1 Motivation

In the last decades information technology advanced significantly and the need for newly designed materials to detect and transport signals grew remarkably. For accurate diagnosis in most cases a trained healthcare worker and diagnostic technology, only affordable in developed countries, is required.[1] Therefore, the demand for analytical tools which are meeting the criteria of the WHO to be affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free and deliverable to users (ASSURED) is increasing to allow for an improvement in medical care in both developed and developing countries. Cellulose paper as a platform for the generation of devices suitable for medical diagnostics is highly attractive due to its cheapness and easy accessibility. An additional important advantage of cellulose substrates is the possibility to transport liquids without the need of a pumping device. Finally, as a natural product it can easily be disposed of without any necessary decontamination.[2] The group of Whitesides demonstrated with the generation of μPADs – microfluidic paper-based analytical devices – the applicability of cellulose based biosensors.[3,4] The scope of the current thesis is to establish a set of modular ligation methodologies for the functionalization of cellulose (and other biosubstrates e.g. hyaluronan) surfaces via the immobilization of facile linking sites on the surface. These ligation points are employed to conjugate functional entities such as polymer and peptide strands as well as proteins. Thus, the work of the thesis is methodologically motivated aiming at the provision of platform technology for the precision design of functional cellulose materials. For the functionalization of the surface highly efficient methodologies are required. In the first part of the current thesis thermally induced hetero Diels-Alder

based ligation will be explored, the cellulose acting both as diene and dienophile. In the second part three photochemically driven ligation methods are applied, which allow for the spatio-temporal control over the functionalization process (e.g. photoenol chemistry, generation of thioaldehyde of phenacyl sulfides or the NITEC process). As the present thesis is methodologically driven it wishes to establish toolbox technologies for the modification of biosurfaces and allow for the synthesis of novel substrates which could be employed in biomedical and biosensing applications.

In the following, the current state of the art in the fields of light and thermally driven ligation technologies as well as biosurface modification will be introduced on selected examples, before the results of the work are discussed in detail.

