactivity and solubility. Therefore, dendrimers are well defined in structure. The same words can be spent for dendrons which are only half of a dendrimer

The hyperbranched polymers are the closest structure to dendrimers, but they are inherently different. Like dendrimers, hyperbranched polymers have three types of structural units. In this case they are called: dendritic units, linear units and terminal units. Owing to the structural randomness the core is totally absent. The terminal units are always located at the terminals like in dendrimers. The dendritic units and linear units are randomly distributed within the macromolecular framework⁸. The structural differences are a direct consequence of the strategy used during the synthesis, because hyperbranched polymers are obtained after a one-pot reaction, while dendrimers are prepared through a highly controlled multistep path (see paragraph 4.3).

4.2 A breath history of dendrimers

The first inspiration and studies on such treelike structures came from D. A. Tomalia¹³, who was trying to replicate the tree branching at molecular level. The first synthesis of a “dendrimer” reported in literature come from the laboratory of Professor Fritz Vögtle in 1978¹⁴. It was the first time that a rational concept of a controlled and repetitive growth with branching was reported. At the time they called it a “cascade synthesis”. In the paper was described the preparation of a polyamine tree-like structure made by iterative Michael addition to acrylonitrile and sequent reduction to primary amine. During time, the preparation was efficiently optimized¹⁵ and today those kinds of structure are known as polyamine dendrimers (*Figure 4.2-left*).

On the same line of Professor Vögtle, in 1981 Robert G. Denkewalter patented a heavily branched and asymmetric macromolecule based on the controlled polymerization of L-lysine (*Figure 4.2-right*). Denkewalter followed an approach for the rationalization, synthesis and characterization of the molecule very similar to what is used now. He divided the structure in few parts, identifying an interior unit, an exterior unit, a source unit and a surface unit¹⁶. For the preparation of this macromolecule, he followed the classic peptide synthesis approach by repeating iteratively steps of activation, coupling and deprotection.

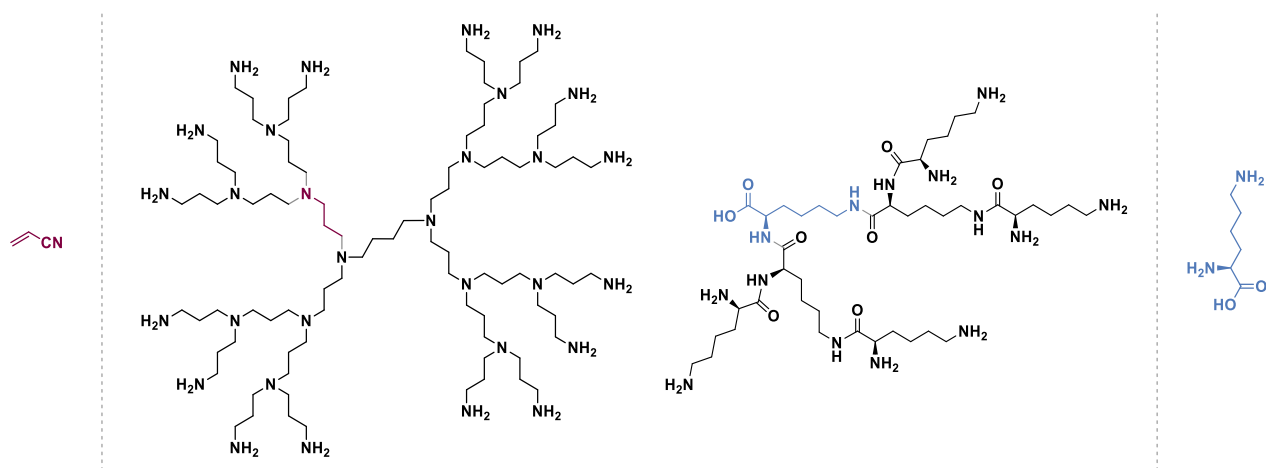


Figure 4.2: Left: example of a third-generation polyamine dendrimer with its monomer highlighted. Right: example of a portion of macromolecule based on the polymerization of L-Lysine with its monomer highlighted.

In 1985 Tomalia *et al.* published the first ever paper showing the word “dendrimer”. It was an arborescent macromolecule in which was possible to identify the central core, the ordered dendritic unit and the external unit. The structure was a polyaminoamide (PAMAM) polymer named “Stardust dendrimer”¹⁷ (*Figure 4.3*). The PAMAM preparation was heavily influenced by the predecessor works, especially from the Vögtle one. The monomer was a combination of an acrylic ester and a bis-amine, and the synthesis was accomplished by repeating iteratively reaction of Michael addition and amidic substitution. This preparation was so well optimized and reproducible that made PAMAM the first commercial dendrimer even in high generations ($G=13$). PAMAM dendrimers became so famous that today this term is used for indicating the class of dendrimer with a similar structure.

In the same year, Newkome et al. published a paper describing the preparation of another type of dendrimer, called the “Arborol System,” from the Latin *arbor* = tree (*Figure 4.3*). Compared to PAMAM dendrimers, it was more complex, yet it still possessed the characteristic dendrimer architecture consisting of a core, dendritic units, and terminal groups. The monomer was synthesized from chloroacetic acid, triethyl methanetricarboxylate, and tris(hydroxymethyl)aminomethane, and the overall dendrimer synthesis was carried out through alternating steps of nucleophilic substitution, reduction, again nucleophilic substitutions reactions and nucleophilic acyl substitution reaction¹⁸.

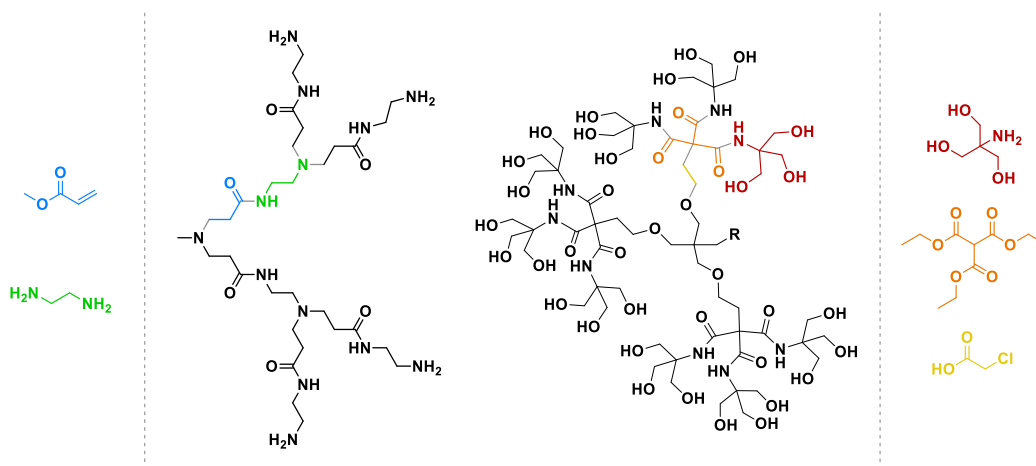


Figure 4.3: Left: example of a “stardust dendrimer” with the monomer highlighted. Right: example of a “arborol system” with the monomer highlighted

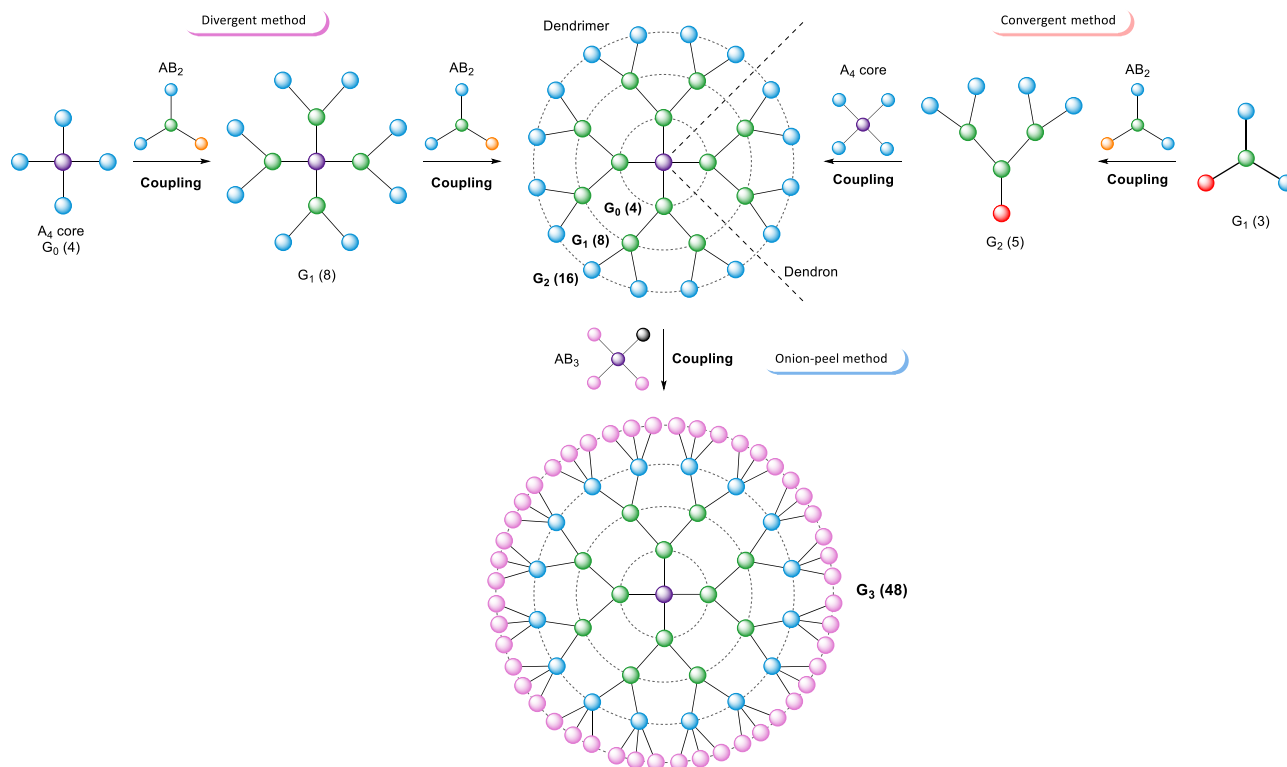
Over time, dendritic structures, particularly dendrimers and dendrons, have gained significant attention, due in part to their broad applicability (see section 4.4). Considerable effort has been done to establishing common rules for defining these macromolecules and to developing models that can predict their final properties by correlating the characteristics of the terminal groups, the dendritic architecture, and the dendrimer (or dendron) generation. To date, more than one hundred dendrimer families have been recognized, and thousands of papers describing their synthesis have been published¹⁹.

4.3 Strategies used for synthesizing a dendrimer

Today, many different strategies can be employed to prepare a dendrimer (or dendron) in the laboratory. Choosing one approach over another can lead to various synthetic challenges, often related to the chemical nature of the monomers. In other cases, the choice is primarily driven by the desired final structure or the specific application being targeted²⁰.

In this paragraph will be discussed the two most important strategies used: the divergent method (*Scheme 4.1*) and the convergent method (*Scheme 4.1*). Over time the strategies for preparing dendrimers evolved and in some cases new methods were created²¹. Among them the “onion-peel method”²² is becoming particularly famous. The name comes from the resemblance to an onion, with concentric layers formed around a central core or, more commonly, around a preformed dendrimer. The main characteristic of this method is the use of different monomers

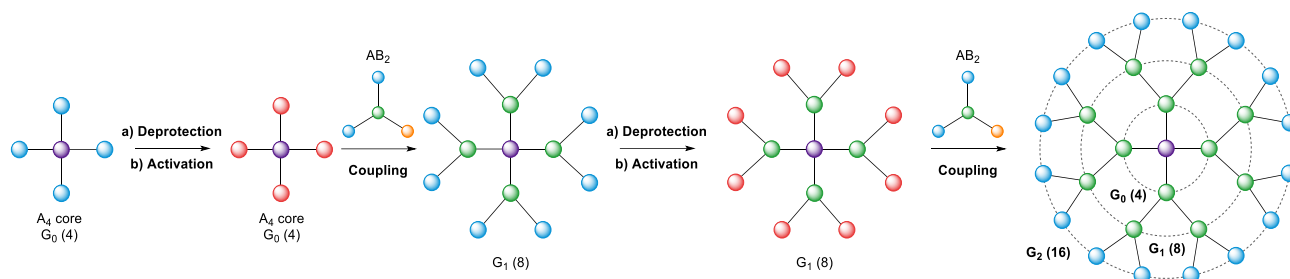
every layer, but, normally, no more than two or three layers are created in this way. It is useful to increase drastically the number of functional groups on the surface of the dendrimer or to give specific chemical and physical properties to the finalized structure, that the normal monomer cannot deliver.



Scheme 4.1: different type of dendron's synthesis. Left: Divergent method; right: convergent method; bottom "onion peel method".

4.3.1 Divergent method

The divergent method, also known as the "classical" approach, is the oldest strategy for dendrimer synthesis. It was used by Vögtle¹⁴ in 1978, Robert G. Denkwalter in 1981¹⁶, Tomalia in 1985¹⁷ and many others. In this approach, the synthesis proceeds in an "inside-out" fashion: it begins from the core, and successive layers of monomers are added (Scheme 4.2), increasing the so-called generation (G_n) by one at each step. This method is ideally suited for medium-generation dendrimers and for systems in which each reaction step is well optimized. As the generation increases, steric hindrance becomes increasingly significant, causing slower reaction rates and requiring larger amount of monomer. Conversely, the growing molecular mass of the macromolecule makes purification from excess monomer easier, and techniques such as filtration and size-exclusion chromatography can be employed. At higher generations ($G_n, n > 4$), the probability of incomplete functionalization or side reactions, most commonly intramolecular cyclization, becomes greater. Both issues lead to a loss of monodispersity, which cannot be recovered by purification.

