The development of a positive feedback loop based on supramolecular ring formation

dan der Haas, R.J.C.

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Roy van der Haas

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The development of a positive feedback loop based on supramolecular ring formation

Graduation report of

R. J. C. van der Haas

Supervisors:
Ir. A. J. P. Teunissen
Dr. Ir. A. R. A. Palmans

Supervising Professor:
Prof. Dr. E. W. Meijer

Advising Committee:
Dr. T. Noël

Eindhoven, November 2014
Laboratory of Macromolecular and Organic Chemistry
Faculty of Chemical Engineering and Chemistry
Eindhoven University of Technology
Summary

The field of supramolecular chemistry focuses on the self-assembly of molecules into larger structures using non-covalent interactions. These interactions play an important role in feedback loops found in nature. Examples are the regulation of the catalytic activity of enzymes and the self-replication of DNA by template directed polymerization. Chemists have tried to mimic these feedback loops artificially. The synthetic positive feedback loops which have been developed so far are generally based on the replication of molecular templates or the replication of physical entities. We would like to propose a new mechanism to obtain a positive feedback loop, based on the release of catalyst from a supramolecular scaffold. Here, the quadruple hydrogen bonding motifs ureidopyrimidinone (UPy) and 2,7-diamido-1,8-naphthyridine (NaPy) will respectively function as supramolecular scaffold and catalyst.

In a system with UPy and NaPy motifs, there is a dynamic equilibrium between UPy dimers, free NaPy and UPy-NaPy complexes. In a certain concentration regime, there is a larger mole fraction of free NaPy in a system containing bifunctional UPys compared to a system containing monofunctional UPys. Separately, the ability of NaPy to catalyze the Michael addition between 2,4-pentanediol and \( \text{trans-}\beta\)-nitrostyrene has been reported. These two phenomena inspired us to develop a positive feedback loop based on supramolecular ring formation. In this system, NaPy catalyzes the coupling between UPy functionalized Michael substrates, leading to the formation of a bifunctional UPy. The high tendency of this bifunctional UPy to form a cycle results in the release of NaPy (i.e. the catalyst). In this way, NaPy catalyzes its own release from a UPy-NaPy complex and a positive feedback loop is obtained.

Chapter 2 describes a detailed analysis of the catalytic activity of NaPy towards the Michael addition. Attempts to reproduce the previously reported NaPy catalyzed Michael addition between 2,4-pentanediol and \( \text{trans-}\beta\)-nitrostyrene failed. Our studies show that the catalytic activity of NaPy is most likely a result of contamination with \( K_2\text{CO}_3 \). Reasons for this are the strong synergy between NaPy and \( K_2\text{CO}_3 \) towards the catalysis of the Michael addition and the fact that \( K_2\text{CO}_3 \) is used in the last step of the synthesis of NaPy. Catalytic experiments in which the Michael acceptor, Michael donor and the inorganic salt have been varied, show that this synergy is very specific. Based on our findings we propose a mechanism in which NaPy functions as a phase transfer catalyst for \( K_2\text{CO}_3 \). These new insights lead us to take another look into the original article, in which the catalytic activity of NaPy was buffered by complexation with bifunctional UPy. We showed that it is possible to improve the buffering of the reaction rate strongly by controlling the amount of \( K_2\text{CO}_3 \) during the concentration dependent catalytic studies.

Chapter 3 describes the synthesis of the components of the positive feedback loop. Several improvements are made to their molecular structure in order to obtain substrates with a higher reactivity. The corresponding bifunctional UPy was successfully formed by the NaPy/\( K_2\text{CO}_3 \) catalyzed reaction. However, significant amounts of side-products were obtained and it was shown that these are likely the result of the high reactivity of the substrates. It was however shown that the reactions can be performed on practical time-scales at the low concentrations that are required. Future work will therefore be focused on optimizing the reaction conditions and substrates in order to minimize the side-reactions.
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Chapter 1: Introduction

1.1 Supramolecular chemistry in nature
Nature provides many examples of highly complex structures formed by the assembly of molecular sub-units via non-covalent interactions. These relatively weak interactions include hydrogen bonding, Van der Waals forces, electrostatic interactions and π-π interactions. The field that focuses on these molecular assemblies is called supramolecular chemistry. As a result of the reversibility of these interactions, non-covalent structures are often much more responsive to external stimuli such as temperature, pH, concentration and solvent polarity, than covalent systems.

A well-known example of non-covalent structures found in nature are proteins. The structure of a protein can be divided into four levels of complexity. Proteins have different levels of structure, which are called the primary, secondary, tertiary and quaternary structure (Figure 1.1). The primary structure of a protein is defined as the linear sequence of the amino-acids. There are twenty natural amino acids, each with different chemical and structural properties. These properties determine the further folding process of the protein. Hydrogen-bonding between the amino-acids of the peptide leads to the assembly into α-helices and β-sheets. This local structural conformation is called the secondary structure. Subsequently, the protein folds into a more stable configuration, which is called the tertiary structure. This folding is mainly the result of hydrogen bonding, hydrophobic interactions, Van der Waals forces and disulphide bridges. Multiple polypeptide chains can associate into a three-dimensional structure which is called the quaternary structure. The final shape of the protein is stabilized by the same interactions as those from the ternary structure.

![Figure 1.1: Schematic representation of the primary, secondary, tertiary and quaternary structure of a protein.](image)

One of the most common functions of proteins lies in enzymatic catalysis, also here supramolecular structures play an important role. Most molecules in nature are formed via a cascade of enzyme catalyzed reactions. Non-covalent interactions between products and earlier enzymes in the cascade give rise to feedback loops, which are important to maintain homeostasis (Figure 1.2).
Another example of a supramolecular structure in nature is Deoxyribonucleic acid (DNA), which functions as carrier of genetic information. It composes two strands of nucleotides, which are coiled around each other to form a double helix. A nucleotide is in turn build up out of a nitrogenous base (adenine (A), thymine (T), cytosine (C) or guanine (G)), a sugar and a phosphate group. Base paring of adenine with thymine and cytosine with guanine by hydrogen bonding results in the formation of a supramolecular polymer.

The ability of organisms to pass genetic information from one generation to another is a prerequisite for the continued existence of a species. DNA can self-replicate by functioning as a template for its own formation (Figure 1.3). The double helix is unwound and each strand acts as a template for the synthesis of a new complementary strand. Free nucleotides match to the unwounded strand and are subsequently polymerized by DNA polymerase. In this way, a replicate of the original DNA molecule is formed via template directed polymerization.

1.2 Supramolecular chemistry in synthetic systems

The elegant supramolecular systems found in nature have inspired chemists to develop synthetic supramolecular systems. A practical application of supramolecular chemistry in materials is the formation of self-healing polymers. Materials which have the ability to heal themselves have a prolonged life-time. Various examples of self-healing materials based on hydrogen bonding and π-...
π interactions\(^8\) have been reported. A typical example of a self-healing mechanism in supramolecular materials is represented in Figure 1.4.\(^5\) The material consists of a network of a supramolecular polymer. The non-covalent character of the network provides reversibility and dynamic behaviour, giving it the ability to restore supramolecular bonds after it is damaged.

![Figure 1.4: Typical example of a self-healing mechanism in supramolecular materials.\(^5\)](image)

A refined supramolecular system found in nature is the allosteric regulation of enzymes. The sub-units of a protein can be brought together by self-assembly, resulting in the formation of a new recognition site for substrate binding. One has tried to mimic this artificially by synthetic metal-ion complexation (Figure 1.5). Here, the coordination of flexible ligands to a metal ion leads to the formation of a second binding site.\(^9\)

![Figure 1.5: Schematic representation of the assembly of an intramolecular binding site.\(^9\)](image)

An example of the assembly of an intramolecular assembly by metal-ion complexation has been reported by Scrimin (Figure 1.5).\(^10\) Here, bis(aminomethyl)pyridine derivative \(1\) coordinates to a \(\text{Cu}^{2+}\) ion. This leads to enhancement of the complexation of a second \(\text{Cu}^{2+}\) ion. The self-assembled structure is capable of catalyzing the hydrolytic cleavage of a β amino-acid by a cooperative effect of the two \(\text{Cu}^{2+}\) ions. The amine of the β amino-acid coordinates to the first \(\text{Cu}^{2+}\) ion and the carbonyl of the ester to the second \(\text{Cu}^{2+}\) ion, which functions as a Lewis acid catalyst for the cleavage of the ester. The cooperative effect leads to enhancement of the rate of hydrolysis.
Supramolecular complex capable of catalyzing the hydrolysis of β amino-acids by a cooperative effect of the Cu\textsuperscript{2+} ions of catalyst 1.\textsuperscript{10}

1.3 Artificial feedback loops

A feedback loop is a system in which the product of the system influences the rate of product formation. In certain cases, where the product is also the catalyst of the reaction, the reaction is called autocatalytic. The replication of matter by positive feedback loops plays an essential role in life. Therefore, chemists have tried to mimic these kind of feedback loops artificially. The artificial feedback loops which have been developed up to now can broadly been divided in two types of self-replicating systems; replication of molecular entities and replication of physical entities.