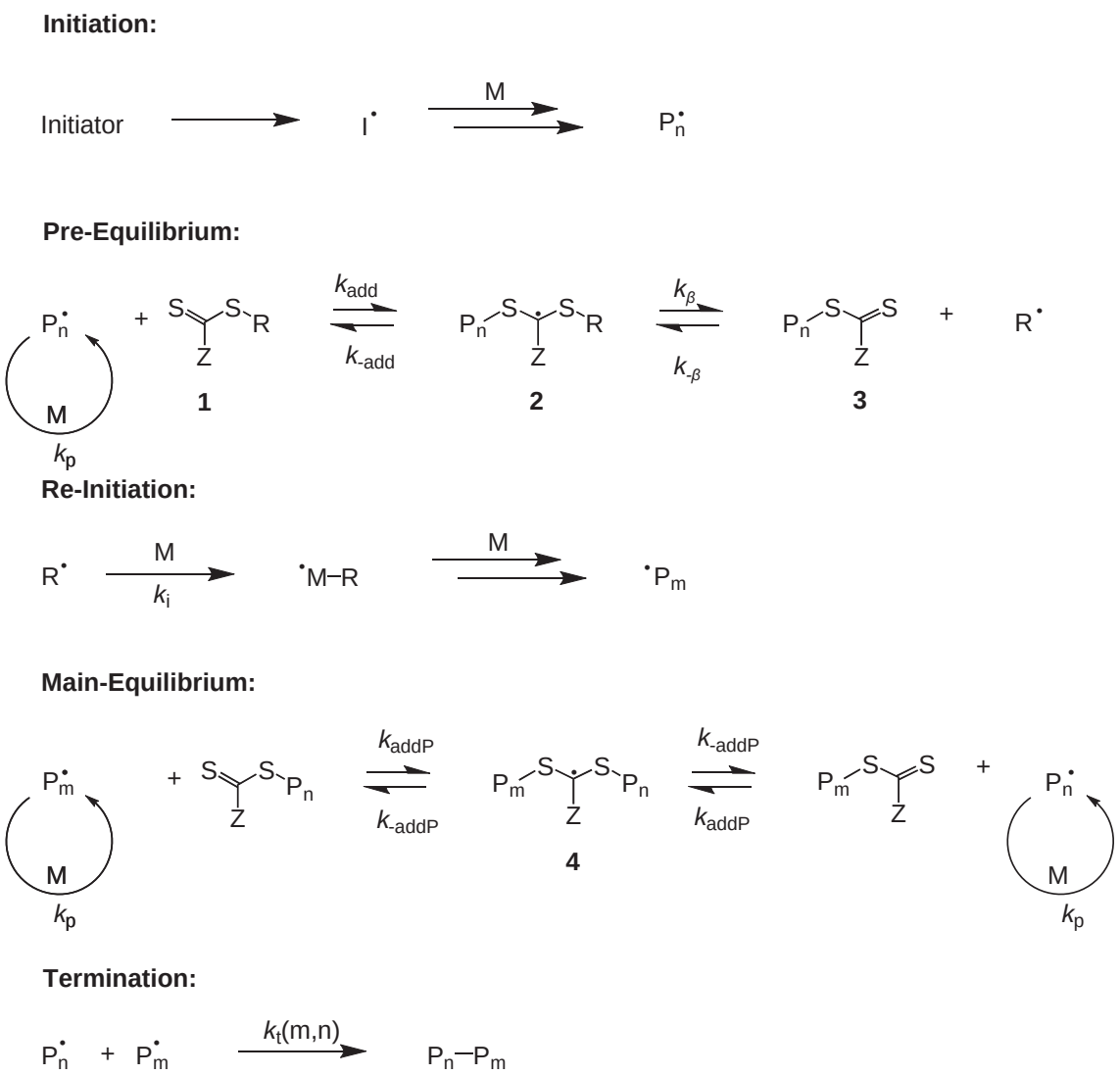
2

Modular Thermal and Photochemical Modifications of Biosurfaces - State of the Art and Theoretical Background

2.1 Reversible Deactivation Radical Polymerization (RDRP)

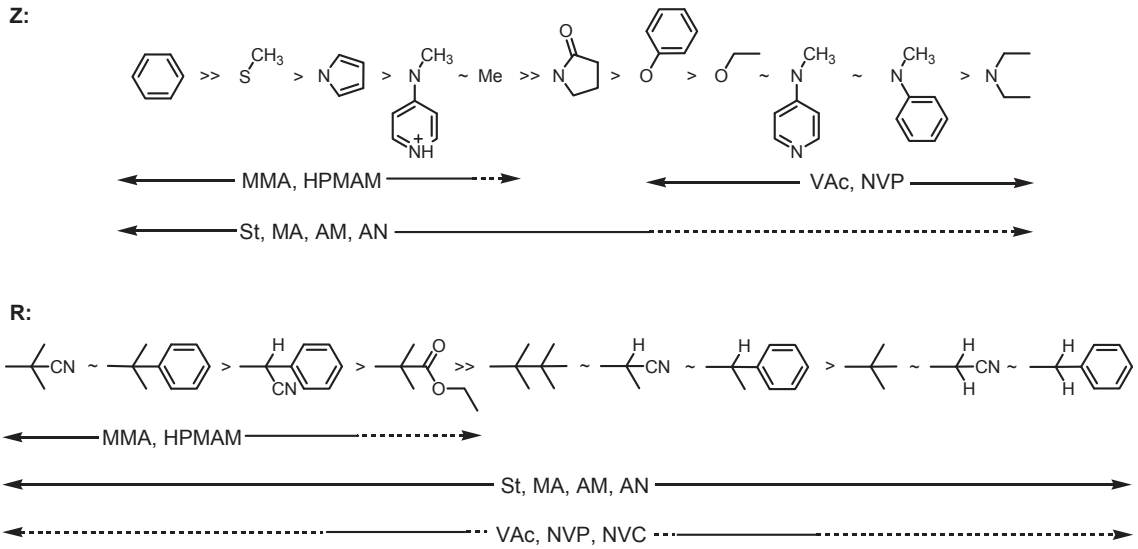
2.1.1 Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerization

The utilization of dithioesters as chain transfer agent (CTA) was published first in 1998.[5] The basic principle of the Reversible Addition Fragmentation Chain Transfer (RAFT) process is depicted in Scheme 2.1 on the following page. Similar to a free radical polymerization, the reaction is started with an initiator (e.g. AIBN). In contrast to other techniques (such as atom transfer radical polymerization (ATRP) described in the following section), the overall number of propagating radicals is not decreased through the RAFT process, however the average lifetime of each radical is greatly enhanced. As the overall propagating radical concentration remains constant, it results in a higher overall polymerization rate compared to ATRP or nitroxide mediated polymerization (NMP).