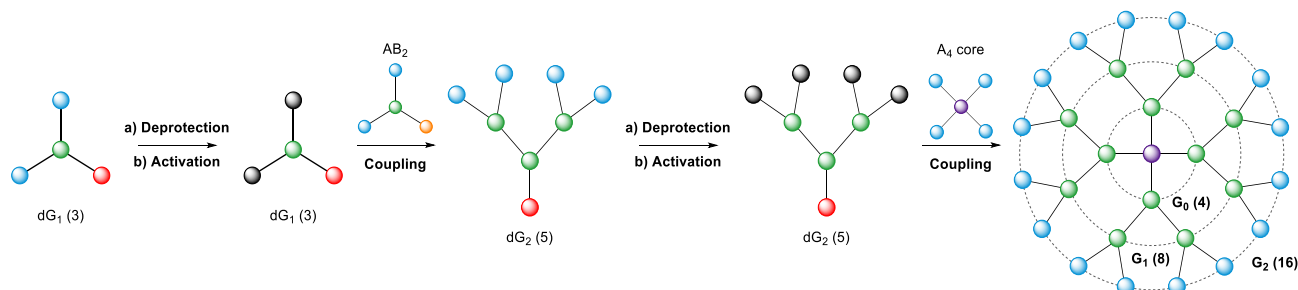


Scheme 4.2: general representation of each synthetic step used in a divergent synthesis

The entire synthesis follows steps of activation, coupling and deprotection that are iteratively repeated. It starts by choosing the core of the dendrimer and by activating its terminal functional groups. The second step is the coupling between the activated core and the complementary functional group present on the monomer. Usually, the other functional groups on the monomer molecule are protected to avoid side reactions, thus the monomer is indicated as an AB_n unit. The first branch point is obtained. After, the terminal functional groups of the prepared dendrimer are subjected to a deprotection and an activation reaction to be further coupled with fresh monomer. After the coupling reaction the dendrimer second generation (G_2) is isolated. In this way new branch points are created. The procedure is repeated to synthesise the G_{n+1} .

4.3.2 Convergent method

In the convergent method the synthesis follows an “outside in” fashion. It was first introduced by Hawker and Fréchet in 1989–1990²³. In this approach, firstly it is obtained the dendron, that carries all the information for the branched and terminal part, and as last step it is added the multifunctional core. The convergent method has many advantages respect to the divergent. Overall, as the molecular mass increases less reactions happen simultaneously, and this leads to lower excess of monomer required to accomplish the reaction and a consecutive easier purification. The dendron is a smaller molecule than the dendrimer, therefore the control on each reaction is higher, even at high generation, delivering monodisperse dendrons lacking defects. The convergent method is problematic when big and complex dendrons are used because the coupling to the core can be negatively influenced by the steric hinderance reducing the yields and making the purification more complex.



Scheme 4.3: general representation of each synthetic step used in a convergent synthesis

The preparation of a dendron follows the same sequence of reactions used in the divergent method for dendrimer synthesis. Specifically, activation, coupling, and deprotection steps are

iteratively repeated (Scheme 4.3). In the first step, a functionality (generally a small molecule) bearing a reactive site complementary to the functional groups on the core is added to the monomer. The molecule obtained is the first generation of the dendron (dG_1). The external functional groups of dG_1 are then deprotected, activated, and coupled with a new monomer (AB_n). During this step, the first branches are introduced, yielding the second generation dendron (dG_2). The same sequence of reactions is repeated on dG_2 to produce dG_{n+1} . Finally, the completed dendron is coupled to the core to obtain the final dendrimer.

This final step in the preparation highlights one of the reasons that contributed to the widespread adoption of the convergent approach. When orthogonal functional groups are installed on the core molecule, it becomes possible to obtain both symmetric and asymmetric dendrimers. These differences in reactivity can be exploited to attach dendrons of different structures to the same core²⁴. An asymmetric dendrimer in which the two halves possess opposite polarities is referred to as a “Janus-type dendrimer”²⁵. The convergent method is therefore particularly well suited for the rapid preparation of libraries of dendrimers whose cores exhibit diverse chemical and physical properties (e.g. photoactive²⁶ or catalytic active molecules²⁷).

4.4 Dendrimers applications and dendritic effect

Owing to their distinctive architecture and the simplicity with which they can be chemically modified, dendrimers have been applied widely in numerous scientific and technological areas.

In 2017 Deraedt *et al.*²⁸ published a paper in which a G_4 hydroxy-terminal PAMAM dendrimer was used as a matrix to enclose metals nanoparticles (Pd, Pt, Rh) to catalyse selective dehydrogenation reaction on nitrogen containing heterocycles and oxygen containing heterocycles. The same dendrimer loaded with Pd nanoparticles was also used to study the C-H activation of indoles. In the same research field in 2013 Peng *et al.*²⁹ published an innovative use of PAMAM dendrimers in asymmetric catalysis. To different generations of PAMAM dendrimers were covalently bound enantiopure Salen Mn (III) complexes to achieve enantioselective epoxidation of conjugated alkenes with high yield and good to acceptable enantiomeric excess.

Dendrimers can be employed in the engineering sector, with applications ranging from light-emitting materials to macromolecules for electron conduction. As a recent example, in 2023 Zhang *et al.*³⁰ published research focussed on the use of conjugated carbazole-based dendrimers capable of delivering a high phosphorescence quantum efficiency for OLED application. It was also demonstrated that those kinds of structure exhibit highly efficient thermally activated delayed fluorescence.

Dendrimers can find uses in the cosmetic world too. Sectorial review reports how different industries patented dendrimers or formulation containing dendrons for specific applications. To cite an example, Revlon demonstrated how PAMAM dendrimers can be used to encapsulate salicylic acid³¹ and riboflavin³² for anti-acne treatment or make-up. They showed how the metals concentration in the final formulation was reduced almost to 0³¹. Another example is L’Oreal that

patented a specific formulation of polyester dendrimers dispersed in a series of film forming polymers to modulate their viscosity and enhance their applicability for skin and nail treatment³³.

Among all their possible applications, dendrimers are particularly prominent in biological and nanomedicine-related fields, partly due to a unique phenomenon known as the “dendrimer or dendritic effect”³⁴. This effect can manifest in two forms: the *multivalency effect* and the *generation effect*. The *multivalency effect* occurs when all generations of a dendrimer display properties that are different or absent in the monomer itself. In contrast, the *generation effect* is observed when the properties of the dendrimer change progressively as the generation increases. This behaviour is primarily associated with the terminal functional groups, although in rare cases it may also arise from the core under the influence of the surrounding branches. The dendritic effect is not always beneficial; in some systems it enhances performance only up to a certain dendrimer size, while in others the opposite trend may be observed³⁴.

The first ever dendritic effect reported in literature was observed studying the interactions in aqueous solution between pyrene and carboxylate-terminated PAMAM dendrimer across generations 1 to 9. It was found that the fluorescence intensity of pyrene increased as the dendrimer generation increased. Researchers attributed this phenomenon to an enhancement of the first and third vibronic peaks of pyrene, resulting from stronger hydrophobic interactions between the two compounds associated with the progressively more apolar nature of the higher-generation dendrimer structures³⁵.

Dendritic structures (G_n , $n \geq 3$) possess dimensions, function, and topologies that are very close to a wide variety of important biological structures (especially proteins³⁶). Moreover, dendrimers are defined as multivalent species. Multivalency can be defined as a property of a structure to establish more than one interaction at the same time with other substrates. Dendrimers possess a wide superficial area and many functional groups in the terminal unit, and this can be translated in the possibility to have more than one interaction at same time³⁷. An example of biological multivalent species is proteins, that can do multiple ligand–receptor interactions.

Dendrimers can be efficiently used for drug delivery, sensing, and imaging, owing to their multivalency effect. In the field of sensing and imaging, the research group of Professor Inestrosa published in 2024 an example demonstrating how dendrimers can enable bimodal imaging (fluorescence and MRI). For this purpose, a specialized polyamide dendrimer was designed, featuring TEMPO (2,2,6,6-tetramethylpiperidinyloxy) groups on the surface and a fluorescent 1,8-naphthalimide core. The water-soluble dendrimer was introduced into cells, and its behaviour was examined using fluorescence microscopy and EPR (electron paramagnetic resonance)⁴.

4.4.1 Dendrimers in drug delivery

The world of pharmaceutical active small molecules drugs is a fascinating topic and most importantly is in constant evolution. This sector is very vast and among all the sub-sectors present, this project was focussed on a specific one: drug delivery. The active compound present in the

drugs that we use every day could be a natural based product, a semi-synthetic or synthetic compounds. These organic molecules in many cases share common problems. They could be unstable and prone to degradation or oxidation. Most of them have poor water solubility and, this is translated in a poor bioavailability at the target site, requiring high doses to overcome the problem. On the other hand, when the water solubility is very high, the issues connected to absorption rise, because they are unable to cross lipid membranes. Moreover, it is important to consider that drug delivery is composed by three main steps, the carriage, targeting and then release³⁸.

Dendrimers can be used as vector to deliver pharmaceutical active compounds at the target site. The conjugation (covalently bonding) a drug to a dendrimer (*Figure 4.4*) can enhance its solubility (for example paclitaxel's water solubility was increased by $9 \cdot 10^3$ times³⁹) and its lifetime (for example methotrexate half-life was increased from 24 min to 24 hours when conjugated³⁹). Despite the tangible advantages, the drug conjugation rises an important issue: the bond between the small molecule and the dendrimer must be cleavage once the target is reached. Different synthetic strategies can be used, however in many cases dendrimer-drug conjugates were classified into new drugs or combinational devices, making the entire procedure for their approval and commercialization longer and more complex⁴⁰.

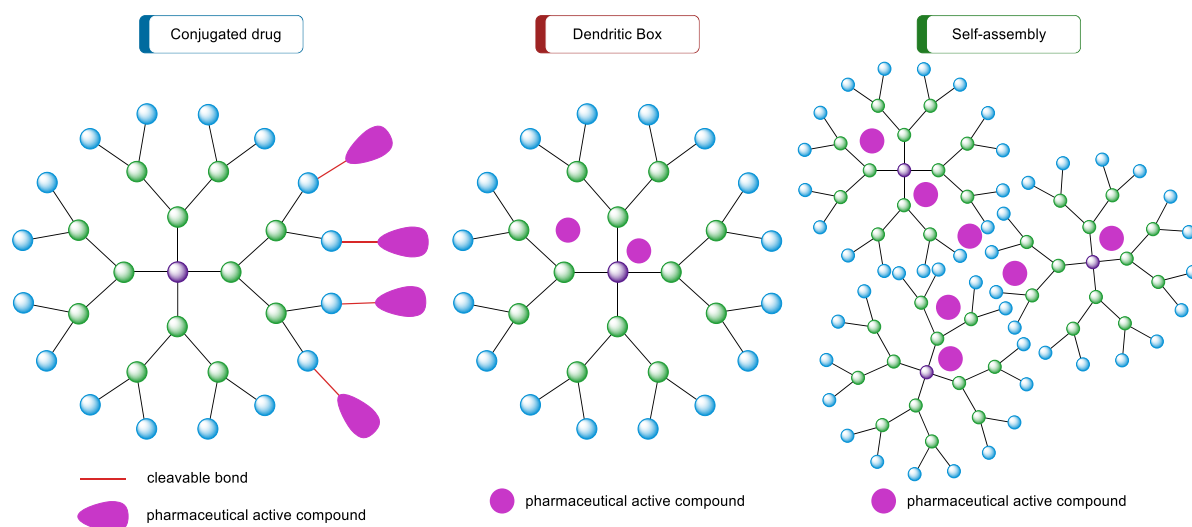


Figure 4.4: General structure of a dendrimer-drug conjugate (left), a dendritic box (centre) and a self-assembly of dendrimers for the drug delivery (right)

Scientists have employed different strategies to overcome these limitations, and one of the most promising is the micro and nanoencapsulation. Nanocarriers can modify the payload, pharmacokinetics and biodistribution, as well as increase solubility, stability, and efficacy of the encapsulated drug creating an “ad-hoc” environment for it⁴¹.

Amphiphilic dendrimer can incorporate through guest-host interaction small organic molecules, placing them inside the branched part or near the core (*Figure 4.4*). These dendrimers are called “dendritic box” or “unimolecular micelle”. The organic molecule is trapped only thanks to weak interactions, and no covalent bonds are created, therefore the release is easier. The

dendritic box can occur only when a certain internal volume is reached, but the dimension and the surface properties influence the toxicity of a dendrimer. PAMAM dendrimer which generation is lower than five can be easily eliminated by the human body through kidneys. Although they show a low toxicity, an efficient drug transport can be obtained only with generations higher than six⁴², which are toxic for the human body. A solution to this problem was found by functionalizing the surface of small dendrimers with PEG (polyethylene glycol) chains. The immediate results was a higher drug loading combined with higher permeation and retention effect. Although the enhancement of the drug loading, the pharmacokinetic was altered and part of the activity was lost.

To avoid all the drawback reported before, it was explored and studied the ability of low generation dendrimers and dendrons to create self-assembled structure able to encapsulate small molecules and deliver them at the active site without needing conjugation, high generation dendrimers and PEG polymers functionalization.

4.5 Aim of the project

Micelles and vesicles are among the most common and widely used nanocarriers for drug delivery. They have received considerable attention in recent decades, mainly because they can enhance solubility, as well as photo- and chemical stability, thereby improving the bioavailability of active compounds. Consequently, both the biodistribution and the therapeutic efficacy of the active principle are also affected. Micelles and vesicles are three-dimension self-assembled structure⁴¹. The self-assembly is a process in which an organization in structure takes place spontaneously and, normally, this arrangement exhibits higher stability due to its physical and mechanical properties. Micelles are monolayers of self-assembled molecules (*Figure 4.5a*), while vesicles are bilayers of self-assembled molecules (*Figure 4.5b*). Both are obtained when the concentration of the organic compound goes over the “critical micelle concentration” (CMC) in a specific solvent (commonly water)⁴³. To create a stable micelle/vesicle it is always necessary to use an amphiphilic compound. In a “classic” micelle/vesicle the hydrophilic part is directed to the aqueous environment (the solvent in which is made the dispersion), while the hydrophobic part is directed to the core of the micelle where hydrophobic interaction can take place. In case of an inverse micelle/vesicle the situation is the opposite.

Dendrimers and dendrons are suitable, and easy to make, material for the creation of those types of self-assembled structures.

The aim of the project was to synthesize a series of amphiphilic dendrimers bearing a hydrophilic dendron part and a hydrophobic core, capable of doing self-assembly in micelles and/or vesicles. The possibility to shift from one structure to the other in response to an external stimulus or simply due to the method of preparation of the dispersion solution was considered. The type of hydrophobic and hydrophilic segments, solvent conditions, solution pH, temperature, and presence of added solutes are the most important factors influencing the morphologies of micelles and vesicles^{44,45}.