1.3.1 Replication of molecular entities

Nature shows that it is possible for a molecule to make copies of themselves by template directed synthesis as described in paragraph 1.1. This has inspired chemists to develop synthetic self-replicating systems. The minimal model of template-directed self-replication is depicted in Figure 1.6.\textsuperscript{11

![Schematic representation of the minimal model of self-replication. The complementary reagents A and B can react through three reaction channels: The uncatalyzed bimolecular reaction, the autocatalytic cycle and the formation of a binary complex.\textsuperscript{11}](image-url)
Here, compound T functions as a template for its own replication. The substrates A and B bear complementary recognition sites and are therefore able to bind template T supramolecularly. In this way, the substrates A and B are brought close together. This leads to bond formation between A and B, resulting in the formation of product T. The complex T-T dissociates into the two individual molecules, which both can function as a new template. In this way, template T has duplicated itself. Ideally, such an autocatalytic cycle results in an exponential increase of the amount of product T.

However, there are a few processes which lower the efficiency of the template-directed synthesis. First of all, the product duplex T-T should have a lower stability than ternary complex A-B-T. The autocatalytic cycle won’t be completed when a stable T-T complex is formed as there will be no release of new templates. This difference in stability is not self-evident. As a result of cooperative binding of the sub-units, duplexes are generally more stable than ternary complexes. Also the entropy penalty of forming ternary complexes is usually higher. Besides the autocatalytic cycle, product T can also be formed by the uncatalyzed reaction. The rate of the autocatalytic cycle should be significantly larger than the bimolecular reaction to get an efficient self-replicating process. Furthermore, there is a third reaction channel for the reagents A and B. A pseudo-intramolecular reaction leads to the formation of an inactive binary complex. All these processes can limit the exponential autocatalytic growth of template T.

Von Kiedrowski demonstrated that a template based self-replicating system can be developed with small organic molecules (Figure 1.7). Here, reactants 1 and 2 can undergo a condensation reaction leading to the formation of product T_{12}. Product T_{12} has complementarity with reactants 1 and 2 and can therefore form ternary complex \([1·2·T_{12}]\) via amidinium-carboxylate salt-bridges. In this way the reactants are brought close together leading to enhancement of the rate of the condensation reaction. The newly formed product T_{12} can again function as a template for the reaction, yielding a self-replicating system.

\[\begin{align*}
\text{H}_2\text{N} \quad \text{H} & \quad \text{H} \quad \text{H} \\
\text{N} & \quad \text{NH}_2 \\
\text{N} & \quad \text{NH}_2 \\
\text{N} & \quad \text{NH}_2 \\
\text{I} & \quad \text{Bu}
\end{align*}\]

\[\begin{align*}
\text{H} & \quad \text{N} \\
\text{N} & \quad \text{NH}_2 \\
\text{N} & \quad \text{NH}_2 \\
\text{N} & \quad \text{NH}_2 \\
\text{I} & \quad \text{Bu}
\end{align*}\]

Figure 1.7: Self-replication via a template-catalyzed condensation reaction.

Replication of templates can also proceed via a reciprocal mechanism. These kind of replicating systems are based on the complementarity of non-identical templates. The templates catalyze the formation of each other via interlinked crosscatalytic cycles (Figure 1.8). The reaction between C and D is catalyzed by template T_{EF} via ternary complex \([C·D·T_{EF}]\) resulting in the formation of T_{CD}. Similarly, the reaction between E and F is catalyzed by template T_{CD} via ternary complex \([E·F·T_{CD}]\)
resulting in the formation of $T_{EF}$. Ideally, this results in an exponential increase of templates $T_{CD}$ and $T_{EF}$. However, the system will be more complex if $C$ can react with $E$ and $D$ with $F$. This would result in the minimal replication of $T_{CE}$ and $T_{DF}$ and the reciprocal replication of $T_{CD}$ and $T_{EF}$.

Figure 1.8: Schematic representation of a reciprocal replicating system. $T_{EF}$ catalyzes the reaction between $C$ and $D$ via ternary complex $[C\cdot D\cdot T_{EF}]$ resulting in the formation of $T_{CD}$. $T_{CD}$ catalyzes the reaction between $E$ and $F$ via ternary complex $[E\cdot F\cdot T_{CD}]$ resulting in the formation of $T_{EF}$.

A well-known example of a reciprocal replicating system in nature is the replication of DNA. Each DNA strand functions as a template for the formation of a complementary strand as described in paragraph 1.1. Due to the complexity of the design of the substrates, these kind of systems have barely been developed artificially. An example of a synthetic reciprocal replicating system has been reported by Philp and collaborators (Figure 1.9). Here, the Diels-Alder reaction between $G$ and $H$ results in the formation of template $T_{GH}$ and 1,3-dipolar cycloaddition between $I$ and $J$ results in the formation of template $T_{IJ}$. These reactions are catalyzed via interlinked crosscatalytic cycles. $T_{IJ}$ functions as a template for the reaction between $G$ and $H$ via ternary complex $[G\cdot H\cdot T_{IJ}]$ and similarly $T_{GH}$ functions as a template between $I$ and $J$ via ternary complex $[I\cdot J\cdot T_{GH}]$. An increase of the reaction rate compared to the uncatalyzed reaction was observed when the reaction was performed in the presence of the templates.
Figure 1.9: Replication as a result of interlinked crosscatalytic cycles. The Diels-Alder reaction between G and H is catalyzed by template $T_{u}$ via ternary complex $[G\cdot H\cdot T_{u}]$ resulting in the formation of $T_{CD}$. The 1,3-dipolar cycloaddition between I and J is catalyzed by template $T_{GH}$ via ternary complex $[I\cdot J\cdot T_{GH}]$ resulting in the formation of $T_{IJ}$.

1.3.2 Replication of physical entities

Besides template directed self-replication of molecules, self-replication can take place via the replication of physical entities. An example of such a positive feedback loop is the mechanosensitive self-replication reported by Otto and collaborators. The system is based on dithiol building blocks, which can form macro-cycles of different sizes by oxidative disulfide formation (Figure 1.10).
The dithiol building blocks are functionalized with a peptide sequence of alternating hydrophobic (leucine) and hydrophilic (lysine) amino-acids. These peptides assemble into β-sheets by non-covalent interactions between the peptide-sequences, which results in stacking of the macro-cycles into one-dimensional fibers (Figure 1.11). These stacks can grow from their ends by the consumption of monomers. When the mixture is agitated by stirring, stacks with a relatively low stability will fragmentate. As a result, the total number of stacks with a low stability increases exponentially, which in turn act as nuclei for further growth of this type of stacks. This autocatalytic behavior was observed as a sigmoidal growth of the less stable hexamer and heptamer units in the system.

Another example of reproduction of physical entities is the autopoietic self-reproduction of fatty acid vesicles reported by Luisi and collaborators. When the pH is approximately equal to the pKₐ of the fatty acid, there will be an equimolar amount of deprotonated and protonated fatty acids.
present in solution. A stable dimer can be formed by hydrogen bonding between a protonated and deprotonated fatty acids. Above the critical aggregation concentration (cac), the assembly of dimers into bilayers leads to the formation of a vesicle (Figure 1.12).\textsuperscript{15}

The initial system composes of two immiscible phases, an anhydride phase and an alkaline aqueous phase. The hydrolysis of the anhydride molecules is catalyzed at the interface with the alkaline solution, which leads to the release of fatty acids into the solution. When the amount of fatty acids present in solution is high enough, vesicles will be formed in the aqueous phase. The anhydride molecules are able to solubilize in the vesicle. The total interfacial area between the anhydride and the alkaline aqueous phase increases by the formation of vesicles. As a consequence the rate of the hydrolysis of anhydride molecules increases, which leads to the formation of even more vesicles.

### 1.4 The supramolecular assembly of 2-ureido-4-pyrimidinone (UPy)

#### 1.4.1 The assembly of monofunctional UPys

Hydrogen bonds are often used to hold the molecular sub-units of supramolecular complexes together. Examples are the double hydrogen bonding between carboxylic acids\textsuperscript{16} and triple hydrogen bonding between imides and melamines\textsuperscript{17}. Larger arrays of hydrogen bonding increase the strength of a complex even more. In the search of a supramolecular building block with higher order hydrogen bonding, the 2-ureido-4-pyrimidinone (UPy) motif was developed.\textsuperscript{18,19}
The UPy motif can undergo tautomerization resulting in different monomeric and dimeric forms (Figure 1.13). The ratio of the tautomeric forms strongly depends on the polarity of the solvent. In chloroform the DDAA configuration is the major tautomeric form. It has self-complementarity in forming stable dimers by quadruple hydrogen bonding \((K_a = 6 \times 10^7 \text{ M}^{-1} \text{ in CDCl}_3)\). Due to their synthetic accessibility and high dimerization constant, the UPy-motif has been used for the development of self-healing materials, bio-active polymeric scaffolds and single-chain polymeric nanoparticles.

Figure 1.13: Equilibria between tautomeric forms of UPy and the formation of dimers by quadruple hydrogen bonding.

1.4.2 Ring-chain equilibria of bifunctional UPys

Bifunctional UPys have the ability to form cyclic oligomers as well as linear supramolecular polymers (Figure 1.14). This behaviour has been confirmed by \(^1\)H-NMR spectroscopy and viscometry.