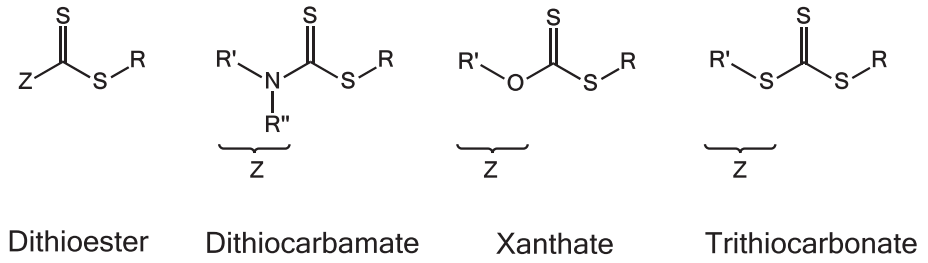
Scheme 2.1: Mechanism of the Reversible Addition Fragmentation Chain Transfer (RAFT) Polymerization. Reproduced with permission from Ref. 6 Copyright 2008, John Wiley and Sons.

The RAFT agent **1** has a leaving group R and a stabilizing group Z. On addition of a growing chain onto **1** ($k_{add} \approx 10^6\,\mathrm{L{\cdot}mol^{-1}{\cdot}\,s^{-1}}$) **2** is formed, which can fragment in a fast equilibrium reaction to **3** and release a new radical R·. The newly generated radical can grow via the addition of monomer units until it finally reacts again with a macro-RAFT agent **3** to yield **4** and release another radical. The determination of the involved rate coefficients is complex, but the following estimation is sufficient to demonstrate the advantages of the RAFT process. The concentration of the growing radical species in a RAFT process is approximately $[\mathrm{R}\cdot] \approx 10^{-8}\,\mathrm{mol{\cdot}L^{-1}}$. The average termination rate coefficient is generally around $k_t \approx 10^8\,\mathrm{L{\cdot}mol^{-1}{\cdot}s^{-1}}$. The employed CTA concentration is significantly higher ($[\mathrm{RAFT}] \approx 10^{-2}\,\mathrm{mol{\cdot}L^{-1}}$) than the radical concentration and therefore a simple kinetic assessment is sufficient to show that a free radical reacts around 10.000 times more likely with a macro-RAFT agent than to terminate. For an effective control over the polymerization the right choice of the Z-group is crucial.



Scheme 2.2: Influence of the R-/Z-group on the control of polymerization of different monomers (dashed: some control (i.e. good control of reaction but bad polydispersity or significant retardation of VAc or NVP); AM: acrylamide, AN: acrylonitrile, HPMAM: *N*-(2-hydroxypropyl) methacrylamide, MA: methacrylate, MMA: methyl methacrylate, NVC: *N*-vinyl carbazole, NVP: *N*-vinyl pyrrolidone, St: styrene, VAc: vinylacetate. Adapted with permission from Ref. 7 Copyright 2012 American Chemical Society.

The macro-RAFT radical (**2** or **4**) must be more stable than the propagation chain radical $P_n\cdot$, but not too stable, otherwise it would inhibit the transformation into **3**. Thus, each monomer class requires the design of a specific RAFT agent with an optimum Z-group. The R-group design is as well crucial to gain control over the polymerization. Factors such as steric hindrance, radical stability and polarity have to be taken into account for the synthesis of the optimal RAFT agent.[8] In Scheme 2.2 a selection of the most common R- and Z-groups is depicted. An alternative possibility is the use of switchable (universal) RAFT agents.[9]