Figure 4.5: representation of how dendrimers can self-aggregate to create a micelle (a) or a vesicle (b)

4.6 Synthesis of the amphiphilic dendrimer

The family of amphiphilic dendrimers ideated is reported in Figure 4.6. In the molecule can be identified three distinct parts. The branched dendron exposes eight terminal -NH_2 groups to the external environment. These groups can interact with water through hydrogen bonding and can also respond to variations in solution pH by modifying their protonation state and, consequently, their behaviour. The hydrophobic core is a tetra-ester of D-mannitol, where the length of the fatty acid chains could range from 8 to 16 ($n = 3-7$). Octanoic acid (8 carbons, $n=3$) was selected as the starting point, because provides the best compromise between controlling the steric hindrance and enhancing the hydrophobic properties of the core, thereby favouring the self-aggregation process. The last portion of the dendrimer is the linker between the core and the branched dendron. A triazole moiety was chosen, that is the product of the CuAAC (Copper Azido Alkyne catalysed Cycloaddition) reaction, a high yielding, fast, reproducible and reliable click reaction. CuAAC reaction proceeds under mild conditions, that are compatible with sterically hindered and sensitive compounds⁴⁶. The synthetic approach chosen followed a *convergent* fashion, therefore core and dendrons were prepared separately and bound as last step.

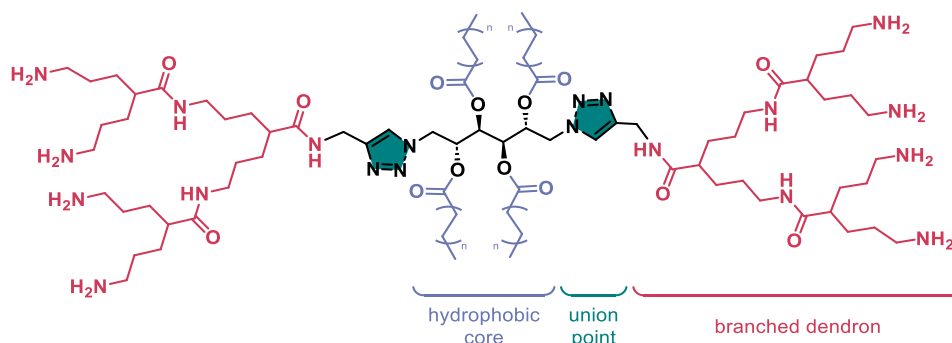
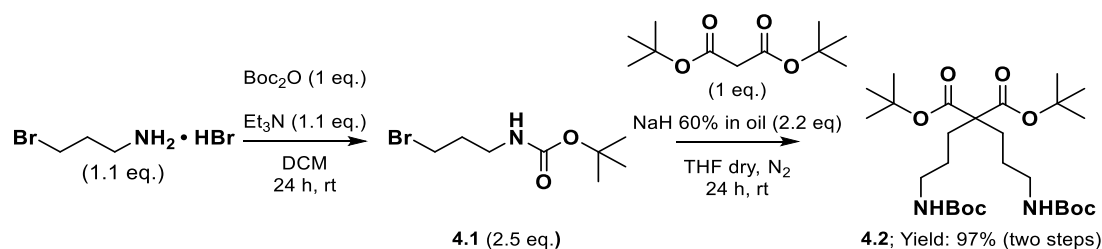


Figure 4.6: structure of the dendron designed for obtaining micelles and/or vesicles

4.6.1 Preparation of the branched dendron

The entire synthetic route followed did not require any optimization because it was used a known and published procedure by the Spanish research group¹. The multigram scale preparation

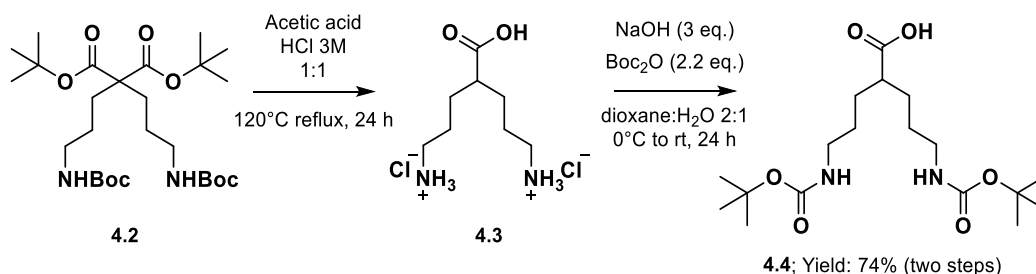
started with the protection with Boc_2O (di-tert-butyl dicarbonate) of 1,3-bromo propylamine hydrobromide, delivering the carbamate (**4.1**) in quantitative yield.



Scheme 4.4: protection of 1,3-bromo propylamine hydrobromide with Boc_2O and sequent $\text{S}_\text{N}2$ reaction with di-tert-butylmalonate

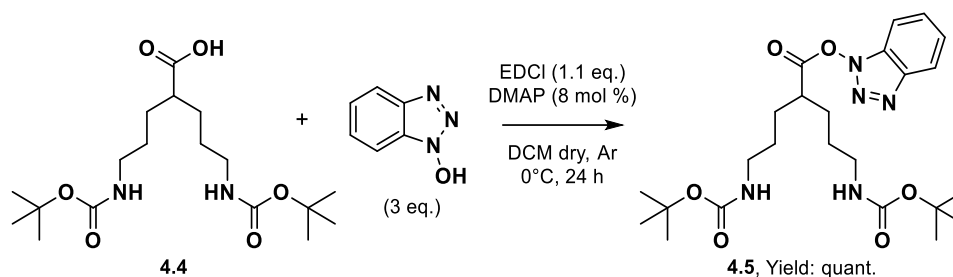
The second reaction was the nucleophilic substitution of the bromine atom of **4.1** by the carbanion obtained by in-situ deprotonation of di-tert-butylmalonate in the presence of NaH. It delivered the desired product, **4.2**, in 97% yield (two steps). Directly after the preparation, the α -diester (**4.2**) underwent a decarboxylation in the presence of acetic acid and HCl 3M aqueous solution delivering the desired product (**4.3**) quantitatively. In this reaction due to the presence of a strong acid and high temperature (120°C) the amino groups were deprotected along with the hydrolysis of the remaining tertbutyl ester.

The two primary amines in the form of salts were protected again, only after quenching with a base, to avoid problems in the further reactions. The di-protected diamino acid (**4.4**) was obtained in a 74% yield (two steps) (Scheme 4.5).

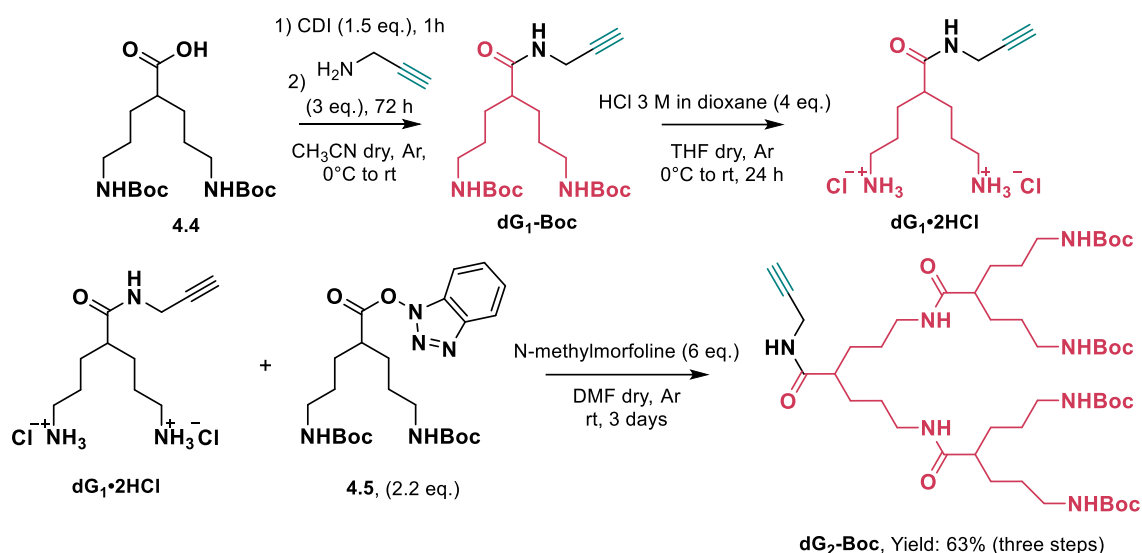


*Scheme 4.5: decarboxylation of diester **4.2** and sequent protection with Boc_2O*

The branched part of the dendrimer reported in Figure 4.6 is a polyamide. However, the direct reaction between a carboxylic acid and an amine to form an amide is almost impossible under standard conditions because the acid–base reaction is strongly favoured. Therefore, it is necessary to properly activate the carboxylic acid to accomplish the transformation. To overcome this issue, the carboxylic moiety of the monomer (**4.4**) was activated in order to increase the rate of the nucleophilic acyl substitution reaction while maintaining mild conditions. The carboxylic acid (**4.4**) was activated with HOBt (1-hydroxybenzotriazole) a common reagent used in peptide synthesis. It was used in combination with DMAP (4-Dimethylaminopyridine) and EDCI (1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride) as coupling agents to accomplish the esterification. The activated ester (**4.5**) was obtained in quantitative yield (Scheme 4.6).



Scheme 4.6: coupling of HOBt to acid (**4.4**) with EDCI and DMAP to obtain the activated ester (**4.5**)



Scheme 4.7: reaction used to prepare the first generation (**dG₁**) and the second generation (**dG₂**) of the dendron

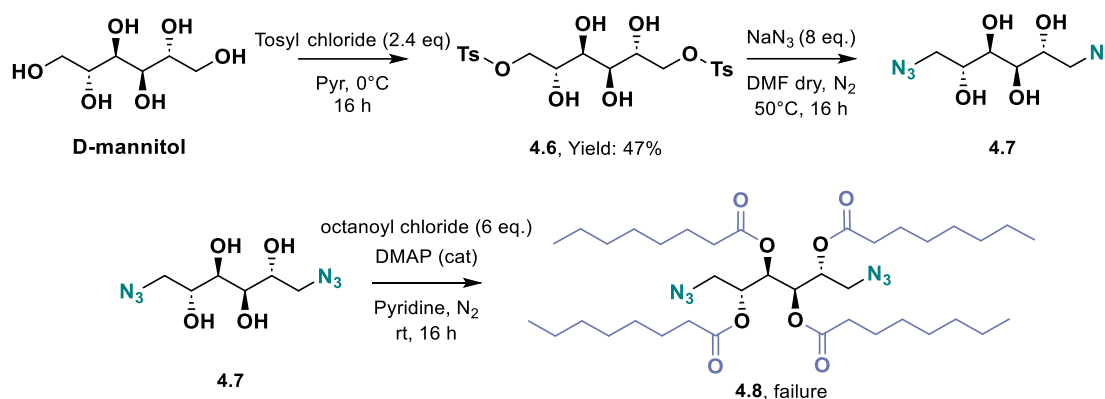
The di-protected amino acid (**4.4**) was used as starting material for the preparation of the first generation of the dendron (**dG₁**). Initially, it was used to prepare **dG₁-Boc** through an amide coupling with propargyl amine in the presence of CDI (1,1'-carbonyldiimidazole) as the coupling agent. The use of propargyl amine was necessary to install the right functional group for the further CuAAC reaction necessary to anchor the dendron to the core. Once obtained, **dG₁-Boc** was deprotected using HCl to isolate the desired **dG₁•2HCl** (Scheme 4.7).

The increase in generation was the final coupling for the synthesis of the second generation dendron (**dG₂**). This step is again a nucleophilic acyl substitution reaction in which is used the previously prepared activated ester (**4.5**) and **dG₁•2HCl** (Scheme 4.7). In this case N-methylmorpholine was used to generate in situ **dG₁**, which could act as the real nucleophile in the substitution of HOBt (Scheme 4.7). After 3 days **dG₂-Boc** was obtained with a yield of 63% (three steps). **dG₂-Boc** was not deprotected to avoid issues correlated to the low solubility and potential side reactions, moreover the free amino groups can chelate the copper ions during the CuAAC reaction prohibiting it.

4.6.2 Preparation of the hydrophobic core

The starting point for the preparation of the core of the dendrimer was to choose a suitable polyalcohol. D-mannitol was chosen because it is a low-cost carbohydrate, available in nature and

easy to prepare from D-mannose. The chirality and the possibility of using an enantiopure substrate are not important factors for the final application.

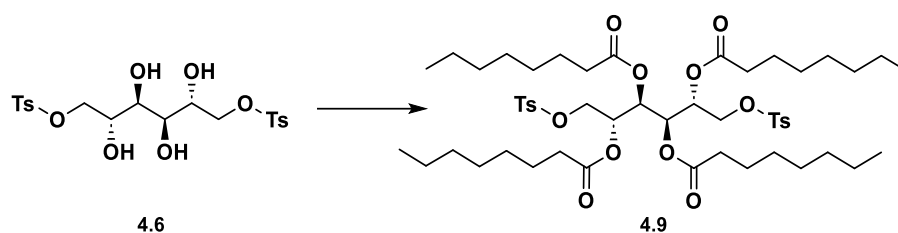


Scheme 4.8: first synthetic approach for the preparation of the core molecule (4.8)

Initially, it was attempted a direct approach to prepare the final structure (**4.8**), which involved a double tosylation of D-mannitol (compound **4.6**), followed by a nucleophilic substitution with NaN₃ (compound **4.7**) and then a direct tetra esterification of the four free hydroxy groups with octanoyl chloride (compound **4.8**) (Scheme 4.8). Unfortunately, although it was the fastest route, it proved to be unusable. During the nucleophilic substitution with NaN₃ (the second reaction in Scheme 4.8) OH groups took part in many side-reactions lowering the yield. The insolubility of bis-azide (**4.7**) made impossible any purification. Moreover, it didn't react as expected in the final step with octanoyl chloride where only side products were isolated after purification.

After the initial failure, it was decided to change the synthetic approach by performing the esterification before the nucleophilic substitution with NaN₃ (Scheme 4.9). The higher solubility given by the tosyl groups (**4.6**) should have helped in the reaction. Unfortunately, the tosyl groups were proved to be incompatible with each reaction conditions attempted due to their high reactivity (Table 4.1).

When octanoyl chloride was used (entry from 1 to 3) a complicated mixture of side-products and unreacted intermediates was found. Entry 4 gave the best result, but the product was isolated along with the triacyl-substituted intermediate and another unknown impurity. Even after 2 chromatographic purifications was not possible to isolate it as a pure compound. In entry 5 a milder octanoyl-HOBt was used, instead of the strongly reactive acyl chloride. Octanoyl-HOBt was quantitatively prepared from octanoic acid using the condition reported in Scheme 4.6. Nonetheless the condition chosen, numerous side-product were detected, mainly due to the degradation of the starting material (compound **4.6**). One of the side products (**4.9a**, reported in Figure 4.7) was isolated through chromatographic purification in 35% yield, which can be formed if the hydroxybenzotriazole acts as a nucleophile substituting the tosyl group.