Figure 1.14: Schematic representation of the ring-chain equilibria of bifunctional UPys.

A theoretical model for ring-chain equilibria has been developed by Jacobson and Stockmayer. It was shown that below a certain critical concentration only rings are present. The concentration of rings remains constant above this critical concentration and each excess monomer is present as a linear chain. Ercolani et al. showed that this cut-off point is only present for very high values of the intramolecular association constant (a criteria met for the UPy motif). He extended the treatment of the theory of macro-cyclization equilibria of Jacobson and Stockmayer to dilute conditions and a large range of association constants. Assuming all rings to be strainlesss and obeying Gaussian
statistics, the equilibrium between rings and linear chains can be described by the effective molarity (EM).

\[
EM = \frac{K_{\text{intra}}}{K_{\text{inter}}}
\]  

(Eq. 1.1)

Here, \( K_{\text{intra}} \) is the dimensionless intramolecular equilibrium constant and \( K_{\text{inter}} \) (M\(^{-1}\)) is the intermolecular equilibrium constant. When the total concentration of the bifunctional monomer in a system is below the effective molarity, the formation of cyclic structures is more favourable. While above the effective molarity, added monomers will form linear chains. The effective molarity of a bifunctional UPy is mainly determined by the length and flexibility of the linker between the associating end-groups. The effective molarity of a bifunctional UPy reaches a maximum when the linker has the optimal length to allow formation of an intramolecular complex and decreases weakly with increasing linker length. Below the optimal linker length, strain of the intramolecular complex decreases the effective molarity.

1.4.3 2-Ureido-4-pyrimidinone (UPy) - 2,7-diamido-1,8-naphthyridine (NaPy) systems

Besides forming stable homo-dimers (\( K_a = 6 \times 10^7 \) M\(^{-1}\) in CDCl\(_3\)), UPy has complementarity with 2,7-diamido-1,8-naphthyridine (NaPy) (\( K_a = 5 \times 10^6 \) M\(^{-1}\) in CDCl\(_3\)).\(^{26}\) There is a dynamic equilibrium between UPy dimers, free NaPy and UPy-NaPy complexes (Figure 1.15).\(^{27}\) The position of the equilibrium is controlled by Le Chatelier’s principle. At high concentrations mainly UPy-NaPy complexes are present. The equilibrium shifts upon dilution, resulting in an increase of the mole fraction of UPy dimers. Further dilution leads to the dissociation of UPy dimers. Finally resulting in a system which mainly consists of UPy monomers and free NaPy.

\[ \begin{align*}
R_1 & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} \\
R_2 & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}}
\end{align*} \]  

UPy dimer

\[ \begin{align*}
& \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \\
& \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \\
& \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \\
& \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}} \quad \overset{N}{\text{H}}
\end{align*} \]  

NaPy

\[ \begin{align*}
R_1 & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} \\
R_2 & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}} & \quad \overset{N}{\text{H}}
\end{align*} \]  

UPy-NaPy complex

**Figure 1.15:** Top: The equilibrium between UPy dimers, NaPy and UPy-NaPy complexes. Bottom: Calculated mole fractions of UPy monomers, UPy dimers and UPy-NaPy complexes in an equimolar ratio of UPy:NaPy units in CHCl\(_3\) at room temperature.\(^{27}\)
The concentration dependence of the mole fraction of free NaPy in a system based on monofunctional UPys is weak (Figure 1.16a).\(^{27}\) In a system formed by NaPy and bifunctional UPy in an equimolar ratio of UPy:NaPy units, the concentration dependence of the mole fraction of free NaPy is mainly determined by the effective molarity of the bifunctional UPy. At concentrations far below the effective molarity the mole fraction of free NaPy \(\approx 1\), while far above the effective molarity the mole fraction of free NaPy approaches zero. There is a strong concentration dependence of the mole fraction of free NaPy, when the total concentration is around the effective molarity (Figure 1.16b).\(^{27}\) This is a result of the competition between intra- and intermolecular interactions at these concentrations. Comparing a UPy-NaPy system containing bifunctional UPys with the system containing monofunctional UPys, there is a large difference in the mole fraction of free NaPy at certain concentrations.

Supramolecular autoregulation
The strong dependence of the concentration of free NaPy on the total NaPy concentration, has been used to develop a system, where the concentration of free NaPy was buffered over a broad concentration regime \((c = 10 - 80 \text{ mM})\) (Figure 1.17).\(^{27}\) It was shown that in a mixture of bifunctional UPy 1 and NaPy 2 in an equimolar ratio of UPy:NaPy units, the decrease of the total concentration of NaPy upon dilution is roughly compensated by the increase of the mole fraction of free NaPy by the shift of the equilibrium.
Figure 1.17: a) Schematic representation of the supramolecular autoregulation of free NaPy. b) Measured concentrations of free NaPy as function of the total concentration of NaPy in the presence of a bifunctional UPy in an equimolar ratio of UPy:NaPy units. Effective molarity of bifunctional UPys: 4 mM (triangles), 8 mM (circles), 10 mM (filled circles).

Besides functioning as a supramolecular group, it has been shown that NaPy can function as an organocatalyst for the Michael addition between 2,4-pentanedione and trans-β-nitrostyrene. Hetero-dimerization of NaPy with UPy leads to inhibition of its catalytic properties. Combining the autoregulation of free NaPy and its organocatalytic properties, yielded a system in which the turnover frequency (TOF) of the NaPy is kept constant over a broad concentration regime (Figure 1.18).

Figure 1.18: Top: The NaPy-catalyzed Michael addition between trans-β-nitrostyrene and 2,4-pentanedione in CDCl₃. Bottom: (a) Catalytic activity at different concentrations of catalyst NaPy. (b) Catalytic activity at different concentrations of catalyst NaPy in the presence of bifunctional UPy (EM = 8 mM) in an equimolar ratio of UPy:NaPy units.

1.6 Objective and outline
The aim of the project described in this thesis is to develop a positive feedback loop based on supramolecular ring formation, using the UPy and NaPy motifs. It has been reported that at some concentrations, a system with bifunctional UPys contains a larger fraction of free NaPy compared to a system with monofunctional UPys (Figure 1.16). Furthermore, it was shown that NaPy can
catalyze the Michael addition between 2,4-pentanedione and trans-β-nitrostyrene. We plan to combine these two phenomena in order to develop a positive feedback loop based on supramolecular ring formation (Figure 1.19).

The starting components of the system will be a UPy functionalized 2,4-pentanediolone, a UPy functionalized trans-β-nitrostyrene and NaPy. At certain concentrations, the mole fraction of free NaPy will be relatively small in a system based on monofunctional UPys (Figure 1.16a). The small fraction of free NaPy will catalyze the Michael addition between 2,4-pentanediolone and trans-β-nitrostyrene, resulting in the formation of a bifunctional UPy. As a result of ring formation, a system based on bifunctional UPys has a larger mole fraction of free NaPy than those with monofunctional UPys (Figure 1.16b). The NaPy catalyzed Michael addition therefore results in the release of free NaPy (i.e., the catalyst), resulting in an increase in reaction rate.

This system differs considerably from other artificial positive feedback loops by the fact that the product of the reaction doesn’t function as a catalyst for its own formation, but as a supramolecular scaffold from which the catalyst is released. As a result it is not autocatalytic; we will therefore refer to the system as a “self-accelerating reaction”. Since the product of the reaction doesn’t function as a catalyst for the reaction itself, modifications to the substrates are not naturally detrimental for the self-accelerating effect, which is often the case in autocatalytic systems.

On the other hand, a drawback of the self-accelerating reaction is the dependence of the self-accelerating effect on the total concentration. Significant acceleration can only be obtained in a small concentration regime. Above the optimal concentration, the tendency of the bifunctional UPy to form a cycle decreases. Consequently, the amount of NaPy released into solution will be decreased. Below the optimal concentration, the rate of the Michael addition decreases. Also, at low concentrations the fraction of free NaPy is already significant at the start of the reaction, thereby decreasing the fraction of free NaPy that is released. As a result of these phenomena, the accelerating reaction will be less efficient under these conditions.

**Figure 1.19:** Schematic representation of the self-accelerating system. Red symbols depict NaPy, blue symbols depict UPy, A and B represent substrates and P represents product.
Chapter 2: Covers our studies of the catalytic activity of NaPy towards the Michael addition and the influence of these results on the previously reported buffering of the catalytic activity of NaPy. Furthermore, various combinations of Michael substrates have been screened in order to find suitable substrates to couple to the UPy motif.