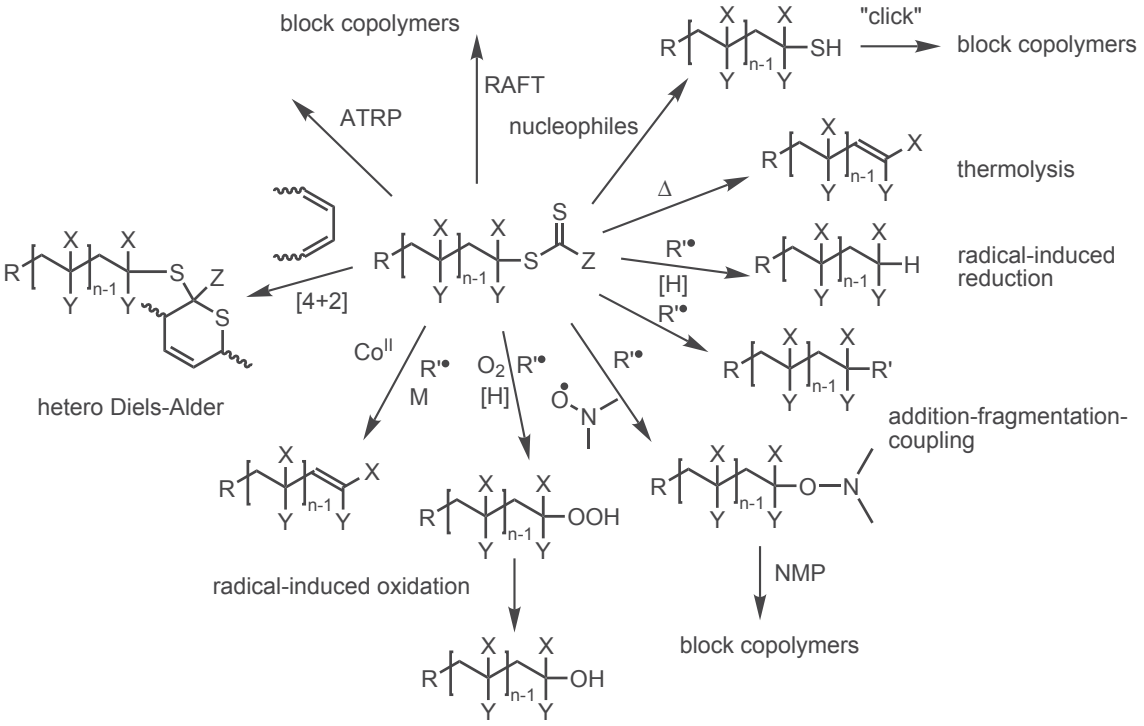


Scheme 2.3: Classes of RAFT agents. Reproduced with permission from Ref. 6 Copyright 2008, John Wiley and Sons.

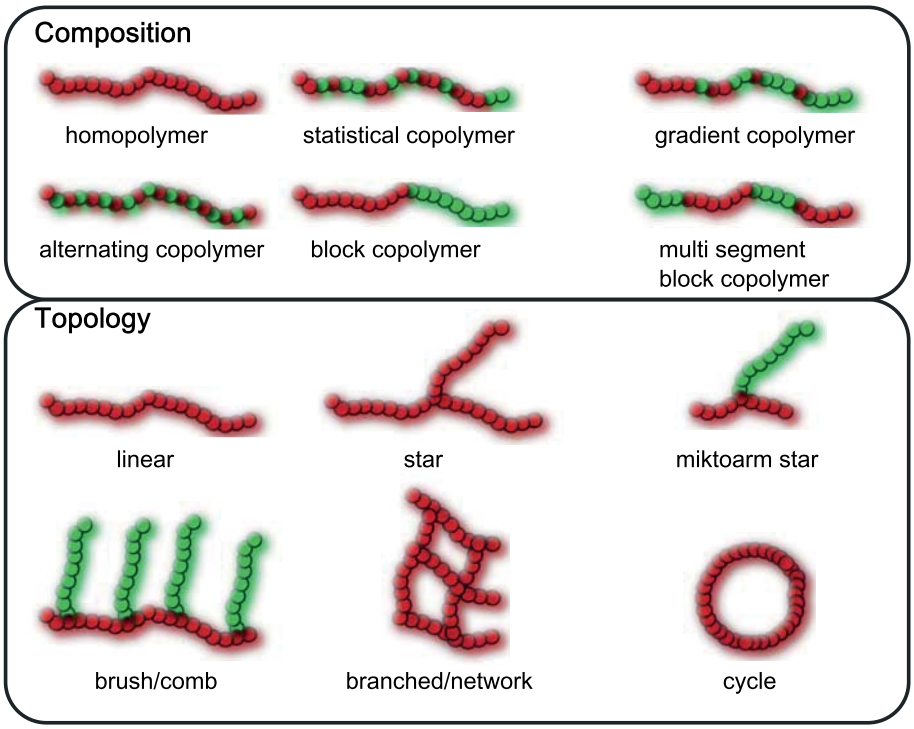
There are four classes of sulfur containing RAFT agents: dithioesters, dithiocarbamates, xanthates and trithiocarbamates (see also Scheme 2.3). These classes allow for different synthetic strategies to generate tailor-made RAFT agents in line with the required design.