Scheme 4.9: alternative synthetic path used to prepare the dendrimer core

Table 4.1: condition used for the tetra-esterification of di-tosyl-mannitol

Entry	Conditions	Yield*
1	Octanoyl chloride (8 eq.), DMAP (5 mol%), pyridine; N ₂ , 0°C to rt, 48 h	Yield: 18%
2	Octanoyl chloride (8 eq. + 2 eq. after 24h), DMAP (5 mol%), pyridine; N ₂ , 0°C to 40°C, 48 h	Yield: 12%
3	Octanoyl chloride (8 eq.), DMAP (5 mol%), Et ₃ N (12 eq.), DMF dry; N ₂ , 0°C to rt, 48 h	Yield: 20-30%
4	Octanoyl chloride (8 eq. + 2 eq. after 24h), DMAP (5 mol%), Et ₃ N (12 eq.), DCM dry; N ₂ , 0°C to rt, 48 h	Yield: 63-69%
5	Octanoyl-HOBt activated (4.8 eq. + 2 eq. after 24h + 2 eq. after 48h), Et ₃ N (5 eq.), DCM dry; N ₂ , 0°C to rt, 76 h	Yield: 31-40%
6	Octanoic acid (4.8 eq. + 2 eq. after 24h + 2 eq. after 48h), EDCl (4.8 eq. + 2 eq. after 24h + 2 eq. after 48h), DMAP (8 mol%), DMF dry; N ₂ , 0°C to rt, 96 h	Yield: 0%

*yield after 2 flash chromatography on silica

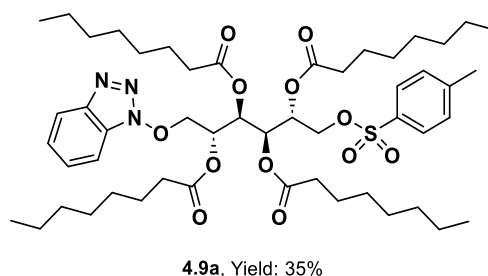
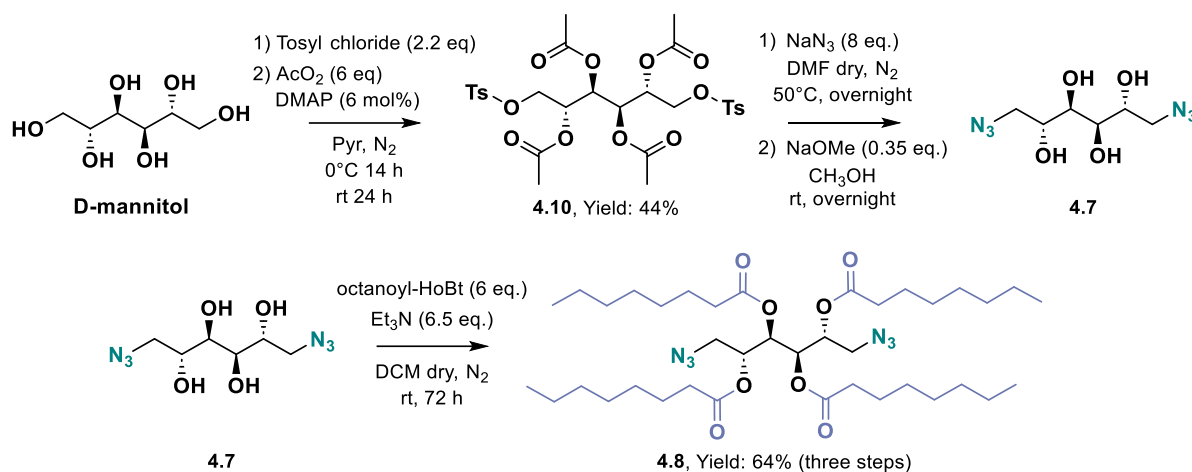


Figure 4.7: structure of the side product isolated during the esterification of compound **4.6** using octanoyl-HOBt

An even milder approach was carried out (entry 6), but it was proved to be inefficient. No side-product were detected, but after 96 hours, a mixture of starting material (the majority of the mass), mono-acyl-substituted and di-acyl-substituted intermediates was detected. The product was absent.

The synthetic approach was changed again based on the results reported. This time, it was decided to protect the secondary OH groups as acetate and then perform the S_N2 reaction with NaN₃. The di-tosyl-tetra-acetyl protected compound (**4.10**) was obtained in a 44% yield in a two steps reaction. Compound **4.10** was used in the S_N2 reaction with NaN₃ and the resulting bis-azide was pure enough to be submitted to the deprotection step. The acetyl groups were removed in MeOH using a catalytic amount of sodium methoxide. Notably, this method offers the advantage of generating methyl acetate as the only byproduct (Scheme 4.10). This strategy provides a more efficient route to compound **4.7**, overcoming the limitations encountered in the initial method.



Scheme 4.10: sequence of reactions optimized for the preparation of the hydrophobic core (**8**)

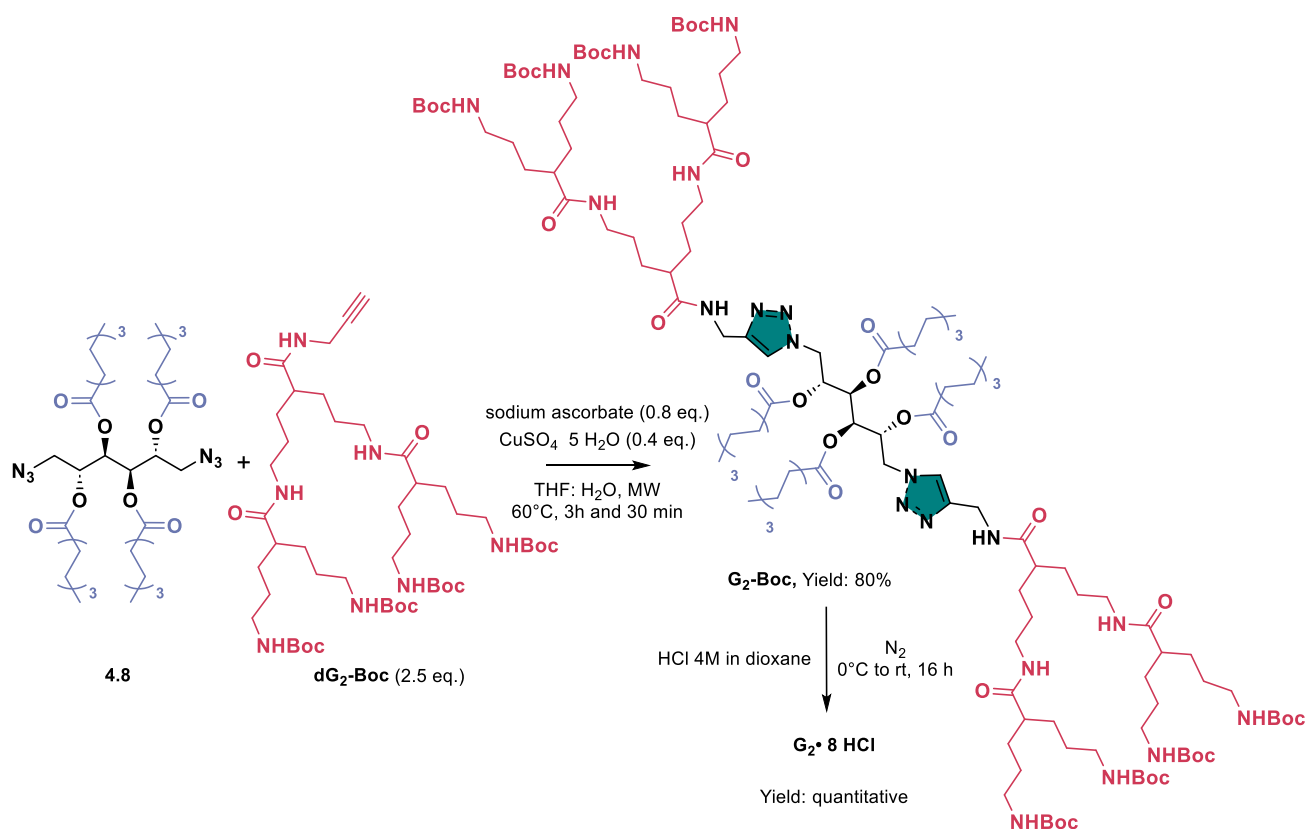
In the last step the activated HOBt ester was used instead of octanoyl chloride, and the bis-azide (**4.7**) was converted into the desired core of the dendrimer **4.8**, with an overall yield of 64% over three steps. Although the starting material was not soluble in DCM, it gradually underwent reaction, leading to complete conversion into more soluble intermediates. Using this new approach, the desired product was successfully isolated following chromatographic purification, with a notable reduction in the formation of side products.

4.6.3 Preparation of the final dendrimer

Once obtained, the tetra-acylated bis-azide (**4.8**) and **dG₂-Boc** were reacted together to obtain the final dendrimer **G₂-Boc**. The chosen reaction was the CuAAC, a very reliable and fast click reaction, that can work with sensitive and bulky compounds like the one reported in Scheme 4.11. The reaction conditions used were already established by the research group. By optimizing the workup and the purification method the yield was improved from an initial 69% to an 80%. In the work-up of the reaction it was preferred to remove in negative pressure the solvents, solubilize the organic compound in THF and filter it over paper to remove the insoluble copper salts, instead of doing a traditional liquidous-liquidous extraction with a complexing agent. For the purification of this macromolecule, size-exclusion chromatography was preferred over other chromatographic techniques.

The final step of the synthesis was the deprotection of **G₂-Boc** (Table 4.2 and Scheme 4.11). Initially, to avoid hydrolysis of the esters promoted by the acid, it was used a stoichiometric amount of 4M HCl in dioxane mixed with THF as solvent (entry 1). The result was a partial conversion of the starting material, where only two Boc groups were removed. Even longer reaction times did not lead to any improvement (entry 2) as the employment of a higher amount of acid (entry 3). It was decided to increase the amount of solvent, to avoid aggregation of the intermediate and favour a better reactivity, but again only partial deprotection was detected after the reaction (entry 4). Following several unsuccessful attempts, a larger amount of acid was employed to promote the deprotection, while retaining a portion of the THF to ensure sufficient dilution and minimizing potential ester hydrolysis (entry 5). Analysis once again revealed intermediates with only two Boc

groups removed. Ultimately, it was decided to use 4 M HCl in dioxane as both solvent and reagent (entry 6). A higher volume was employed to prevent dendrimer aggregation. The new conditions delivered **G₂-8HCl** dendrimer quantitatively without the need for purification.



Scheme 4.11: Click reaction between di-azide (**4.8**) and **dG₂-Boc** and final deprotection to deliver **G₂-8HCl**

Table 4.2: the conversion of the starting material was measured using ¹H-NMR

Entry	Conditions	Result
1	HCl 4M in dioxane (18 eq.), THF dry 0.15M, N ₂ , 0°C to rt, 4 h	No product
2	HCl 4M in dioxane (18 eq.), THF dry 0.15M, N ₂ , 0°C to rt, 16 h +16 h	No product
3	HCl 4M in dioxane (60 eq.), THF dry 0.15M, N ₂ , 0°C to rt, 16 h	No product
4	HCl 4M in dioxane (60 eq.), THF dry 0.001M, N ₂ , 0°C to rt, 16 h	No product
5	1:1 4M HCl in dioxane/THF dry, final conc. G₂-BOC 30 mg/mL, N ₂ , 0°C to rt, 16 h	No product
6	HCl 4M in dioxane 6.5 mg/mL, 0°C to rt, N ₂ , 16 h	Quantitative yield

Due to all the problems encountered during the synthesis of the first dendrimer, there was no time left to start the preparation of a new one and enlarge the family of amphiphilic macromolecules. The time left was used to analyse the **G₂-8HCl** and study its ability to self-aggregate.

4.7 Experimental analyses

G₂-8HCl was analysed with the classical techniques like HPLC-HRMS and NMR to assess the structure of the compound. Parallel to those, DLS (dynamic light scattering) was used to evaluate its capability to do self-aggregation.

DLS analysis was performed to measure the hydrodynamic diameter and ζ (zeta-potential) was used to confirm the positive charge on the surface of the formed self-assembled structure. For this analysis 4 different molar concentration of **G₂-8HCl** were used: 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} M. Deionized water was chosen as solvent, to avoid possible ions interference in the aggregation process (Kadiri *et al.* reported an entire study on how the presence of external ions can influence the self-aggregation process⁴⁷). Each solution was analysed directly after the preparation and after 24 hours. In this way, it was possible to evaluate possible collapses, enlargement or stabilization of the structures. 10^{-6} M solution was found to be the limit of detection of the instrument and more diluted solutions were not prepared. All the results are reported in Table 4.3. All the small peaks over 4 μm , related to the dust suspended in the cuvettes, were not considered.

Table 4.3: hydrodynamic diameter (D_h [nm]) and zeta potential (ζ [mV]) measurement of **G₂-8HCl** in deionized water solutions.

Concentration	D_h [nm] (Rel. Intensity %)		ζ (mV)
$1 \cdot 10^{-6}$ M	t_{0h}	117 (1%)	+1.45
	t_{24h}	381 (100%)	
$1 \cdot 10^{-5}$ M	t_{0h}	116 (15%), 571 (78%)	+5.3
	t_{24h}	351 (50%), 672 (45%)	
$1 \cdot 10^{-4}$ M	t_{0h}	368 (99%)	+5.1
	t_{24h}	509 (95%)	
$1 \cdot 10^{-3}$ M	t_{0h}	50 (13%), 366 (87%)	+7.5
	t_{24h}	35 (4%), 459 (80%)	

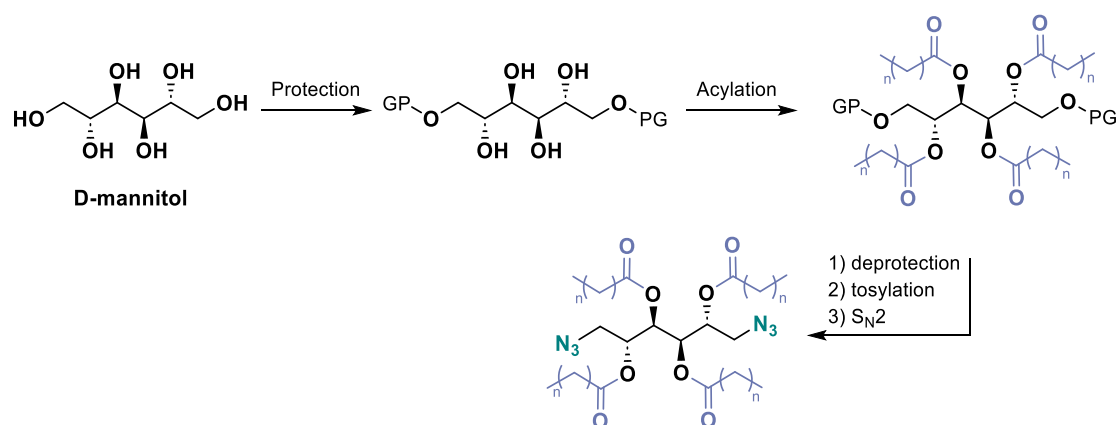
Two factors influence the dimensions of the aggregates: the operating concentration and the stabilization time. In the 1×10^{-5} M solution at t_0 , aggregates with two distinct sizes (116 nm and 571 nm) were present, both of which increased in size over time. A similar trend was observed in the 1×10^{-4} M solution, although in this case, aggregates of only a single size were detected. $1 \cdot 10^{-3}$ M solution was too concentrated for the formation of monodisperse aggregates. The aggregates with small diameters are probably formed after the collapse of bigger structures, because they are totally absent in the more diluted solutions. In a freshly prepared solution, the most stable structure exhibits an average diameter of 366 nm. However, upon allowing the system to reach equilibrium, the aggregate size stabilizes at approximately 459 nm. Aggregates with a bigger diameter were not found. The dimension increase over time can be clearly seen in all the solutions. The analysis of $1 \cdot 10^{-6}$ M solution is the one most influenced by the background noise. At t_0 no aggregates were detected but after 24 hours a single type of structure with a diameter of 381 nm

was formed. Due to the high dilution, the Z-potential could not be accurately determined. For all the other samples, the potential is comparable with the value reported in literature. For example, a G₄ PAMAM (polyaminoamide) aggregate possesses a ζ of +8.7 mV⁴⁸.