Chapter 3: Describes the synthesis of the components of the self-accelerating reaction. The reactivity of the UPy functionalized Michael substrates is measured and improvements are made upon changing the molecular structure. Furthermore, the coupling of the UPy functionalized substrates have been tested.
1.7 References

2) Particle sciences, Technical brief 2009, 8.
4) OpenStax College, The Nucleus and DNA Replication, June 26, 2013, http://cnx.org/content/m46073/1.4/.
Chapter 2: Investigating the catalytic activity of 2,7-diamido-1,8-naphthyridine towards the Michael addition

2.1 Introduction
Organocatalysis is an emerging field in catalysis and provides an alternative for metal catalyzed reactions.\textsuperscript{1,2} Advantages of the use of organic molecules as catalysts are their synthetic accessibility, robustness, high efficiency, selectivity and insensitivity towards water and air.\textsuperscript{3,4} Organocatalysts are applied in a broad range of reactions, such as aldol reactions\textsuperscript{5}, Michael additions\textsuperscript{6} and Mannich reactions\textsuperscript{7}. Organic molecules are able to catalyze the Michael addition in various ways. Examples are the promotion of the conjugate addition between the reactants by activation of the Michael acceptor via hydrogen bonding\textsuperscript{8}; covalent activation by the reversible formation of an iminium-ion or enamine\textsuperscript{9,10} or functioning as a phase transfer catalyst\textsuperscript{11}.

Various examples of the use of organic molecules as phase transfer catalyst for solid bases (e.g. $\text{K}_2\text{CO}_3$ or KOH) in the catalysis of Michael additions are known.\textsuperscript{12-14} Typically, the first step is proton abstraction of the phase transfer catalyst at the surface of the solid base, leading to formation of a complex with the anion (Figure 2.1)\textsuperscript{15}. The anion is highly reactive due to its poor solvation in the organic phase. The reactive complex deprotonates the substrate leading to its activation, thereby reforming the phase transfer catalyst. Benefits of phase transfer catalysts are their high reactivity and selectivity, mild reaction conditions and inexpensive reactants.\textsuperscript{16}

![Figure 2.1: Example of a mechanism of a phase transfer catalyzed reaction.\textsuperscript{15}](image)

Recently, the ability of NaPy to catalyze the Michael addition between 2,4-pentanedione 1 and \textit{trans}-\beta-nitrostyrene 2 has been reported.\textsuperscript{17} The catalytic activity of NaPy towards the Michael addition was attributed to its basic character. A strong decrease of the reactivity was observed by the addition of 1 equivalent of UPy (Figure 2.2). The addition of another equivalent of UPy leads to complete inhibition of the catalytic activity of NaPy. The ability to control the catalytic activity of an organocatalyst by supramolecular inhibition makes it an interesting building block for the development of complex catalytic systems. In this chapter the catalytic activity of NaPy towards the Michael addition is further investigated in order to optimize the conditions for the self-accelerating reaction.
2.2 Investigating the mechanism of the NaPy catalyzed Michael addition

One of the key elements that inspired us to develop the self-accelerating reaction was the reported ability of NaPy to catalyze the Michael addition between 2,4-pentanedione 1 and nitrostyrene 2. However, attempts to reproduce the catalytic experiments reported in literature failed. Our NaPy showed no catalytic activity towards the Michael addition between 2,4-pentanedione 1 and nitrostyrene 2.

Since the substrates are commercially obtained and therefore assumed to be pure, the difference in observed catalytic activity is likely a result of the presence of impurities in our NaPy or the original batch of NaPy. The last step of the synthesis of NaPy is the Pd-catalyzed amidation of N-(7-chloro-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide with Pd(OAc)$_2$, Xantphos and K$_2$CO$_3$ in dioxane (Figure 2.3). After filtration at room temperature, the conventional work up proceeds via recrystallization in ethanol and toluene. This method appeared to be ineffective at the large scale we performed the synthesis of NaPy. Therefore, NaPy was additionally purified by column chromatography.

![Figure 2.3: The last step towards the synthesis of NaPy. Reaction conditions: Dodecanamide, Pd(OAc)$_2$, Xantphos, K$_2$CO$_3$, dioxane, 80 °C, overnight.](image)

K$_2$CO$_3$ is used as a base to catalyze the formation of NaPy. Various examples are known of Michael additions catalyzed by K$_2$CO$_3$. We hypothesized that incomplete removal of K$_2$CO$_3$ could have led to the high rates of the Michael addition reported in literature, while the work up by column chromatography would most likely have removed K$_2$CO$_3$. In order to investigate this hypothesis, the Michael addition between 2,4-pentanedione 1 and nitrostyrene 2 has been performed in the presence of either K$_2$CO$_3$, NaPy or a combination of these (Figure 2.4).
Figure 2.4: The conversion of the Michael addition between 2,4-pentanedione (500 mM) and nitrostyrene (100 mM) in the presence of K$_2$CO$_3$ (5 mM), NaPy (20 mM) and UPy (20 mM) in CDCl$_3$ after 2 hours at room temperature.

The presence of column purified NaPy doesn’t lead to the formation of the Michael adduct of 2,4-pentanedione 1 and nitrostyrene 2 for up to 7 days. K$_2$CO$_3$ does catalyze the Michael addition, although the rate of Michael adduct formation is low. Remarkably, a combination of both leads to a more than 100-fold increase in product formation under the same conditions. Further studies showed that 100 % conversion is obtained already after roughly 45 minutes, indicating a strong synergy between NaPy and K$_2$CO$_3$ towards the catalysis of the Michael addition. These experiments strongly support our hypothesis that contamination of NaPy with K$_2$CO$_3$ has led to the high reaction rates reported in literature. Complexation of NaPy by the addition of 1 eq. UPy leads to inhibition of its catalytic activity, confirming that NaPy only has the ability to catalyze the Michael addition when it is in the unbounded state.

To investigate the dependence of the reaction rate on the concentration K$_2$CO$_3$, the Michael addition has been performed at a constant concentration of 2,4-pentanedione 1, nitrostyrene 2 and NaPy and increasing amounts of K$_2$CO$_3$ (Figure 2.5).

Figure 2.5: The conversion of the Michael addition between 2,4-pentanedione 1 (100 mM) and nitrostyrene 2 (100 mM) in the presence of NaPy (20 mM) and different amounts of K$_2$CO$_3$ in CDCl$_3$ after 0.5 hour and 1.0 hour. Error bars depict the standard deviation of two measurements.

There is roughly a linear dependence of the reaction rate on the concentration K$_2$CO$_3$. The relatively low conversion after 0.5 hour compared to the conversion after 1 hour, suggests an initial lag period for the formation of the Michael adduct.
In order to obtain more insight in what is causing the synergy and possibly find a co-catalyst with a higher catalytic activity, the NaPy catalyzed Michael addition has been performed in the presence of various salts (Figure 2.6). The rate of the Michael addition follows the general trend of solubility ($\text{Cs}_2\text{CO}_3 > \text{K}_2\text{CO}_3 > \text{Na}_2\text{CO}_3 > \text{CaCO}_3$). Replacing $\text{K}_2\text{CO}_3$ for KCl doesn’t result in a high reaction rate for the Michael addition. The reaction still takes place when NaPy is replaced by the phase transfer catalyst 18-crown-6.

![Conversion after 2 hours (%)](image)

**Figure 2.6:** The conversion of the Michael addition between 2,4-pentanedione 1 (500 mM) and nitrostyrene 2 (100 mM) in the presence of NaPy or 18-crown-6 (20 mM) and a carbonate (5 mM) or KCl (10 mM) in CDCl$_3$ after 2 hours.

As mentioned, several examples are known in which K$_2$CO$_3$ functions as a solid base in solid/liquid phase transfer catalyzed reactions. Typical phase transfer catalysts are crown-ethers and tetraalkylammonium salts. The catalyst facilitates the migration of potassium into the organic phase, followed by proton abstraction of the substrate. In this way the phase transfer catalyst facilitates the reaction between the substrate in the organic phase and the solid K$_2$CO$_3$. Here, we would like to propose a mechanism in which NaPy functions as a phase transfer catalyst for K$_2$CO$_3$ in the Michael addition between 2,4-pentanedione 1 and nitrostyrene 2 (Figure 2.7).

![Proposed mechanism](image)

**Figure 2.7:** The proposed mechanism of the NaPy/K$_2$CO$_3$ catalyzed Michael addition between 2,4-pentanedione 1 and nitrostyrene 2.

In our proposed mechanism, NaPy reacts first with K$_2$CO$_3$, resulting in the formation of KHCO$_3$ and the potassium salt of NaPy. The NaPy salt deprotonates the acidic 2,4-pentanedione, leading to its activation and reformation of NaPy. The activated 2,4-pentanedione attacks the electrophilic $\beta$-
carbon of trans-β-nitrostyrene, thereby forming the potassium salt of the Michael adduct. Finally, the Michael product is formed after taking up a proton of another 2,4-pentanedione. The newly activated 2,4-pentanedione undergoes the Michael addition following the same reaction path.

The proposed mechanism rationalizes the results of the earlier studies of the synergy between NaPy and K$_2$CO$_3$. (i) The observed initial lag phase is a result of the need to form the potassium salt of NaPy before the Michael addition can take place. (ii) It agrees with the catalytic studies of the individual components. (iii) Full conversion can be reached in the presence of only catalytic amounts of K$_2$CO$_3$, due to the activation of another 2,4-pentanedione in the last step of the formation of the Michael product. (iv) The high rates of the Michael addition catalyzed by Cs$_2$CO$_3$ and K$_2$CO$_3$ compared to Na$_2$CO$_3$ and CaCO$_3$ are a result of their higher solubility.