RAFT polymers can be subjected to numerous post-polymerization reactions as is depicted in Scheme 2.4.[10] The thiocarbonyl thio moieties can readily undergo reactions with

nucleophiles – such as amines,[11–13] thiols[14] or hydroxy groups[15] – to yield thiol groups, whereas the treatment with a radical initiator in the presence of oxygen provides a hydroxy terminated polymer chain.[10,16] If the thiocarbonyl thio moiety is electron deficient it can undergo thermally induced hetero Diels-Alder reactions,[17] but even it is not activated the thiocarbonyl thio moiety can act as a dienophile in a photo-induced hetero Diels-Alder ligation with suitable reactive dienes.[18] Scheme 2.5 on the opposite page depicts compositions and topologies accessible through the use of the RAFT process.

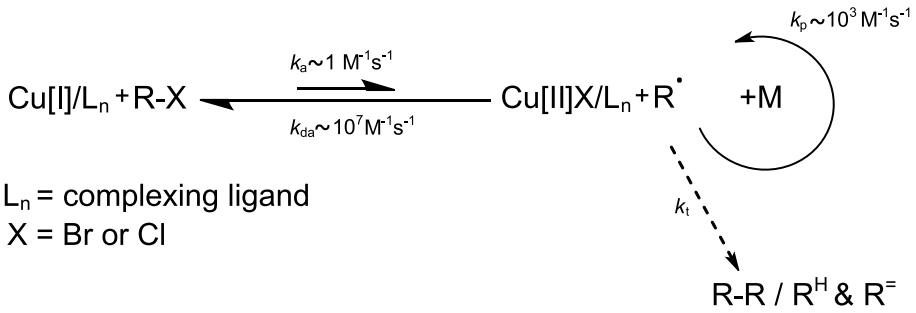


Scheme 2.4: Processes for RAFT end-group transformation (R'• : radical; [H] : hydrogen donor; M : monomer). Reproduced with permission from Ref. 10 Copyright 2011, John Wiley and Sons.



Scheme 2.5: Different polymer compositions and topologies accessible with the RAFT process and ATRP.[19]