4.8 Conclusions and future plans

In conclusion, the first dendrimer of a new family of amphiphilic macromolecules for biomedical applications was synthesized, and several synthetic steps were optimized. The double tosylation of D-mannitol, however, remains problematic and low-yielding, and requires further optimization. A better control of the reaction temperature using a thermostatic bath should be implemented, particularly to maintain constant cooling overnight, in order to reduce the formation of tri-tosylated mannitol. Other parameters that could be evaluated include shortening the reaction time or adding tosyl chloride more slowly over time using a syringe pump.

Alternatively, the synthetic approach could be changed again, as reported in *Scheme 4.12*. The proposed route is less divergent and less atom-economic, but it reduces the number of purification steps required and eliminates the issues associated with the double tosylation. The primary hydroxyl groups of D-mannitol could be protected with two bulky silicon-based protecting groups such as triphenylsilyl or tert-butyldiphenylsilyl. Triphenylsilyl chloride and tert-butyldiphenylsilyl chloride are known to provide selective quantitative protection of primary hydroxyl groups⁴⁹. A high-yielding tetra-acylation with acyl chlorides can then follow, with improved efficiency due to the chemical stability of the protecting groups. The acylated product can subsequently undergo a reaction sequence consisting of deprotection (using tetra-*n*-butylammonium fluoride, TBAF), tosylation, and nucleophilic substitution with NaN₃ under the same conditions described in the previous sections, followed by a single purification step after the three transformations.



Scheme 4.12: alternative synthetic path for the preparation of the super hydrophobic core (PG: protecting group)

In the future, new type of fatty acid chains will be bound to the core, to study how steric hindrance can affect the self-assembly capability. **G₂·8HCl** will be analysed using other techniques like cryo-TEM (Cryogenic Transmission Electron Microscopy) to visualize the structure of the aggregates and DOSY-NMR (Diffusion Ordered Spectroscopy) to measure how the

aggregates diffuse in solution. If the resulting data will be promising, tests in cellular culture will be performed.

4.9 Experimental procedures

1.9.1 General aspects

Solvents and reagents

Commercial solvents and reagents were used directly without any other purification. Inert gases were taken directly from the original tanks. When inert atmosphere was used, commercially available dry solvents were employed, and every possible precaution was taken.

Routine procedure

Analytical TLC was performed on Merck silica gel 60 F254 0.25 mm TLC aluminium sheets and visualized by UV (254 nm) and developed by dipping and heating with KMnO_4 (1,5 g of KMnO_4 , 10 g of K_2CO_3 in 200 mL of deionized water). R_f were measured after an elution of 7–9 cm. After extractions, the aqueous phases were always re-extracted three times with the appropriate organic solvent, and the organic extracts were always dried over MgSO_4 and filtered before evaporation to dryness. Concentration under reduced pressure was performed by rotavapor setting temperature and pressure on the solvent used. Chromatographic purification was performed as flash chromatography (FC) on MERCK Geduran® Si 60 (40–63 μm , 230–400 mesh) under positive pressure and the crude mixtures were loaded as solution in the eluent. Purified compounds were dried further under high vacuum ($\sim 10^{-2}$ torr). Purification by exclusion dimension chromatography was performed using as stationary phase the Sephadex™ LH-20 resin loaded and packed into a 1.5 cm in diameter chromatographic column. The solvent used was a mixture 1:1 of dichloromethane and methanol. The elution was performed by gravity, and the crude mixtures were loaded as solution in the solvent mixture. Maximum amount loaded was 80 mg and it was solubilized in 1 mL of solvent. Purified compounds were dried further under high vacuum ($\sim 10^{-2}$ torr).

All products were characterized by ^1H , ^{13}C , HPLC-ESI-HRMS (high resolution mass spectrometry).

Nuclear magnetic resonance spectroscopic analysis (NMR)

^1H and ^{13}C NMR spectra were recorded on a Bruker Ascend 400 MHz (at 400 MHz ^1H) and on a Bruker Ascend 500 M (at 500 MHz ^1H and 126 MHz ^{13}C). Chemical shifts (δ) are reported in parts per million (ppm) using as internal standard tetramethylsilane (TMS, 0.00 ppm) or the residual solvent signal (CDCl_3 at 7.26 ppm for ^1H and 77.16 ppm for ^{13}C , D_2O at 4.79 ppm for ^1H , $\text{DMSO-}d_6$ at 2.50 ppm for ^1H and 39.52 for ^{13}C and CD_3OD at 3.31 ppm for ^1H and 49.00 for ^{13}C). The data are reported as follow: chemical shift, multiplicity (indicated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad signal and combinations thereof), coupling constants (J), in Hertz (Hz) integration, and assignation (when possible). Peak assignments were done with the aid of gCOSY

(gradient correlation spectroscopy), 135-DEPT (Distortionless Enhancement by Polarization Transfer, 135 spectroscopy), gHMQC (gradient heteronuclear multiple-quantum correlation spectroscopy) and gHMBC (gradient heteronuclear multiple-bond correlation spectroscopy).

All the NMR spectrums were analysed using the software MestReNova (Mestrelab Research® v. 14.2).

High Performance Liquid Chromatography (HPLC)

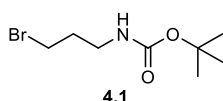
High Performance Liquid Chromatography (HPLC) analysis was performed on a Thermo Scientific UltiMate 3000 (Thermo Fisher Scientific, Waltham, MA, USA). High-resolution mass spectra (HRMS) were acquired using a Thermo Scientific Q Exactive Orbitrap mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive and negative ion mode.

Dynamic Light Scattering (DLS) and Zeta Potential (ζ) Measurements

Particle size and Zeta potential measurements were carried out in a Malvern Analytical Zetasizer Nano ZS 90 system (Malvern Instruments, UK). Particle sizes were measured in single use PS cuvettes registering scattering at 173° upon incident laser beam (623.8 nm wavelength, 4.0 mW). The analysis was performed only after stabilizing the internal temperature to 25°C. The amount of solution used was 1 mL. Zeta potential was registered directly in the PS cuvettes using a ZEN1002 Dip cell zeta potential probe. The analysis was performed only after stabilizing the internal temperature to 25°C. The amount of solution used was 1 mL. Each sample was analysed in cumulative mode using the integrated Zetasizer software (Malvern Zetasizer software v.8.02). Only the size distribution based on intensity was considered.

4.9.2 Preparation of the dendrimer

Synthesis of tert-butyl (3-bromopropyl)carbamate (**4.1**)



3-Bromopropylamine hydrobromide (15.00 g, 68.52 mmol), was suspended in CH₂Cl₂ (50 mL). A white and thin suspension was obtained. To the crude was added a solution of di-*tert*-butyl dicarbonate (13.60 g, 62.29 mmol) in CH₂Cl₂ (30 mL). After 1 minute triethylamine (9.5 mL, 68.52 mmol) was slowly injected with a syringe. Intense bubbling and warming of the reaction flask were observed. After 16 h at room temperature, the organic layer was extracted twice with deionized water, washed with 1M sodium bicarbonate, and Brine, dried (MgSO₄) and the solvent removed under vacuum. The crude, a slightly yellow oil (14.82 g), was considered to be sufficiently pure to be used directly in the subsequent reaction.

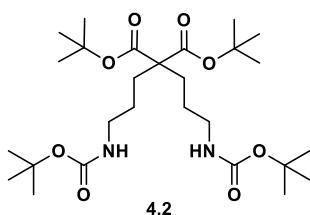
Molecular weight: 283.13 g/mol (C₈H₁₆BrNO₂)

TLC: R_f 0.66 (hexane:EA 8:2, basic KMnO₄)

¹H NMR (500 MHz, CDCl₃): δ 4.65 (bs, 1H, NH), 3.44 (t, *J* = 6.5 Hz, 2H, Br-CH₂CH₂CH₂-NH), 3.27 (q, *J* = 6.2 Hz, 2H, Br-CH₂CH₂CH₂-NH), 2.05 (p, *J* = 6.3 Hz, 2H, Br-CH₂CH₂CH₂-NH), 1.44 (s, 9H, C(CH₃)₃).

All the other data were in accordance with literature

Synthesis of di-*tert*-butyl 2,2-bis(3-((*tert*-butoxycarbonyl)amino)propyl)malonate (**4.2**)



NaH (60% in mineral oil, 1.320 g, 55.2 mmol) was suspended in dry THF (40 mL) under nitrogen atmosphere. Di-*tert*-butylmalonate (5.6 mL, 25.1 mmol) was added slowly dropwise. Intense bubbling was observed. The grey and white foam was left stirring for 30 minutes. To the reaction crude was slowly added a solution of *N*-Boc-3-bromopropylamine (**4.1**) (14.94 g, 62.72 mmol) in THF dry (40 mL). The reaction mixture was cooled in an ice bath to prevent intense heating. After 15 minutes the reaction was warmed to room temperature. After 16 h the solvent was removed in reduced pressure, and the residue was dissolved in ethyl acetate and extracted using deionized H₂O (50 mL). The organic phases are washed 3 times with distilled H₂O, dried (MgSO₄) and the solvent removed under vacuum. The crude was purified by trituration in *n*-hexane. A fine and white solid (12.881 g, yield 97% two steps) was obtained.

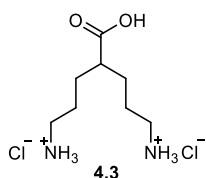
Molecular weight: 530.36 (C₂₇H₅₀N₂O₈)

TLC: R_f 0.28 (hexane:EA 8:2, KMnO₄ basic)

¹H NMR (400 MHz, CDCl₃): δ 4.58 (bs, 2H, 2x NH), 3.10 (q, *J* = 4.7 Hz, 4H, 2x C-CH₂CH₂CH₂-NH), 1.82 – 1.74 (m, 4H, 2x C-CH₂CH₂CH₂-NH), 1.45 – 1.41 (m, 36H, 4x C(CH₃)₃), 1.40 – 1.30 (m, 4H, 2x C-CH₂CH₂CH₂-NH).

All the other data were in accordance with literature

Synthesis of 4-carboxyheptane-1,7-diaminium chloride (**4.3**)



Di-*tert*-butyl 2,2-bis(3-((*tert*butoxycarbonyl)amino)propyl)malonate (**4.2**) (12.880 g, 24.3 mmol) was suspended in acetic acid (21.5 mL) and 3 N hydrochloric acid (21.5 mL). The solid was dispersed through sonication. The reaction crude was heated to 100°C. As soon as the crude reached 100°C, the solid was dissolved obtaining a clear solution. After 16 h the solvent was removed under vacuum. Fresh 100 mL of distilled water were added, freezed with liquid N₂ and the reaction flask was plugged to the freeze drier. It was obtained a sticky colourless amorphous solid (5.86 g). It was considered to be sufficiently pure to be used in the sequent reaction without any further purification.

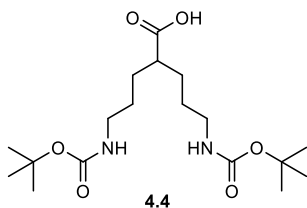
Molecular weight: 246.09 g/mol (C₈H₂₀Cl₂N₂O₂)

TLC: R_f 0.05 (9:1 DCM:CH₃OH, KMnO₄ basic)

¹H NMR (500 MHz, D₂O): δ 3.02 (s, 4H, 2x CH-CH₂CH₂CH₂-NH₃⁺), 2.52 (s, 1H, CH-CH₂CH₂CH₂-NH₃⁺), 1.69 (s, 8H, 2x C-CH₂CH₂CH₂-NH₃⁺).

All the other data were in accordance with literature

Synthesis of 5-((tert-butoxycarbonyl)amino)-2-(3-((tert-butoxycarbonyl)amino)propyl)pentanoic acid (**4.4**)



4-carboxyheptane-1,7-diaminium chloride (**4.3**) (6.304 g, 25.5 mmol) was solubilized in a mixture of dioxane : H₂O (2:1, 35 mL). The crude was cooled to 0°C and NaOH in powder (3.062 g, 76.5 mmol) was added in portions. After 15 minutes, when NaOH was solubilized, a solution of di-*tert*-butyl dicarbonate (12.256 g, 56.1 mmol) in dioxane:H₂O (2:1, 15 mL) was added dropwise. After 24 h the solvent was removed under vacuum and the residue was dissolved in H₂O (50 mL). The aqueous phase was extracted with ethyl acetate twice. The aqueous layer was acidified to pH 1-2 with 1M HCl and extracted again with ethyl acetate three times. Only the organic layers from the second extraction were dried (MgSO₄) and the solvent was removed under vacuum. After the double extraction it was obtained the clean product as colourless foamy solid (6.758 g, 74% yield).

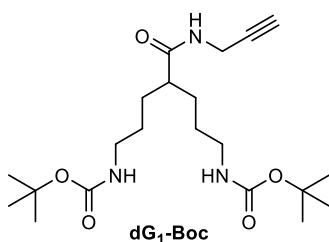
Molecular weight: 374,48 g/mol (C₁₈H₃₄N₂O₆)

TLC: R_f 0.50 (hexane:EA 9:1, KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 4.62 (s, 2H, 2x NH), 3.12 (s, 4H, 2x NH-CH₂CH₂CH₂-CH), 2.42 (s, 1H, NH-CH₂CH₂CH₂-CH), 1.71 – 1.61 (m, 2H, NH-CH₂CH₂CH₂-CH), 1.60 – 1.37 (m, 24H, NH-CH₂CH₂CH₂-CH, 2x NH-CH₂CH₂CH₂-CH and 2x C(CH₃)₃).