### 2.3 Screening of Michael substrates

Different combinations of substrates have been screened for the NaPy/K$_2$CO$_3$ catalyzed Michael addition in order to find suitable for the self-accelerating reaction (Table 2.1). Remarkably, only four of the sixteen tested combinations resulted in the formation of a Michael adduct.

**Table 2.1:** The conversion of the Michael addition between various Michael donors (500 mM) and Michael acceptors (100 mM) in the presence of NaPy (20 mM) and K$_2$CO$_3$ (5 mM) in CDCl$_3$ after 2 hours at room temperature.

<table>
<thead>
<tr>
<th>Michael donor</th>
<th>Michael acceptor</th>
<th>O C</th>
<th>H S</th>
<th>O C</th>
<th>NH$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>0 %</td>
<td>0 %</td>
<td>0 %</td>
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<td>100 %</td>
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<td>41 %</td>
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</table>

The individual contribution of NaPy and K$_2$CO$_3$ as catalyst for the Michael addition for these four combinations of substrates was investigated to find out if there is a similar synergistic relation between NaPy and K$_2$CO$_3$ (Figure 2.8). This only the case for two combinations: 2,4-pentanediol with trans-β-nitrostyrene and 2,4-pentanediol with maleimide. For the other two combinations, thiol with trans-β-nitrostyrene and aniline with trans-β-nitrostyrene, the formation of product is simply the sum of the individual contributions of NaPy and K$_2$CO$_3$. 
Figure 2.8: The conversion of the Michael addition between various Michael donors (500 mM) and Michael acceptors (100 mM) in the presence of NaPy (20 mM) and/or K$_2$CO$_3$ (5 mM) in CDCl$_3$ after 2 hours at room temperature.

In order to achieve a self-accelerating effect (as described in paragraph 1.7) during the progress of the NaPy/K$_2$CO$_3$ catalyzed Michael addition between the UPy functionalized substrates, an increase of the free NaPy concentration should lead to a large increase of the catalytic activity. Therefore, the two combinations for which the product formation is a result of the synergy between NaPy and K$_2$CO$_3$ are the most suitable substrates. The highest rate of Michael adduct formation is achieved using 2,4-pentanedione 1 and nitrostyrene 2, therefore these substrates are most suited for use in the self-accelerating reaction.

2.4 Supramolecular autoregulation revisited
A system in which a bifunctional UPy buffers the concentration of free NaPy over a broad concentration regime has been reported (Paragraph 1.6; Figure 1.17). As a result the catalytic activity of NaPy towards the Michael addition between trans-β-nitrostyrene and 2,4-pentanedione increases for an unbuffered system (Figure 2.9a), while it remains constant in the presence of bifunctional UPy 3 (Figure 2.9b).
Figure 2.9: (a) Catalytic activity of NaPy towards the Michael addition between trans-β-nitrostyrene and 2,4-pentanedione at different concentrations of catalyst NaPy.\(^{17}\) (b) Catalytic activity of NaPy towards the Michael addition between trans-β-nitrostyrene and 2,4-pentanedione at different concentrations of catalyst NaPy in the presence of bifunctional UPy 3 (EM = 8 mM) in an equimolar ratio of UPy:NaPy units.\(^{17}\)

In the absence of bifunctional UPy 3, the initial reaction rates increase exponentially with the total NaPy concentration (Figure 2.10a). In contrast, the presence of bifunctional UPy 3 results in a linear increase of the initial reaction rate (Figure 2.10b).

Figure 2.10: Conversion of the Michael addition between 2,4-pentanedione 1 (500 mM) and nitrostyrene 2 (100 mM) in the presence of various concentrations NaPy in CDCl\(_3\).\(^{17}\) b) Conversion of the Michael addition between 2,4-pentanedione 1 (500 mM) and nitrostyrene 2 (100 mM) in the presence of various concentrations NaPy and bifunctional UPy 3 (EM = 8 mM) in an equimolar ratio of UPy:NaPy units in CDCl\(_3\).\(^{17}\)

In the original article, the catalytic activity of NaPy towards the Michael addition was attributed to its basic character. Our findings show that the catalytic activity of NaPy is actually a result of contamination with K\(_2\)CO\(_3\), where NaPy functions as a phase transfer catalyst. Here we report the influence of our findings on the reported autoregulation.

Buffering of the TOF (mol product / (mol catalyst*h)) was reported. However, the initial reaction rates still increase with the total NaPy concentration. Since it was shown that the concentration of free NaPy is buffered in this concentration regime, one would expect constant reaction rates under these conditions. Since the observed catalytic activity is actually a result of contamination with...
K$_2$CO$_3$, an increase of the total NaPy concentration also led to an increase of the concentration K$_2$CO$_3$. The constant concentration of substrates and free NaPy, but increasing amounts of K$_2$CO$_3$, likely resulted in the increasing reaction rates. The reason there is still a constant TOF is a result of the used definition. The catalyst concentration has been equated to the total NaPy concentration, while NaPy only functions as a catalyst when it is in the unbounded state. Therefore, autoregulation of the catalytic activity is less pronounced using the correct definition of TOF.

Presumably, it is possible to achieve better buffering of the catalytic activity when the concentration K$_2$CO$_3$ is kept constant. We therefore repeated the reported catalytic experiments in the presence of constant amounts of K$_2$CO$_3$. First the NaPy/K$_2$CO$_3$ catalyzed Michael addition between 2,4-pentanediione 1 and nitrostyrene 2 in the absence of bifunctional UPy 3 was examined. When the concentration K$_2$CO$_3$ is kept constant at 1 mM, an increase of the NaPy concentration from 1 mM to 5 mM results in an increase of the reaction rate (Figure 2.11a). Performing the reaction at a higher NaPy concentration doesn’t result in a significant increase of the reaction rate. Likely, the K$_2$CO$_3$ concentration becomes rate limiting in this concentration regime.

When the same reaction is performed in the presence of bifunctional UPy 3, the initial reaction rates are nearly constant as function of the total NaPy concentration (Figure 2.11b). Remarkably, there seems to be a trend in this concentration regime. An increase of the total NaPy increase leads to a decrease of the catalytic activity. Furthermore, the reaction rate is roughly a five-fold lower compared to a system in which the concentration of free NaPy is buffered at 1 mM. These phenomena suggest that the presence of K$_2$CO$_3$ influences the UPy-NaPy equilibrium slightly, thereby lowering the free NaPy concentration.

Figure 2.11: a) Conversion of the Michael addition between 2,4-pentanediione 2 (500 mM) and nitrostyrene 2 (100 mM) in the presence of K$_2$CO$_3$ (1 mM) and various concentrations of NaPy in CDCl$_3$. b) Conversion of the Michael addition between 2,4-pentanediione 2 (500 mM) and nitrostyrene 2 (100 mM) in the presence of K$_2$CO$_3$ (1 mM), bifunctional UPy 3 (EM = 8 mM) and various concentrations of NaPy in CDCl$_3$. 

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2.5 Conclusions

The catalytic activity of NaPy towards the Michael addition has been investigated, in order to optimize the conditions for the self-accelerating reaction. Attempts to reproduce the reported NaPy catalyzed Michael addition between 2,4-pentanedione and trans-β-nitrostyrene failed. Further studies showed that the catalytic activity of NaPy is most likely a result of contamination with K₂CO₃, due to its incomplete removal in the last step of its synthesis. We have discovered a strong synergy of NaPy and K₂CO₃ towards the Michael addition for certain substrates. Our findings lead us to propose a mechanism in which NaPy is functioning as a phase transfer catalyst for K₂CO₃.

The synergy between NaPy and K₂CO₃ towards the Michael addition appeared to be very selective. Only four of sixteen tested combinations of Michael substrates resulted in product formation, while only two combinations display a synergy between NaPy and K₂CO₃. Based on their high reactivity, 2,4-pentanodione and nitrostyrene were selected as substrates for the self-accelerating system.

As the catalytic activity of NaPy appeared to be a result of contamination with K₂CO₃, the influence of these new findings on the previously reported buffering of its catalytic activity have been investigated. The independence of the TOF towards the total NaPy concentration appeared to be a result of the way that the TOF has been defined. Buffering of the catalytic activity is much less pronounced when the TOF is defined using free NaPy as catalyst instead of the total NaPy concentration as was done in the original article. As a consequence of the contamination by K₂CO₃, an increase of the total NaPy concentration also led to an increase of the concentration K₂CO₃. The same catalytic studies have been performed in the presence of a constant concentration K₂CO₃. Under these conditions the reaction rate has become independent of the total NaPy concentration, yielding a system in which the free NaPy concentration and reaction rates are buffered over a broad concentration regime.
2.6 Experimental section

All chemicals were purchased from Sigma Aldrich and used as received, unless stated otherwise. All solvents were obtained from BioSolve. Deuterated solvents were purchased from Cambridge Isotope Laboratories. \( \text{K}_2\text{CO}_3 \) was grinded prior to use. \( ^1\text{H}-\text{NMR} \) spectra were recorded on a Varian Mercury 400 MHz or Varian Mercury 500 MHz. The synthetic procedure of NaPy and UPy 3 are described in paragraph 3.7. Bifunctional UPy 3 was kindly provided by Tim Paffen.