2.1.2 Atom Transfer Radical Polymerization (ATRP)



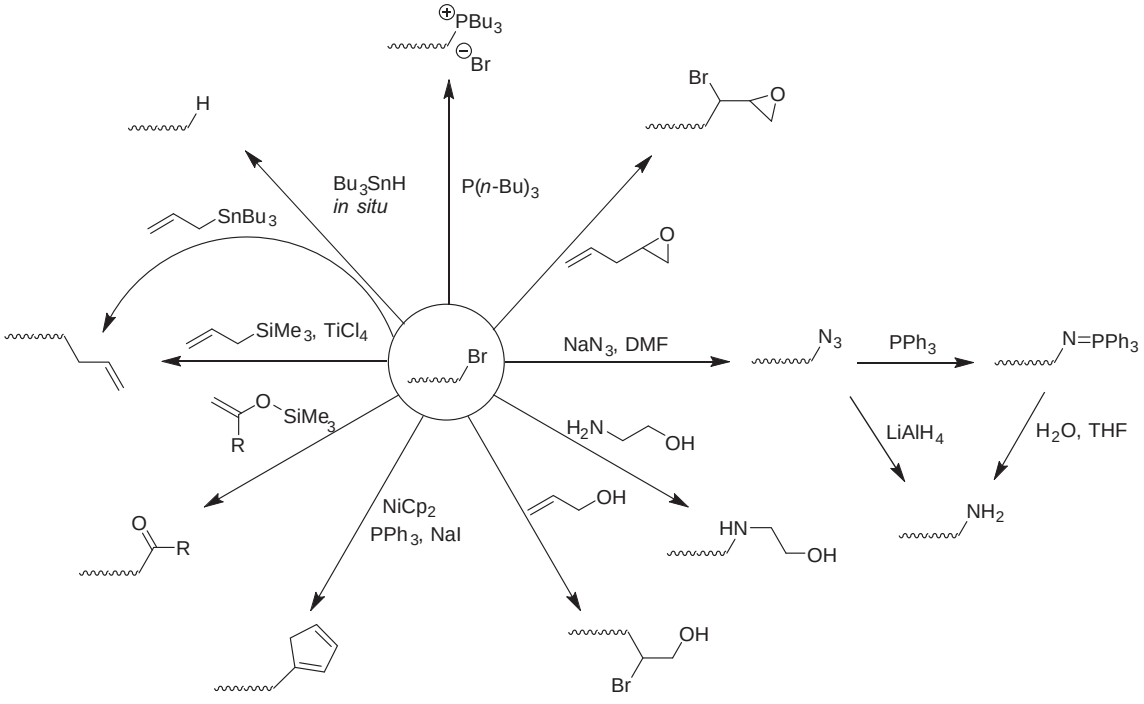
Scheme 2.6: Scheme of ATRP with typical rate coefficients. Reprinted with permission of Ref. 19 Copyright 2001 American Chemical Society.

In contrast to RAFT polymerization, ATRP relies on the equilibrium of radicals in an active and a dormant form.[20,21] In contrast to the RAFT process, the overall radical concentration is reduced and thus the polymerizaion is retarded compared to a comparable conventional free radical polymerization. Typically copper salts are employed for the ATRP process. As demonstrated in Scheme 2.6, a change of the oxidation state of the copper species transforms the macro radical R· in a dormant species.

An α-bromoester often serves as initiator, although several sulfonyl chlorides can be employed as well. The resulting polymer exhibits a terminal halogen atom. As can be en-

visioned by the noted typical rate coefficients, the equilibrium lies nearly on the dormant side. This leads to the noted lower overall radical concentration in the polymerization mixture compared to an analogous free radical polymerization process.[19] Since Cu(I) species are employed for the ATRP process, the reaction is sensitive to moisture and air, which increases the complexity of the reaction setup (i.e. the use of Schlenk technique is required). Furthermore, the toxicity of Cu is hampering the application in products of the pharmaceutical industry. The usage of other transition metals such as iron, nickel, palladium, rhodium or ruthenium are only solving the problem in some cases.[19] An alternative possibility to generate the Cu(I) species is based on the activators regenerated by electron transfer (ARGET) approach.[22,23] In this approach a reducing agent is employed to reduce the inactive Cu(II) species, allowing to conduct the polymerization in the presence of air. Typical monomers polymerized via ATRP are, but not limited to, styrenes, (meth)acrylates, acrylonitrile, (meth)acrylamides and (meth)acrylic acids.[19]

The presence of a halide as endgroup renders the ATRP polymers an excellent target for post polymerization transformations. The terminal bromine (or chlorine) atom can be exchanged e.g. via nucleophilic substitution reactions as shown in Scheme 2.7. For a general review on the post-functionalization of polymers the reader is referred to the review of Goldmann et al.[24]

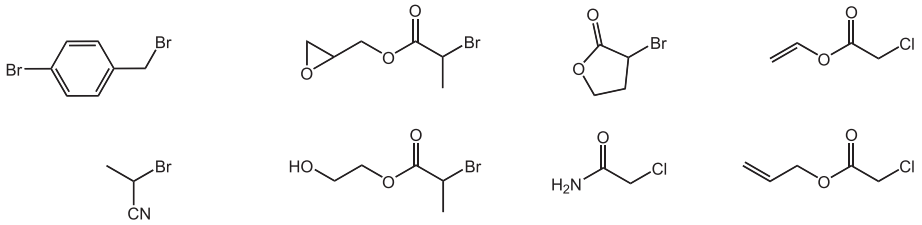


Scheme 2.7: Scheme of possible post-ATRP endgroup transformations. Adapted with permission of Ref.19 Copyright 2001 American Chemical Society.