All the other data were in accordance with literature

Synthesis of di-*tert*-butyl (4-(prop-2-yn-1-ylcarbamoyl)heptane-1,7-diyl)dicarbamate (**dG₁-Boc**)



Carboxylic acid (**4.4**) (2.156 g, 5.76 mmol) and 1,1-carbonildiimidazol (1.400 g, 8.63 mmol) were solubilized in dry CH₃CN (15 mL) under inert atmosphere, Ar. After 1 h, propargyl amine (1.10 mL, 17.3 mmol) was added dropwise. The crude changed colour to slightly yellowish. After 3 days, the solvent was removed under vacuum and the crude was diluted with DCM and HCl 0,05M. The organic phase was extracted 4 times, dried (MgSO₄) and the solvent removed. The crude, a colourless amorphous solid (2.271 g), was considered to be enough pure and it was used in the next reaction without any further purification.

Molecular weight: 411,54 g/mol (C₂₁H₃₇N₃O₅)

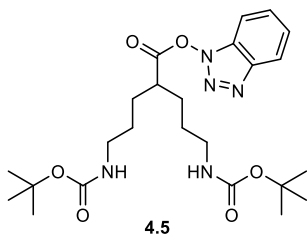
TLC: R_f 0.25 (hexane:EA 9:1, KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 6.33 (s, 1H, NHCH₂C≡CH), 4.62 (s, 2H, 2x NH), 4.02 (dd, *J* = 5.1, 2.3 Hz, 2H, NHCH₂C≡CH), 3.21 – 3.10 (m, 2H, NH-CH₂CH₂CH₂-CH), 3.11 – 2.98 (m, 2H, NH-CH₂CH₂CH₂-CH), 2.33 – 2.14 (m, 2H, NH-CH₂CH₂CH₂-CH and CH₂C≡CH), 1.68 – 1.61 (m, 2H, NH-

CH₂CH₂CH₂-CH), 1.50 – 1.40 (m, 24H, NH-CH₂CH₂CH₂-CH and 2x NH-CH₂CH₂CH₂-CH and 2x C(CH₃)₃).

All the other data were in accordance with literature

Synthesis of 1H-benzo[d][1,2,3]triazol-1-yl-5-((tert-butoxycarbonyl)amino)-2-(3-((tert-butoxycarbonyl)amino)propyl)pentanoate (**4.5**)



Carboxylic acid (**4.4**) (4.603 g, 0.0123 mmol), hydroxy benzotriazole (HOBt, 4.983 g, 12.3 mmol) and 4-dimethylaminopyridine (DMAP, 120 mg, 0.983 mmol) were solubilized in dry DCM (40 mL), under inert atmosphere, Ar. The reaction crude was cooled to 0°C and after 5 minutes a solution of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCl, 2.592 g, 13.5 mmol) in dry DCM (25 mL) was slowly added. The reaction was let to warm slowly to room temperature. After 24 h at rt, the crude was diluted with H₂O and DCM. The organic phase was extracted with deionized water twice, 3 times with HCl 0.5 M and three times with s.s. NaHCO₃, dried (MgSO₄) and the solvent removed. A foamy colourless solid was obtained (5.621 g). The crude was considered to be enough pure to not require a purification.

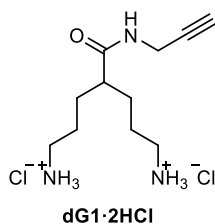
Molecular weight: 491,59 g/mol (C₂₄H₃₇N₅O₆)

TLC: R_f 0.62 (DCM:CH₃OH 9:1, KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 8.07 (d, *J* = 9.0 Hz, 1H, CH_{arom}), 7.55 (t, *J* = 7.5 Hz, 1H, CH_{arom}), 7.47 – 7.39 (m, 2H, 2x CH_{arom}), 4.68 (bs, 2H, 2x NH), 3.23 (d, *J* = 6.2 Hz, 4H, 2x NH-CH₂CH₂CH₂-CH), 2.98 (s, 1H, NH-CH₂CH₂CH₂-CH), 1.96 – 1.84 (m, 2H, NH-CH₂CH₂CH₂-CH), 1.83 – 1.66 (m, 6H, NH₂-CH₂CH₂CH₂-CH and 2x NH₂-CH₂CH₂CH₂-CH), 1.49 – 1.38 (m, 18H, 2x C(CH₃)₃).

All the other data were in accordance with literature

Synthesis of 4-(prop-2-yn-1-ylcarbamoyl)heptane-1,7-diaminium chloride (**dG₁ · 2 HCl**)



Di-tert-butyl (4-(prop-2-yn-1-ylcarbamoyl)heptane-1,7-diyl)dicarbamate (**dG₁-Boc**) (1.529 g, 3.72 mmol) was solubilized in dry THF (15 mL). The reaction crude was cooled to 0°C and after 10 minutes HCl 4M in dioxane (3.7 mL, 14.9 mmol) was slowly added. After 15 minutes the reaction was warmed to room temperature. After 16 h at room temperature, the reaction solvent was completely removed under vacuum. Then, the crude was solubilized in deionized water and extracted 3 times with DCM. The aqueous layer was freeze dried until dryness. It was obtained a glue like amorphous solid (770 mg). The product was considered to be enough pure to be used directly in the next reaction.

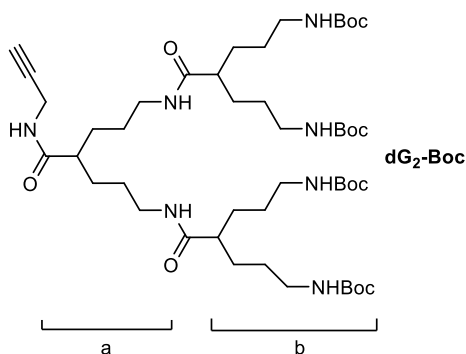
Molecular weight: 284,23 (C₁₁H₂₃Cl₂N₃O)

TLC: Rf 0.04 (DCM:CH₃OH 9:1, KMnO₄)

¹H NMR (500 MHz, D₂O) δ 4.00 (d, *J* = 2.5 Hz, 2H, CH₂C≡CH), 3.06 – 2.93 (m, 4H, 2x NH-CH₂CH₂CH₂-CH), 2.63 (t, *J* = 2.5 Hz, 1H, CH₂C≡CH), 2.42 – 2.28 (m, 1H, NH-CH₂CH₂CH₂-CH), 1.73 – 1.51 (m, 8H, 2x NH-CH₂CH₂CH₂-CH and 2x NH-CH₂CH₂CH₂-CH).

All the other data were in accordance with literature

Synthesis of di-tert-butyl 4-((7-(5-((tert-butoxycarbonyl)amino)-2-(3-((tert-butoxycarbonyl)amino)propyl)pentanamido)-4-(prop-2-yn-1-ylcarbamoyl)heptyl)carbamoyl)heptane-1,7-diyl dicarbamate (**dG₂-Boc**)



4-(prop-2-yn-1-ylcarbamoyl)heptane-1,7-diaminium chloride (**dG₁ · 2 HCl**) (0.770 g, 2.71 mmol) was dissolved in dry DMF (8 mL) under inert atmosphere, Ar. Later, *N*-methylmorpholine (1.8 mL, 16.2 mmol) was slowly added. After 15 minutes 1H-benzo[d][1,2,3]triazol-1-yl 5-((tert-butoxycarbonyl)amino)-2-(3-((tert-butoxycarbonyl)amino)propyl)pentanoate (**4.5**) was added as a solution in dry DMF (12 mL). After 3 days at room temperature, the reaction solvent was removed under vacuum. The crude was diluted with DCM and extracted 4 times with deionized water, 4 times with 0.05M HCl solution and 4 times with s.s. NaHCO₃. The organic phase was dried (MgSO₄) and the solvent was removed. To purify the crude from the unreacted activated monomer (**4.5**), it was suspended in a 1M NaOH solution (50 mL) and stirred. After 16 hours at room temperature, the crude was diluted with DCM, and the two phases were separated. The organic one was extracted 4 times with 0.2M NaOH solution, dried (MgSO₄) and the solvent was evaporated. This purification gave the clean second generation dendron (**dG₂-Boc**) as yellowish foamy solid (2.166 g, 63% yield over 3 steps).

Molecular weight: 924,24 g/mol (C₄₇H₈₅N₇O₁₁)

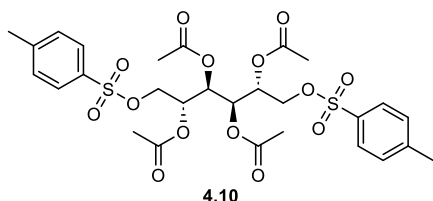
TLC: Rf 0.52 (EA:CH₃OH 9:1, KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃) δ 6.93 (s, 1H, NHCH₂C≡CH), 6.32 (s, 2H, 2x NH^[a]), 4.97 – 4.73 (m, 4H, 4x NH^[b]), 4.00 (dd, *J* = 5.1, 2.4 Hz, 2H, NHCH₂C≡CH), 3.36 – 3.22 (m, 2H, NH-CH₂CH₂CH₂-CH^[a]), 3.23 – 2.99 (m, 10H, 5x NH-CH₂CH₂CH₂-CH), 2.32 – 2.25 (m, 1H, NH-CH₂CH₂CH₂-CH^[a]), 2.22 (t, *J* = 2.5 Hz, 1H, NHCH₂C≡CH), 2.19 – 2.13 (m, 2H, 2x NH-CH₂CH₂CH₂-CH^[b]), 1.68 – 1.57 (m, 4H, NH-CH₂CH₂CH₂-CH^[a] and NH-CH₂CH₂CH₂-CH^[b]), 1.50 – 1.34 (m, 54H, 3x NH-CH₂CH₂CH₂-CH, 6x NH-CH₂CH₂CH₂-CH and 4x C(CH₃)₃).

All the other data were in accordance with literature

4.9.3 Preparation of the core

Synthesis of (2R,3R,4R,5R)-1,6-bis(tosyloxy)hexane-2,3,4,5-tetraol tetraacetate (**4.10**)



D-mannitol (1.500 g, 8.23 mmol) was solubilized in pyridine (23 mL) under inert atmosphere, N₂. The flask was cooled to 0°C and after 5 minutes *p*-toluensulfonylchloride (3.454 g, 18.1 mmol) was slowly added. After 16 h at 0°C acetic anhydride (6.2 mL, 68.84 mmol) was slowly injected and lastly 4-dimethylaminopyridine (60 mg, 0.494 mmol) was added. After 1 h the flask was warmed to room temperature. After 16 h HCl 1M was added until acid pH. The mixture was diluted with AcOEt and transferred in a separatory funnel. The aqueous phase was extracted 3 times with ethyl acetate, the reunited organic phases were washed with brine, dried (MgSO₄), filtered and evaporated to dryness. The crude was purified by FC on silica (DCM to DCM+6% EA) to give the product as a slightly yellow foam (790.6 mg, 44% yield).

Molecular weight: 658.67 g/mol (C₂₈H₃₄O₁₄S₂)

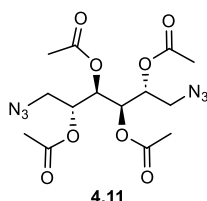
TLC: R_f 0.35 (DCM+5% EA, UV and KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 7.80 – 7.72 (m, 4H, 4x -CCHCH_{arom}C-S), 7.39 – 7.30 (m, 4H, 4x -CCH_{arom}CHC-S), 5.36 (d, *J* = 8.7 Hz, 2H, 2x O-CHHCHCH-), 4.99 – 4.93 (m, 2H, 2x O-CHHCHCH-), 4.13 (dd, *J* = 11.4, 3.0 Hz, 2H, 2x O-CHHCHCH-), 4.00 (dd, *J* = 11.4, 4.5 Hz, 2H, 2x O-CHHCHCH-), 2.45 (s, 6H, 2x Ar-CH₃), 2.01 (s, 6H, 2x CH₃-COO), 2.00 (s, 6H, 2x CH₃-COO).

¹³C NMR (126 MHz, CDCl₃): δ 169.9 (2x -C=OO-), 169.5 (2x -C=OO-), 145.3 (2x -C_{quat,arom}CHCHC-S), 132.7 (2x -CCHCHC_{quat,arom}-S), 130.0 (4x CCH_{arom}CHC-S), 128.2 (4x CCHCH_{arom}C-S), 67.5 (2x O-CHHCHCH-), 67.3 (2x O-CHHCHCH-), 67.0 (2x O-CHHCHCH-), 21.8 (2x CH₃-Ar), 20.9 (2x CH₃COO-), 20.6 (2x CH₃COO-).

HPLC-ESI-HRMS: Rt= 0.73 min, exact mass expected: 658.1390 m/z (C₂₈H₃₄O₁₄S₂), observed 681.1243 (M+Na)⁺

Synthesis of (2R,3R,4R,5R)-1,6-diazidohexane-2,3,4,5-tetraol tetraacetate (**4.11**)



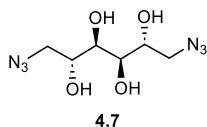
Tetra-acetylated compound (**4.10**) (653 mg, 0.991 mmol) and sodium azide (79 mg, 1.22 mmol) were suspended in dry DMF (20 mL), under inert atmosphere (N₂). The flask was heated to 50°C. After 16 h at 50°C the reaction was cooled to room temperature. Later, the solvent was removed in negative pressure, the crude mixture was diluted with AcOEt and s.s. NH₄Cl and transferred in a separatory funnel. The aqueous phase was extracted 3 times with EA, the reunite organic phase were washed with brine, dried (MgSO₄), filtered and evaporated to dryness. The crude, a yellowish oil (394 mg), was considered to be enough pure to be directly used in the next reaction.

Molecular weight: 400.35 g/mol (C₁₄H₂₀N₆O₈)

TLC: Rf 0.30 (DCM+3% EA, KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 5.45 – 5.39 (m, 2H, 2x N₃-CHHCHCH), 5.04 (ddd, *J* = 8.7, 5.6, 3.5 Hz, 2H, 2x N₃-CHHCHCH-), 3.48 (dd, *J* = 13.5, 3.4 Hz, 2H, 2x N₃-CHHCHCH-), 3.27 (dd, *J* = 13.5, 5.6 Hz, 2H, 2x N₃-CHHCHCH-), 2.11 (s, 6H, 2x CH₃), 2.11 (s, 6H, 2x CH₃).