Procedure of Michael additions paragraph 2.1-2.3:

The Michael donor and Michael acceptor were dissolved in CDCl₃ (2 mL). NaPy was added to the mixture and sonicated. Subsequently, the mixture was added to the inorganic salt and vigorously stirred. The conversion was determined using \( ^1\text{H}-\text{NMR} \).

Procedure of Michael additions paragraph 2.4:

A mixture of NaPy and \( \text{K}_2\text{CO}_3 \) in CDCl₃ (10 mL) was vigorously stirred overnight. The Michael donor and Michael acceptor and bifunctional UPy 3 were dissolved in CDCl₃ (10 mL) and added to the mixture. After every time interval, a sample of 0.6 mL was taken and the conversion was determined using \( ^1\text{H}-\text{NMR} \). After the measurement, the sample was returned to the mixture.

Determination of conversion using \( ^1\text{H}-\text{NMR} \):

The conversion of the Michael adduct of 2,4-pentanedione and trans-\( \beta \)-nitrostyrene was determined by integration of peak b versus peak x (Figure 2.12). The conversion of other Michael adducts are determined by integration of a characteristic peak of the product versus a characteristic peak of the Michael acceptor.

Figure 2.12: \( ^1\text{H}-\text{NMR} \) spectrum of the coupling between 2,4-pentanedione (500 mM) and trans-\( \beta \)-nitrostyrene (100 mM) using NaPy (20 mM) and \( \text{K}_2\text{CO}_3 \) (5 mM) at full conversion in CDCl₃.
$^1$H-NMR spectrum of bifunctional UPy:

Figure 2.13: $^1$H-NMR spectrum of bifunctional UPy 3 in CDCl$_3$. 
2.7 References

Chapter 3: Synthesis of the components of the self-accelerating reaction

3.1 Introduction

As mentioned in paragraph 1.5, the self-accelerating reaction is based on the release of NaPy from a UPy-NaPy complex as a result of the NaPy catalyzed formation of bifunctional UPys. This reaction is preferably performed at a concentration where the difference in the mole fraction of free NaPy between the initial and final system is as large as possible (i.e. the optimal concentration). However, it would be beneficial to perform the reaction at a higher concentration to have a higher reaction rate.

The concentration dependence of the mole fraction of free NaPy in the system with monofunctional UPys is controlled by Le Chatelier’s principle, while in the system with bifunctional UPys it is mainly determined by the effective molarity of the bifunctional UPy. By increasing the effective molarity of the bifunctional UPy, both the optimal concentration of the self-accelerating reaction and the absolute difference of the mole fraction of free NaPy between the initial and final system are increased (Figure 3.1).

![Figure 3.1: Mole fraction of free NaPy as function of the total NaPy concentration in a UPy-NaPy system with an equimolar ratio of UPy-NaPy units in CHCl₃ at room temperature for a system with monofunctional UPys (red), system with bifunctional UPys (blue) and absolute difference between the systems (black) with respectively an effective molarity of 1 mM, 10 mM and 50 mM of the bifunctional UPy.]

As has been shown by Ercolani, the most adequate way to increase the effective molarity is to decrease the linker length between the associating end-groups. Typically, UPy motifs are functionalized on the 1-position. However, this requires a large linker length of the bifunctional UPy to allow the formation of the intramolecular complex (Figure 3.2a). For this reason we plan to functionalize one of the monofunctional UPys with the Michael substrate on the 4-position. The corresponding bifunctional UPy likely requires a much smaller linker length to allow the formation of the intramolecular complex and consequently a higher effective molarity will be obtained (Figure 3.2b).
Figure 3.2: a) Bifunctional UPy with large linker length (low EM). b) Bifunctional UPy with small linker length (high EM).

In summary, the UPy functionalized Michael substrates should ideally be soluble in chloroform, highly reactive towards the Michael addition and the corresponding bifunctional UPy has preferably an as high as possible effective molarity. NaPy 1, UPy functionalized 2,4-pentanedione 2, UPy functionalized nitrostyrene 3 and UPy functionalized nitrostyrene 4 are chosen as target compounds for the development of the self-accelerating reaction (Figure 3.3). Modifications to the molecular structure of the starting compounds will be made if they don’t fulfill the required properties.

Figure 3.3: Target compounds: NaPy 1, UPy functionalized 2,4-pentanedione 2, UPy functionalized nitrostyrene 3 and UPy functionalized nitrostyrene 4.

3.2 Synthesis of the starting compounds

3.2.1 Synthesis of NaPy

Scheme 3.1: Synthetic route towards NaPy 1. (i) malic acid, H₂SO₄, 110 °C, 3 h; (ii) dodecanoyl chloride, pyridine, 110 °C, overnight; (iii) POCl₃, 95 °C, 4 h; (iv) dodecanamide, Pd(OAc)₂, Xantphos, K₂CO₃, dioxane, 80 °C, overnight.

NaPy 1 was prepared according to Scheme 3.1. First pyridine-2,6-diamine 5 was reacted with malic acid in concentrated sulphuric acid to yield 6. Subsequent acylation with dodecanoyl chloride results in the formation of 7, then chlorination using POCl₃ afforded compound 8. Finally, NaPy 1 was
obtained via the Pd-catalyzed amidation of 8 using Pd(OAc)$_2$, Xantphos and K$_2$CO$_3$. The crude product was purified by recrystallization in ethanol and column chromatography. The formation of the product was confirmed by $^1$H-NMR (Figure 3.4).

![1H-NMR spectrum of NaPy 1 in CDCl$_3$.](image)

**Figure 3.4: $^1$H-NMR spectrum of NaPy 1 in CDCl$_3$.**

### 3.2.2 Synthesis of UPy functionalized 2,4-pentanedione

**Scheme 3.2: Synthetic route towards UPy functionalized 2,4-pentanedione 2.** (i) 1,1'-carbonyldiimidazole (CDI), CHCl$_3$, room temperature, overnight; (ii) 8-amino-octanoic acid, TEA, CHCl$_3$, overnight; (iii) Benzyl bromide, NaH, room temperature, overnight; (iv) CBr$_4$, PPh$_3$, CH$_2$Cl$_2$, room temperature, overnight; (v) 2,4-pentanedione, NaH, n-BuLi, THF, room temperature, overnight; (vi) Pd/C, H$_2$, EtOAc, room temperature, 18 h; (vii) Novozym 435, toluene, using a rotovap (65 °C, 250 mbar), 8 hours.
In order to obtain a UPy functionalized 2,4-pentanedione with a high solubility in chloroform, target compound 2 has been prepared (Scheme 3.2). UPy functionalized 2,4-pentanedione compounds with respectively a methyl or tridecyl group on the 4-position turned out to be insoluble in chloroform.

Isocytosine 9 was CDI activated and coupled to 8-aminoctanoic acid via standard synthetic procedure to obtain carboxylic acid functionalized UPy 11.\(^5\) Alcohol functionalized 2,4-pentanedione 16 was prepared via William ether synthesis of butane-1,4-diol 12 with benzyl bromide to afford monofunctional protected compound 13. Bromination of the alcohol yielded compound 14. Subsequent, coupling of 2,4-pentanedione and 4 using NaH and BuLi afforded 15.\(^6,7\) Deprotection of 15 using Pd/C under H\(_2\)-atmosphere yielded 16. Target compound 2 was afforded by the esterification of carboxylic acid functionalized UPy 11 and alcohol functionalized 2,4-pentanedione 16 using Novozym 435. The formation of the product was confirmed by \(^1\)H-NMR (Figure 3.5).

![Figure 3.5: \(^1\)H-NMR spectrum of UPy functionalized 2,4-pentanedione 2 in CDCl\(_3\).](image)

**3.2.3 Synthesis of UPy functionalized nitrostyrene**

![Scheme 3.3: Synthetic route towards UPy functionalized nitrostyrene 3. (i) guanidine carbonate, ethanol, reflux, 18 h; (ii) 1-isocyanatobutane, DMF, 70 °C, 6 h; (iii) KOH, H\(_2\)O, 80 °C, overnight; (iv) (E)-4-(2-nitrovinyl)phenol, EDC, DMAP, DMF, room temperature, overnight.](image)
UPy functionalized nitrostyrene 3 was prepared according to Scheme 3.3. First diethyl 2-acetylpentanedioate 17 was condensated with guanidine carbonate to yield cytosine 18. Subsequent acylation of the amine using 1-isocyanatobutane affords UPy 19, then saponification in an aqueous solution of KOH yielded UPy 20. UPy functionalized nitrostyrene 3 was obtained by coupling UPy 20 to (E)-4-(2-nitrovinyl)phenol using EDC and DMAP.

\[ \text{Scheme 3.4: Synthetic route towards UPy functionalized nitrostyrene 4. (i) (E)-4-(2-nitrovinyl)phenol, dibutyltin dilaurate, CHCl}_3, 40 \degree \text{C, overnight.}} \]

UPy functionalized nitrostyrene 4 was afforded by coupling UPy synthon 21 to (E)-4-(2-nitrovinyl)phenol using dibutyltin dilaurate (Scheme 3.4).