Inglis et al.[25] demonstrated in 2010 an innovative endgroup transformation of bromine terminal polymer strands prepared by ATRP, which is of central importance for the current study. The usage of nickelocene instead of the commonly used sodium cyclopentadienide

allows for a mild reaction protocol suitable for ambient temperature conditions, without any danger of ester side group degradation/alteration. The Cp introduction has been employed on several substrates such as microspheres[26] or cellulose[27] and will be further discussed in Chapter 3.

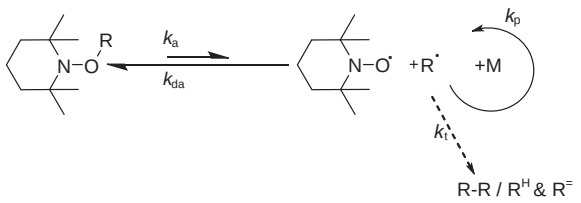
In addition, post polymerization modification of ATRP polymers can not only be conducted on the ω-, but also on the α-end of the polymer strand. For this purpose specific initiators are employed, a selection of which is displayed in Scheme 2.8. The use of these initiators allows for the introduction of e.g., hydroxy, epoxy, allyl, vinyl, γ-lactone and carboxylic acid moieties, which can be readily employed in post polymerization reactions, such as those depicted in Scheme 2.8.



Scheme 2.8: Selection of functional initiators for the polymerization of polystyrene allowing for post functionalization at the α-position. Adapted with permission of Ref.19 Copyright 2001 American Chemical Society.

2.1.3 Nitroxide Mediated Polymerization (NMP)

The nitroxide mediated polymerization (NMP) relies – similar to ATRP – on the equilibrium of the activation/deactivation of the radical species.[28] It is based on persistent radicals in alkoxyamine species and was patented in 1986.[29,30] These radicals are relatively stable due to bulky moieties surrounding the radical center. The nitroxide radical e.g. in the tetramethyl piperidinyl-oxy (TEMPO) radical reversibly couples on the propagating chain end and prevents therefore its termination. Upon decoupling the chain can add monomer units. Georges et al. demonstrated the use of TEMPO for the controlled polymerization of poly(styrene).[31] For the latest developments in NMP, the reader is referred to the very comprehensive review of Nicolas et al.[30]



Scheme 2.9: General scheme for the nitroxide mediated polymerization

2.2 Modular Ligation Chemistry

The term click chemistry was initially defined by Sharpless and co-workers in 2001.[32] Reactions are allowed to be named "click reactions" when they fulfill certain criteria: (*i*) modularity, (*ii*) applicability in a wide range, (*iii*) high yields (90% and higher), (*iv*) unreactive by-products, which can be removed without chromatographic methods, (*v*) stereo specifity, (*vi*) simple reaction sequences, (*vii*) easy accessible reagents, (*iix*) no or unharmful solvents which are readily removable and (*ix*) easy product isolation. Barner-Kowollik et al. adapted these criteria to be applicable for polymer science: The reaction should employ in addition equimolar reactants, purification in large-scale should be possible, conversion should proceed on a fast timescale, in high yields and provide stable compounds.[33]

Certain classes of reaction suffice the characteristics defined above:[32]

- Cycloadditions, as the above mentioned 1,3-dipolar cycloaddition or the Diels-Alder conjugation
- Nucleophilic substitution reactions, such as ring-opening reactions of strained heterocyclic electrophiles
- Carbonyl chemistry of non-aldol type, such as the formation of (thio)ureas, oxime ethers and amides
- Addition to carbon-carbon double or triple bonds, e.g. the formation of epoxidation

An excellent example for a reaction that suffice these criteria is the independently from Sharpless and Meldal investigated improvement of the Huisgen 1,3-dipolar cycloaddition.[34,35] These findings demonstrated that the use of coppper species not only accelerates the conversion but also introduced regiospecifity. While Meldal and co-workers could demonstrate the use of Cu(I) to mediate the generation of triazoles on peptides on a polar solid support, Sharpless and co-workers reported the regiospecifity of the reaction in water employing $CuSO_4$ and sodium ascorbate.