Synthesis of (2R,3R,4R,5R)-1,6-diazidohexane-2,3,4,5-tetraol (**4.7**)



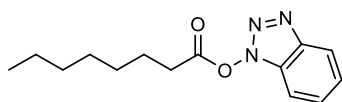
(2R,3R,4R,5R)-1,6-diazidohexane-2,3,4,5-tetraol tetraacetate (**4.11**) (394 mg, 0.985 mmol) was solubilized in methanol (39 mL). After 5 minutes, sodium methoxide (19 mg, 0.345 mmol) was added. After the addition the pH rose to 9. After 16 h at rt, to the crude was added 5 mg of Amberlite IR-120 (H) and it was left stirring for 15 minutes. Then, the suspension was filtered and the solvent removed. The crude, a white solid (233 mg) was considered to be enough pure to be used directly in the sequent step.

Molecular weight: 232,20 g/mol (C₆H₁₂N₆O₄)

TLC: Rf 0.08 (DCM:MeOH 8:2, KMnO₄ basic)

¹H NMR (400 MHz, CD₃OD) δ 3.80 (ddd, *J* = 8.9, 6.3, 2.6 Hz, 2H, 2x N₃-CHHCHCH), 3.74 (d, *J* = 8.7 Hz, 2H, 2x N₃-CHHCHCH), 3.55 (dd, *J* = 12.7, 2.6 Hz, 2H, 2x N₃-CHHCHCH-), 3.39 (dd, *J* = 12.7, 6.3 Hz, 1H, 2x N₃-CHHCHCH-).

Synthesis of 1H-benzo[d][1,2,3]triazol-1-yl octanoate



Octanoic acid (300 mg, 2.08 mmol), 4-(dimethylamino)pyridine (20 mg, 0.166 mmol) and hydroxy benzotriazole (562 mg, 4.16 mmol) were suspended in dry DCM (10 mL) under inert atmosphere (N₂). The reaction flask was cooled to 0°C and after 5 minutes dimethylaminopropyl-N'-ethylcarbodiimide hydrochloride (EDCl, 439 mg, 2.29 mmol) was slowly added. After 15 minutes the reaction was warmed to room temperature. After 16 hours at room temperature, HCl 0.5 M was added to stop the reaction. The crude was diluted with fresh DCM. The phases were separated, and the organic one was extracted twice with HCl 0.5 M, 3 times with s.s. NaHCO₃, dried (MgSO₄) and the solvent removed. The product, a white solid (544 mg), was considered to be enough pure to be used directly in the sequent reaction.

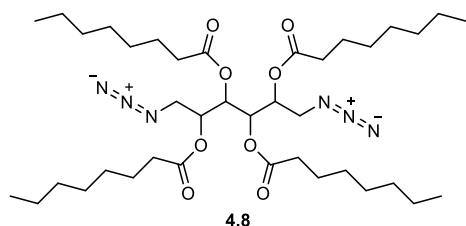
Molecular weight: 261,33 g/mol (C₁₄H₁₉N₃O₂)

TLC: Rf 0.45 (hexane:EA 9:1 KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃) δ 8.42 (d, *J* = 8.4 Hz, 1H, CH_{arm}), 8.01 (d, *J* = 8.4 Hz, 1H, CH_{arm}), 7.78 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H, CH_{arm}), 7.57 (ddd, *J* = 8.2, 7.0, 0.8 Hz, 1H, CH_{arm}), 3.12 (t, *J* = 7.5 Hz, 2H, O=CCH₂CH₂CH₂-), 1.84 (p, *J* = 7.5 Hz, 2H, O=CCH₂CH₂CH₂-), 1.48 – 1.40 (m, 2H, O=CCH₂CH₂CH₂-), 1.39 – 1.26 (m, 6H, 3x CH₂), 0.89 (t, *J* = 6.9 Hz, 3H, CH₃).

All the other data were in accordance with literature

Synthesis of 1,6-diazidohexane-2,3,4,5-tetraol tetraoctanoate (**4.8**)



(2R,3R,4R,5R)-1,6-diazidohexane-2,3,4,5-tetraol (**4.7**) (166 mg, 0.717 mmol) was suspended in dry DCM (2.3 mL) under inert atmosphere, N₂. Triethylamine (0.65 mL, 4.66 mmol) was injected. After 5 minutes, a solution of 1H-benzo[d][1,2,3]triazol-1-yl octanoate (1.124 g, 4.30 mmol) in dry DCM (3 mL) was slowly added. After 96 h at rt, s.s. NH₄Cl was added and the organic solvent was removed in vacuum. The mixture was diluted with AcOEt and transferred in a separatory funnel. The aqueous phase was extracted 3 times with EA, the reunite organic phase were washed with brine, dried (MgSO₄), filtered and evaporated to dryness. The crude was purified by FC on silica (hexane:EA 12:1) to give the product as a yellowish oil (337.4 mg, 64% yield three steps).

Molecular weight: 737.00 g/mol (C₃₈H₆₈N₆O₈)

TLC: R_f 0.52 (hexane:EA 9:1 KMnO₄ basic)

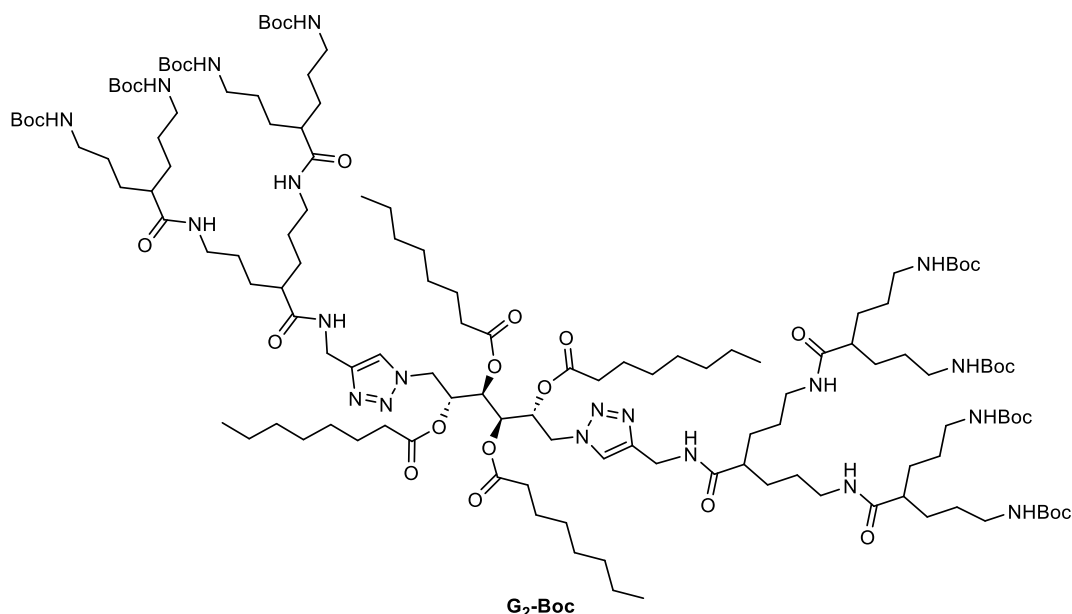
¹H NMR (500 MHz, CDCl₃): δ 5.42 (d, *J* = 8.0 Hz, 2H, 2x N₃-CHHCHCH), 5.01 (dt, *J* = 8.1, 3.5 Hz, 2H, 2x N₃-CHHCHCH), 3.47 (dd, *J* = 13.5, 3.3 Hz, 2H, 2x N₃-CHHCHCH), 3.26 (dd, *J* = 13.5, 5.7 Hz, 2H, 2x N₃-CHHCHCH), 2.42 – 2.28 (m, 8H, 4x OOC-CH₂CH₂), 1.68 – 1.58 (m, 8H, 4x OOC-CH₂CH₂), 1.38 – 1.22 (m, 32H, 8 CH₂), 0.94 – 0.84 (m, 12H, 4x CH₃).

¹³C NMR (126 MHz, CDCl₃): δ 172.8 (2x -COO-), 172.5 (2x -COO-), 68.8 (2x N₃-CH₂CHCH), 68.2 (2x N₃-CH₂CHCH), 50.8 (2x N₃-CH₂CHCH), 34.1 (2x OOC-CH₂CH₂), 34.0 (2x OOC-CH₂CH₂), 31.8 (4x CH₂), 29.2 (2x CH₂), 29.2 (2x CH₂), 29.1 (2x CH₂), 29.1 (2x CH₂), 24.8 (2x OOC-CH₂CH₂), 24.6 (2x OOC-CH₂CH₂), 22.7 (4x CH₂), 14.2 (4x CH₃).

HPLC-ESI-HRMS: Rt= 6.62 min, exact mass expected: 736.5099 m/z (C₃₈H₆₈N₆O₈), observed 759.5017 (M+Na)⁺

4.9.4 Preparation of the dendrimer

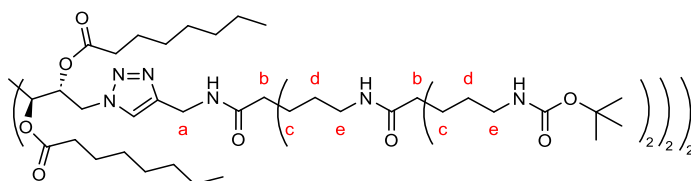
Synthesis of (2R,3R,4R,5R)-1,6-bis(4-(4-(3-(5-((tert-butoxycarbonyl)amino)-2-(3-((tert-butoxycarbonyl)amino)propyl)pentanamido)propyl)-10-(3-((tert-butoxycarbonyl)amino)propyl)-17, 17-dimethyl-3,9,15-trioxo-16-oxa-2,8,14-triazaoctadecyl)-1H-1,2,3-triazol-1-yl)hexane-2,3,4, 5-tetrayl tetraoctanoate (**G₂-Boc**)



In a microwave vessel **dG₂-Boc** (245 mg, 0.265 mmol), CuSO₄ · 5H₂O (10 mg, 0.0407 mmol) and sodium ascorbate (16 mg, 0.0814 mmol) are suspended in THF (6.5 mL). Later, compound **4.8** (75 mg, 0.102 mmol) was added as a solution in THF (1 mL). After, 7.5 mL of deionized H₂O were added. After mixing a yellow/blue suspension was obtained. The vessel was closed with a cup and then placed inside the microwave and heated at 60°C. After 3h, the crude was cooled and the two solvents removed in vacuum. The crude mixture was solubilized in THF and filtered through a paper filter and the solvent removed. The crude was purified by exclusion dimension chromatography (Sephadex resin, eluent: 1:1 DCM:MeOH), delivering the clean product as a colourless amorphous solid (211 mg, 80% yield).

Molecular weight: 2585,47 g/mol (C₁₃₂H₂₃₈N₂₀O₃₀)

TLC: R_f 0.45 (EA + 4% MeOH, KMnO₄ basic) it degrades on silica

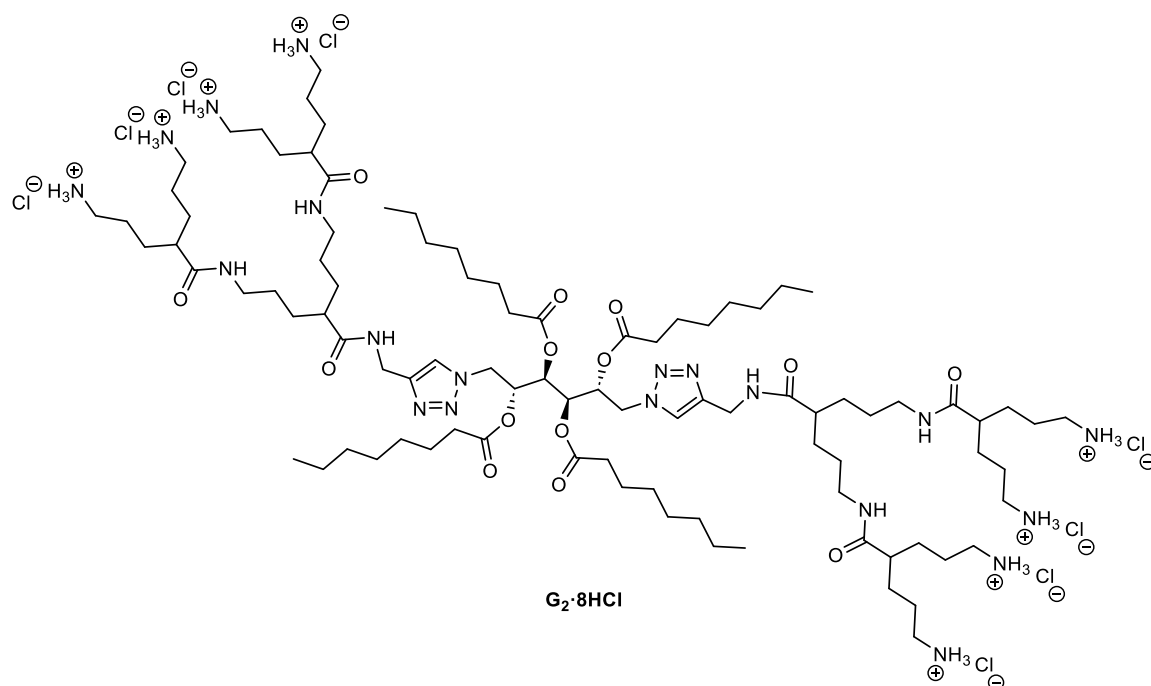


¹H NMR (500 MHz, CDCl₃): δ 7.63 (s, 2H, 2x -CH=C-), 6.87 – 6.23 (m, 6H, 6x -NHCO-), 5.30 (s, 4H, 2x -CHCHCHH- and 2x -CHCHCHH-), 5.16 – 4.84 (m, 8H, 8x -NHCOO-), 4.65 (d, *J* = 13.4 Hz, 2H, 2x -CHCHCHH-), 4.45 (s, 6H, 2x -CHCHCHH- and 2x CH₂^[a]), 3.27 – 2.95 (m, 24H, 12x CH₂^[e]), 2.42 – 2.34 (m, 4H, 2x -OOC-CH₂CH₂-), 2.23 (t, *J* = 7.4 Hz, 4H, 2x -OOC-CH₂CH₂-), 2.15 (s, 6H, 6x CH^[b]), 1.60 (s, 16H, 2x -OOC-CH₂CH₂- and 6 x CH₂^[c]), 1.49 (d, *J* = 6.7 Hz, 4H, 2x -OOC-CH₂CH₂-), 1.42 (s, 108H, 6x CH₂^[c] and 12x CH₂^[d] and 8x O-C(CH₃)₃), 1.33 – 1.24 (m, 32H, 8x -CH₂CH₂-), 0.91 – 0.84 (m, 12H, 4x -CH₃).