### 3.2.4 Characterization of the coupling efficiency of the UPy functionalized Michael substrates

In order to determine the optimal concentration of the self-accelerating reaction, the effective molarity of the corresponding bifunctional UPy needs to be determined. In an attempt to form bifunctional UPys 22 and 23, UPy functionalized 2,4-pentanedione 2 was coupled to respectively UPy functionalized nitrostyrene 3 and 4 using NaPy and K$_2$CO$_3$ (Scheme 3.5). A conversion of 4% was obtained after 7 days for the formation of bifunctional UPy 22, while the formation of bifunctional UPy 23 didn’t take place at all.

\[ \text{Scheme 3.5: The formation of bifunctional UPys 22 and 23 by the NaPy/K}_2\text{CO}_3 \text{ catalyzed Michael addition in CDCl}_3.} \]
In order to find out what causes the low reactivity, the NaPy/K$_2$CO$_3$ catalyzed Michael addition was performed with unfunctionalized Michael substrates 24 and 25 (Figure 3.6). The reaction took place with high rates in the presence of NaPy (0.5 eq.), as well as in the presence of NaPy (2.5 eq.) and UPy 18 (2.0 eq.). This indicates that the problems during the Michael addition between the UPy functionalized Michael substrates are not caused by the presence of the UPy.

![Figure 3.6: Top: Michael addition between nitrostyrene 24 (40 mM) and 2,4-pentanediione 25 (40 mM) in the presence of NaPy 1 (0.5 eq.) and K$_2$CO$_3$ (0.1 eq.). Bottom: Michael addition between nitrostyrene 24 (40 mM) and 2,4-pentanediione 25 (40 mM) in the presence of NaPy 1 (2.5 eq.), UPy 18 (2.0 eq.) and K$_2$CO$_3$ (0.1 eq.).](image)

The Michael addition only proceeds with low rates when the substrates are directly coupled to the UPy motif, which suggests that the low reactivity of the UPy functionalized substrates are possibly a result of the presence of functional groups next to the Michael substrates. In order to investigate this, we coupled the UPy functionalized substrates to the corresponding unfunctionalized substrates.

The coupling of UPy functionalized 2,4-pentanediione 2 with unfunctionalized nitrostyrene 24 results in 62% conversion after 21 hours (Figure 3.7), which is slightly lower compared to unfunctionalized 2,4-pentanediione 25. Nevertheless, the reaction still takes place with an acceptable rate.

![Figure 3.7: Michael addition between UPy functionalized 2,4-pentanediione 2 (40 mM) and nitrostyrene 24 (40 mM) in the presence of NaPy 1 (2.5 eq.), UPy 18 (1.0 eq.) and K$_2$CO$_3$ (0.1 eq.).](image)

The coupling of UPy functionalized nitrostyrene 3 with unfunctionalized 2,4-pentanediione 25 results in 7% conversion after 21 hours and the coupling of UPy functionalized nitrostyrene 4 with unfunctionalized 2,4-pentanediione 25 doesn’t take place at all (Figure 3.8). Thus, the problems during the formation of the bifunctional UPys seems to be caused by the low reactivity of UPy functionalized nitrostyrene 3 and 4 due to electronic effects.
**Figure 3.8:** Top: Michael addition between UPy functionalized nitrostyrene 3 (40 mM) and 2,4-pentanedione 25 (40 mM) in the presence of NaPy 1 (2.5 eq.), UPy 18 (1.0 eq.) and K$_2$CO$_3$ (0.1 eq.).

Bottom: The Michael addition between UPy functionalized nitrostyrene 4 (40 mM) and 2,4-pentanedione 25 (40 mM) in the presence of NaPy 1 (2.5 eq.), UPy 18 (1.0 eq.) and K$_2$CO$_3$ (0.1 eq.).

A resonance structure for the unfunctionalized nitrostyrene can be drawn in which the β-carbon has a positive charge (Figure 3.9a). For this reason, the deprotonated form of 2,4-pentanedione will attack the nitrostyrene on the β-carbon, resulting in the formation of the Michael adduct.

The functionalized nitrostyrenes have respectively a urethane and an ester with their oxygen connected to the aromatic ring. A resonance structure can be drawn in which the β-carbon is slightly nucleophilic, which makes the attack of the deprotonated form of 2,4-pentanedione on the β-carbon unfavourable (Figure 3.9b). Likely, these mesomeric effects resulted in a low reactivity of the functionalized nitrostyrene compared to the unfunctionalized nitrostyrene.

**Figure 3.9:** a) Resonance structure of the unfunctionalized nitrostyrene motif. b) Resonance structure of the ester functionalized nitrostyrene motif.

### 3.3 Synthesis of UPy functionalized substrates with improved reactivity

In order to obtain more reactive substrates, UPy functionalized 2,4-pentanedione 30 and UPy functionalized nitrostyrene 31 were synthesized (Figure 3.10). The presence of the ester with its
carbonyl directly connected to the aromatic ring should lead to activation of the nitrostyrene. As the nitrostyrene is coupled to the UPy at the 1-position, a UPy functionalized 2,4-pentanedione with the 2,4-pentanedione on the 4-position was made.

Figure 3.10: Target compounds: UPy functionalized 2,4-pentanedione 30 and UPy functionalized nitrostyrene 31.

3.3.1 Synthesis of UPy functionalized substrates

Scheme 3.6: Synthetic route towards UPy functionalized 2,4-pentanedione 30. (i) EDC, DMAP, DMF, room temperature, overnight.

UPy functionalized 2,4-pentanedione 30 was prepared according to Scheme 3.6. The product was obtained by the coupling of 16 and 20 using EDC and DMAP. The formation of the product was confirmed by 1H-NMR (Figure 3.11).

Figure 3.11: 1H-NMR spectrum of UPy functionalized 2,4-pentanedione 30 in CDCl3.
Scheme 3.7: Synthetic route towards UPy functionalized nitrostyrene 31. (i) 4-vinylbenzoic acid, EDC, DMAP, DMF, 50 °C, overnight; (ii) AgNO₃, Ag₂CO₃, TEMPO, CHCl₃, 60 °C, 36 hours.

UPy functionalized 2,4-pentanediol 31 was prepared according to Scheme 3.7. First UPy functionalized alcohol 32 was coupled to (E)-4-(2-nitrovinyl)phenol using EDC and DMAP. The reaction was performed multiple times, none of them reaching full conversion. Therefore, the product was isolated from the starting compounds by column chromatography. Subsequently, the introduction of the nitrostyrene moiety was attempted by stereoselective nitration of 33 with AgNO₃ and TEMPO following a literature procedure.⁸

First a nitro radical is generated from AgNO₃ (Figure 3.12). A radical will then be generated on the most substituted carbon, followed by interception of the radical by TEMPO. Anti-elimination leads to the formation of the trans-β-nitrostyrene motif selectively. Subsequently, excess AgNO₃ oxidizes TEMPOH back to TEMPO. AgNO₃ has thus a dual role; it functions as a source of nitro radicals and as oxidizing agent.

Attempts to repeat the literature procedure failed. Following the progress of the reaction by ¹H-NMR showed that the product was formed and proceeds well till roughly 80 % conversion. However, the product precipitates when the reaction continues. It was not possible to obtain the product by stopping the reaction before it reaches full conversion, since the separation of the product from the starting material by column chromatography was not successful.

Besides the observed formation of the nitrostyrene motif, nitration possibly also occurred on the more sterically hindered alkylidene of the pyrimidinone ring. Since AgNO₃ has a dual role as source of nitro radicals and as oxidizing agent, the nitration was performed in the presence of an excess of AgNO₃. In order to prevent any unwanted nitration by the excess AgNO₃, it can be replaced by another source of oxidizing agent such as Ag₂CO₃. Performing the reaction in the presence of 1 eq. of AgNO₃ as source of nitro radicals and 0.5 eq. of Ag₂CO₃ as oxidizing agent, leads to the formation of the UPy functionalized nitrostyrene 31 with 72 % yield. The stereoselective formation of UPy functionalized nitrostyrene 31 was confirmed by ¹H-NMR (Figure 3.13).⁹
3.3.2 Formation of bifunctional UPy

The UPy functionalized nitrostyrene $31$ was coupled to unfunctionalized 2,4-pentanedione $25$ in order to have an indication of its reactivity (Figure 3.14). A conversion of 100 % after 21 hours was obtained compared to respectively a conversion of only 7 % and 0 % using UPy functionalized nitrostyrene $3$ and $4$ respectively. Thus, UPy functionalized nitrostyrene $31$ is much more reactive than previously synthesized UPy functionalized nitrostyrene $3$ and $4$.

Now that reactive UPy functionalized Michael substrates have been obtained, we set out to couple $30$ and $31$ in order to measure the effective molarity of corresponding bifunctional UPy $35$ (Figure 3.15). The formation of product was confirmed by $^1$H-NMR and MALDI-ToF MS. However, the occurrence of side reactions was observed. Also UPy functionalized 2,4-pentanedione $30$ is not fully converted during the reaction. The conversion of the reaction is related to the amount of $K_2CO_3$, since higher conversions are obtained upon decreasing the amount of $K_2CO_3$. This is possibly a consequence of the occurrence of side-reactions catalyzed by $K_2CO_3$. Also the reaction mixture became cloudy during the reaction. These observations might be explained by polymerization of nitrostyrene, which has previously been reported.  