^{13}C NMR (126 MHz, CDCl_3): δ 175.9 (2x $\text{O}=\text{CO}-\text{R}$), 175.8 (2x $\text{O}=\text{CO}-\text{R}$), 172.8 (6x $\text{O}=\text{CNH}-\text{R}$), 156.4 (8x $-\text{HNCOO}-\text{R}$), 145.3 (2x $-\text{CH}=\text{C}-$), 123.5 (2x $-\text{CH}=\text{C}-$), 79.2 (8x $\text{O}-\text{C}(\text{CH}_3)_3$), 69.4 (2x $-\text{CHCHCHH}-$), 68.7 (2x $-\text{CHCHCHH}-$), 49.6 (2x $-\text{CHCHCHH}-$), 46.2 (6x $\text{CH}^{[b]}$), 40.3 (8x $\text{CH}_2^{[e]}$), 38.8 (4x $\text{CH}_2^{[e]}$), 34.9 (2x $\text{CH}_2^{[a]}$), 34.1 (2x $\text{OOC}-\text{CH}_2\text{CH}_2-$), 33.9 (2x $\text{OOC}-\text{CH}_2\text{CH}_2-$), 31.8 (6x CH_2), 30.1 (6x $\text{CH}_2^{[c]}$), 29.2 (2 CH_2), 29.11 (2 CH_2), 29.08 (6x $\text{CH}_2^{[c]}$), 28.6 (8x $\text{O}-\text{C}(\text{CH}_3)_3$), 28.1 (6x $\text{CH}_2^{[d]}$), 27.5 (6x $\text{CH}_2^{[d]}$), 24.7 (2x $\text{OOC}-\text{CH}_2\text{CH}_2-$), 24.5 (2x $\text{OOC}-\text{CH}_2\text{CH}_2-$), 22.7 (6x CH_2), 14.29 (2x CH_3), 14.22 (2x CH_3).

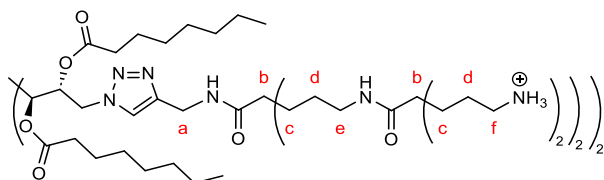
HPLC-ESI-HRMS: $R_t = 5.96$ min, exact mass expected: 2583.7713 m/z ($\text{C}_{132}\text{H}_{238}\text{N}_{20}\text{O}_{30}$), observed 1293.4022 ($\text{M}+2\text{H}$) $^{2+}$

Synthesis of 4,4',4'',4'''-((((((((((2R,3R,4R,5R)-2,3,4,5-tetrakis(octanoyloxy)hexane-1,6-diyl)bis(1H-1,2,3-triazole-1,4-diyl))bis(methylene))bis(azanediyl))bis(carbonyl))bis(heptane-4,1,7-triyl))tetrakis(azanediyl))tetrakis(formyl))tetrakis(heptane-1,7-diaminium) chloride (**G₂·8HCl**):



A reaction flask containing the protected dendrimer (**G₂-Boc**) (150 mg, 0.0580 mmol) under inert atmosphere, N_2 , was cooled to 0°C . After 5 minutes HCl 4M in dioxane (22 mL) was slowly added. The flask was slowly brought to room temperature and left running 16 h at room temperature. The solvent was fully removed in negative pressure, and the crude was solubilized in deionized H_2O . It was frozen and totally dried by freeze-drying. At the end was obtained a yellow amorphous solid (115 mg, quantitative yield) that was enough pure to not require any purification.

Molecular weight: 2076.19 g/mol ($\text{C}_{92}\text{H}_{182}\text{Cl}_8\text{N}_{20}\text{O}_{14}$)



^1H NMR (500 MHz, $\text{DMSO}-d_6$): δ 8.64 (s, 2H, 2x $-\text{NHCO}-$), 8.21 (s, 4H, 4x $-\text{NHCO}-$), 8.06 (s, 24H, 8x NH_3), 7.87 (s, 2H, $-\text{CH}=\text{C}-$), 5.34 (s, 2H, 2x $-\text{CHCHCHH}-$), 5.25 (s, 2H, 2x $-\text{CHCHCHH}-$), 4.72 (d, $J = 12.6$ Hz, 2H, 2x $-\text{CHCHCHH}-$), 4.56 (dd, $J = 13.4, 8.7$ Hz, 2H, 2x $-\text{CHCHCHH}-$), 4.25 (s, 4H, 2x $\text{CH}_2^{[a]}$), 3.08 – 3.00 (m, 4H, 2x $\text{CH}_2^{[e]}$), 3.00 – 2.92 (m, 4H, 2x $\text{CH}_2^{[e]}$), 2.74 (s, 16H, 8x $\text{CH}_2^{[f]}$), 2.47 – 2.40 (m, 2H, 2x $-\text{OOC}-\text{CHH}$), 2.38 – 2.28 (m, 2H, 2x $-\text{OOC}-\text{CHH}$), 2.23 (s, 6H, 6x $\text{CH}^{[b]}$), 2.19 – 2.14 (m,

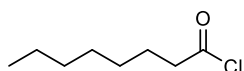
4H, 2x -OOC-CH₂), 1.47 (s, 28H, 2x CH₂ and 12x CH₂^[c]), 1.40 – 1.16 (m, 60H, 12x CH₂^[d] and 18x CH₂), 0.89 – 0.81 (m, 12H, 4x CH₃).

¹³C NMR (126 MHz, DMSO-D₆): δ 175.2 (2x O=CO-R), 173.9 (4x O=CNH-R), 172.1 (2x O=CO-R), 171.5 (2x O=CNH-R), 144.8 (2x -CH=C-), 123.7 (2x -CH=C-), 69.0 (2x -CHCHCHH-), 68.2 (2x -CHCHCHH-), 49.2 (2x -CHCHCHH-), 44.9 (2x CH₂^[b]), 44.1 (4x CH₂^[b]), 38.6 (2x CH₂^[e] and 8x CH₂^[f]), 38.4 (2x CH₂^[e]), 34.1 (2x CH₂^[a]), 33.2 (2x -OOC-CHH), 33.1 (2x -OOC-CH₂), 31.1 (4x CH₂), 30.0 (2x CH₂), 29.2 (12x CH₂^[d]), 28.5 (2x CH₂), 28.41 (2x CH₂), 28.37 (2x CH₂), 28.2 (2x CH₂), 27.2 (2x CH₂), 24.8 (12x CH₂^[c]), 22.1 (4x CH₂), 13.9 (4x CH₃).

ESI-HRMS: exact mass expected: 1783.3518 m/z (C₉₂H₁₈₂N₂₀O₁₄), observed 1784.3591 (M+H)⁺

4.9.5 Characterization of compound from alternative routes

Characterization of octanoyl chloride



Following the procedure published by Cailotto et al.⁵⁰. Octanoic acid (2.800 g, 19.6 mmol) was added to a reaction flask under inert atmosphere, N₂. The flask was cooled to 0°C and thionyl chloride (7.1 mL, 97.9 mmol) was slowly injected. After 5 minutes the reaction was warmed to room temperature and after other 5 minutes it was heated to reflux. After 16 h at reflux, the crude was cooled to rt and SOCl₂ was removed in vacuum using a rotavapor. An NMR check showed the presence of the desired octanoyl chloride (3.200 g), and the slightly yellow oil was directly used in the sequent reaction.

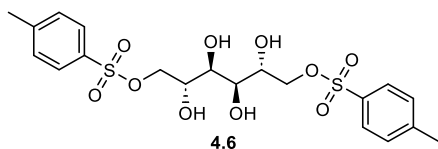
Molecular weight: 162,66 g/mol (C₈H₁₅ClO)

TLC: it was deposited the corresponding methyl ester, by quenching with MeOH, Rf: 0.62 (hexane:EA 8:2, KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 2.88 (t, *J* = 7.3 Hz, 2H, O=CCH₂CH₂-), 1.71 (p, *J* = 7.3 Hz, 2H, O=CCH₂CH₂-), 1.39 – 1.23 (m, 8H, 4 CH₂), 0.88 (t, *J* = 6.9 Hz, 3H, CH₃).

All the other data were in accordance with literature

Characterization of (2R,3R,4R,5R)-2,3,4,5-tetrahydroxyhexane-1,6-diyl bis(4-methylbenzene sulfonate) (**4.6**)



D-mannitol (2.000 g, 11.0 mmol) was solubilized in pyridine (30 mL). The flask was cooled to 0°C and after 5 minutes tosylchloride (5.023 g, 26.4 mmol) was slowly added. After 16 h at 0°C, HCl 1M was added until acid pH. The mixture was diluted with AcOEt and transferred in a separatory funnel. The organic phase was extracted 3 times with HCl 1M, washed with s.s. NaHCO₃, dried (MgSO₄), filtered and evaporated to dryness. The crude was purified by FC on silica (DCM:EA 4:6 to EA) to give the product as a colorless solid (2.379 g, 47% yield).

Molecular weight: 490.54 g/mol (C₂₀H₂₆O₁₀S₂)

TLC: Rf 0.35 (DCM:EA 4:6, UV and KMnO₄ basic)

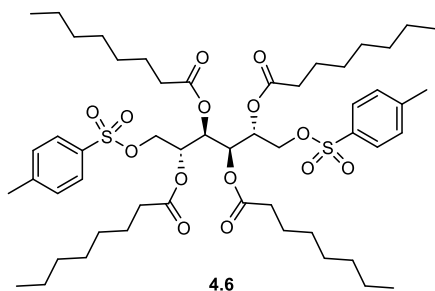
¹H NMR (500 MHz, CD₃OD): 7.81 (d, *J* = 8.3 Hz, 4H, 4x -CCHCH_{arom}C-S), 7.43 (d, *J* = 8.1 Hz, 4H, 4x -CCH_{arom}CHC-S), 4.27 (dd, *J* = 10.0, 2.3 Hz, 2H, 2x O-CHHCHCH-), 4.06 (dd, *J* = 10.0, 6.3 Hz, 2H, 2x

O-**CHH**CHCH-), 3.77 (ddd, $J = 8.7, 6.3, 2.3$ Hz, 2H, 2x O-CH₂**CH**CH-), 3.66 (d, $J = 8.8$ Hz, 2H, 2x O-CH₂CH**CH**), 2.45 (s, 6H, 2 **CH**₃)

¹³C NMR (126 MHz, CD₃OD): δ 146.4 (2x -**C**_{quat,arom}CHCHC-S), 134.4 (2x -CCHCH**C**_{quat,arom}-S), 131.0 (2x -**CCH**_{arom}CHC-S), 129.1 (2x -CCH**CH**_{arom}C-S), 74.1 (2x O-**CHH**CHCH-), 70.1 (2x **CH**), 70.1 (2x **CH**), 21.6 (2x **CH**₃).

HPLC-ESI-HRMS: Rt= 5.59 min, exact mass expected: 490.5380 m/z (C₂₀H₂₆O₁₀S₂), observed 491.5390 m/z (M+H)⁺

Characterization of (2R,3R,4R,5R)-1,6-bis(tosyloxy)hexane-2,3,4,5-tetraol tetraoctanoate (**4.9**)



1,6-tosylmannitol (**4.6**) (200 mg, 0.408 mmol) and 4-(dimethylamino)pyridine (3 mg, 0.0204 mmol) were suspended in DCM (4.0 mL). Triethylamine (0.68 mL, 4.89 mmol) was injected. The solubilization of the starting material was complete. The flask was cooled to 0°C and after 5 minutes octanoyl chloride (0.56 mL, 3.26 mmol) was slowly added. After 48 h at rt, HCl 1M was added until acid pH. The mixture was diluted with AcOEt and transferred in a separatory funnel. The organic phase was extracted 3 times with HCl 1M, washed with s.s. NaHCO₃, dried (MgSO₄), filtered and evaporated to dryness. The crude was purified by FC on silica (hexane:EA 8:2). For the characterization was used the only tube containing the clean product (yellowish oil).

Molecular weight: 995.33 g/mol (C₅₂H₈₂O₁₄S₂)

TLC: Rf 0.38 (hexane:EA 8:2, UV and KMnO₄ basic)

¹H NMR (500 MHz, CDCl₃): δ 7.75 (d, $J = 8.3$ Hz, 4H, 4x **CH**_{arom}), 7.32 (d, $J = 8.0$ Hz, 4H, 4x **CH**_{arom}), 5.34 (d, $J = 8.4$ Hz, 2H, 2x O-CH₂CH**CH**-), 4.96 (ddd, $J = 8.1, 4.7, 3.0$ Hz, 2H, 2x O-CH₂CH**CH**-), 4.13 (dd, $J = 11.3, 2.9$ Hz, 2H, 2x O-**CHH**CHCH-), 3.96 (dd, $J = 11.3, 4.9$ Hz, 2H, 2x O-**CHH**CHCH-), 2.44 (s, 6H, 2x **CH**₃-Ar), 2.32 – 2.14 (m, 8H, 4x -OOC-**CH**₂), 1.55 – 1.48 (m, 8H, 4x -OOC-CH₂**CH**₂), 1.34 – 1.22 (m, 32H, 16x **CH**₂), 0.89 (td, $J = 6.9, 3.0$ Hz, 12H, 4x **CH**₃).

¹³C NMR (126 MHz, CDCl₃) δ 172.5 (2x OOC-CH₂), 172.1 (2x OOC-CH₂), 145.1 (2x **C**_{quat,arom}), 132.8 (2x **C**_{quat,arom}), 130.0 (4x **CH**_{arom}), 128.2 (4x **CH**_{arom}), 67.5 (2x O-CH₂CH**CH**-), 67.2 (2x O-CH₂CH**CH**), 67.0 (2x O-CH₂CHCH-), 34.0 (2x -OOC**CH**₂), 33.9 (2x -OOC**CH**₂), 31.8 (2x **CH**₂), 31.8 (2x **CH**₂), 29.2 (2x **CH**₂), 29.14 (2x **CH**₂), 29.11 (2x **CH**₂), 29.08 (2x **CH**₂), 24.7 (2x -OOCCH₂**CH**₂), 24.5 (2x -OOCCH₂**CH**₂), 22.8 (2x **CH**₂), 22.8 (2x **CH**₂), 21.8 (2x **CH**₃-Ar), 14.24 (2x **CH**₃), 14.22 (2x **CH**₃).

ESI-HRMS: exact mass expected: 994,5146 m/z (C₅₂H₈₂O₁₄S₂), observed 1017.5048 (M+Na)⁺

References

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