---

Figure 3.13: $^1$H-NMR spectrum of UPy functionalized nitrostyrene $31$ in CDCl$_3$.

Figure 3.14: The Michael addition between UPy functionalized nitrostyrene $31$ (40 mM) and 2,4-pentanedione $25$ (40 mM) in the presence of NaPy $1$ (2.5 eq.), UPy $18$ (1.0 eq.) and $K_2CO_3$ (0.1 eq.).
Figure 3.15: Formation of bifunctional UPy 35 by the NaPy/K$_2$CO$_3$ catalyzed Michael addition.

In addition to the mentioned side reactions, ester peak b partly disappeared and a new peak appeared slightly upfield (3.9 ppm) (Figure 3.16). In order to find the origin of the unidentified peak, reference reactions have been performed. The coupling of reference compounds 40 and 36 resulted in the partial disappearance of the ester peak and the appearance of the unidentified peak at 3.9 ppm (Reaction 2). When nitrostyrene reference compound 40 is coupled to the unfunctionalized 2,4-pentanedione 25, the appearance of the new peak is not observed (Reaction 3).

Figure 3.16: The coupling of various Michael substrates (20 mM) in the presence, 0.5 eq. K$_2$CO$_3$ and 2.5 eq. NaPy (reaction 1) or 0.5 eq. NaPy (reaction 2-3) in CDCl$_3$ and the corresponding $^1$H-NMR spectra of the crude reaction mixture at full conversion.
These results could be interpreted as the presence of two conformations or the occurrence of side reactions. However, the presence of two conformations for the product of reaction 2 is unlikely. This was also indicated by the absence of changes in the $^1$H-NMR spectrum upon increasing the temperature from room temperature to 50 °C. Therefore, the occurrence of side-reactions on the ester of the UPy functionalized 2,4-pentanedione 30 is the most rational explanation. In order to investigate this theory, the product has been analysed by $^1$H-$^{13}$C HSQC NMR (Figure 3.17). The unidentified peak has the same chemical shift in the $^{13}$C-NMR-spectrum as the peaks corresponding to the esters in the $^1$H-NMR spectrum. This indicates that the unidentified peak at 3.9 ppm in the $^1$H-NMR spectrum likely corresponds to an ester. This might suggest the occurrence of transesterification during the NaPy/K$_2$CO$_3$ catalyzed Michael addition.

![Figure 3.17: $^1$H-$^{13}$C HSQC NMR spectrum of bifunctional UPy 35.](image)

### 3.4 Prevention of possible transesterification reactions

![Scheme 3.8: Synthetic route towards UPy functionalized 2,4-pentanedione 37. (i) dibutyltin dilaurate, CHCl$_3$, RT.](image)

In order to prevent the possible occurrence of transesterification and gain more insight in the origin of the peak at 3.9 ppm in the $^1$H-NMR spectrum, a UPy functionalized 2,4-pentanedione was synthesized where the ester was replaced for a urethane (Scheme 3.8). UPy functionalized 2,4-pentanedione 37 was prepared by the coupling of 21 and 16 using dibutyltin dilaurate. The formation of the product was confirmed by $^1$H-NMR (Figure 3.18).
UPy functionalized 2,4-pentanedione 31 and UPy functionalized 2,4-pentanedione 37 were coupled to form the corresponding bifunctional UPy (Figure 3.19). Similar to the formation of bifunctional UPy 35, the peak at 3.9 ppm in the $^1$H-NMR spectrum was also here observed. In order to find the origin of this peak, various reference reactions have been performed.

The appearance of the unidentified peak was only observed when the urethane was covalently attached to the 2,4-pentanedione motif. $^1$H-$^{13}$C HSQC NMR analysis of the product of reaction 2, show that the peaks at 4.1 ppm and 3.9 ppm have the same chemical shift in the $^{13}$C spectrum, suggesting that both belong to proton g (Figure 3.20). The absence of the peak in the product of reaction 4 indicates that it is not a result of side-reactions on the urethane. The appearance of the peak was also observed using an unfunctionalized nitrostyrene in reaction 5. Upon increasing the temperature from room temperature to 50 °C, no changes are observed in the $^1$H-NMR spectrum of the product of reaction 2. Thus, the unidentified peak is likely not a result of a different conformation. These results suggest that the two peaks at 4.1 ppm and 3.9 ppm originate from the stereocenters. Likely, the two peaks corresponds to the two diastereomeric pairs (RS, SR and SS, RR) in the Michael adduct. This was further supported by the fact that the purified product of reaction 2 gave identical integrals for both peaks.

**Figure 3.18:** $^1$H-NMR spectrum of UPy functionalized 2,4-pentanedione 37 in CDCl$_3$. 
Figure 3.19: The coupling of various Michael substrates (20 mM) in the presence, 0.5 eq. K₂CO₃ and 2.5 eq. NaPy (reaction 1) or 0.5 eq. NaPy (reaction 2-5) in CDCl₃ and the corresponding \(^1\)H-NMR spectra of the crude reaction mixture at full conversion.
Subsequently, UPy functionalized substrates 31 and 37 were coupled to form corresponding bifunctional UPy 39. In contrast to the formation of ester functionalized bifunctional UPy 35, the reaction mixture doesn’t become cloudy. Though, full conversion was not obtained. Purification by column chromatography allowed us to remove NaPy and starting materials (Figure 3.21). Although all the peaks in the \(^1\)H-NMR spectrum can be assigned, the integrals of the Michael Adduct (i.e. protons h, i and j) are roughly 30% too low. This is very similar as was observed for the coupling of the reference compounds (reaction 2, Figure 3.19). Since the Michael addition was performed in CDCl\(_3\), the slightly acidic protons of the Michael adduct could be deuterated, causing the low integrals. However, refluxing the product in CHCl\(_3\) didn’t result in recovery of the signals. It is therefore likely that an unknown side-product with a very similar structure is formed, which is further supported by MALDI-ToF MS.
3.5 Testing the self-accelerating reaction

Although the synthesis of the bifunctional UPy results in the formation of considerable amounts of side-product, we wished to perform a test of the self-accelerating reaction. The effective molarity of the bifunctional UPy was estimated on 10 mM based on the linker length between the UPy motifs. This means that the optimal concentration to perform the reaction would be roughly 0.5 mM (Figure 3.1b). A coupling of the reference compounds at a NaPy concentration of respectively 5 mM and 0.5 mM was performed in order to estimate the time required to reach full conversion. Using 2 eq. of NaPy and 6 eq. of K₂CO₃ compared to the reference compounds, it took two days to reach 90% conversion for the 5 mM reaction, while it took approximately eight days to reach a similar conversion at 0.5 mM. Similar side-products were observed as before.

These results show that the reaction can be performed on practical timescales at the required concentrations. Future work should therefore be focussed on identifying the side-products and optimizing the reaction conditions. Of especial interest is the concentration of K₂CO₃; when a large excess is used the reaction proceeds at a fast rate, however the occurrence of side-reactions becomes more prominent. Reducing the K₂CO₃ concentration results in less side product formation. However, the reaction becomes impractically slow. Furthermore, we have shown that at least 1 equivalent of K₂CO₃ is necessary for the self-accelerating reaction, since K₂CO₃ otherwise becomes rate limiting and a self-accelerating effect won’t be obtained (Figure 2.11a).
3.6 Conclusions

We synthesized NaPy, UPy functionalized 2,4-pentanediol and UPy functionalized nitrostyrenes as starting compounds for the development of a self-accelerating reaction. The coupling of the UPy functionalized substrates using NaPy and K$_2$CO$_3$ results in very slow product formation. Reference reactions indicate that the problems during the formation of the bifunctional UPy are caused by the low reactivity of the UPy functionalized nitrostyrene compounds, due to the presence of functional groups next to the nitrostyrene motif it is deactivated by mesomeric effects. A new UPy functionalized nitrostyrene have been synthesized with an activating functional group next to the nitrostyrene motif. Reference reactions show that this resulted in a strong increase of the reactivity compared to the previously synthesized UPy functionalized nitrostyrenes.

The coupling of UPy functionalized substrates took place with high rates, however side-reactions occur during the Michael addition. The reaction mixtures became cloudy and UPy functionalized 2,4-pentanediol doesn’t get fully converted. These phenomena are likely a result of the polymerization of UPy functionalized nitrostyrene, which has previously been reported. Furthermore, the ester peak of the UPy functionalized 2,4-pentanediol partly disappeared during the reaction, while a new peak appeared slightly upfield. $^1$H-$^1$C HSQC NMR indicates that the unidentified peak corresponds to an ester. The absence of changes in the $^1$H-NMR spectrum upon increasing the temperature suggests that the unidentified peak is not a result of another conformation. These results suggest the possible occurrence of transesterification.

A UPy functionalized 2,4-pentanediol have been synthesized which contains a urethane instead of an ester. The corresponding bifunctional UPy was formed using NaPy and K$_2$CO$_3$. The results of the coupling of various reference compounds suggest that the unidentified peak in the $^1$H-NMR spectrum is a result of the presence of the stereocenter of the Michael adduct. All the peaks in the $^1$H-NMR spectrum of the bifunctional UPy can be assigned. However, the integrals corresponding to the motif of the Michael adduct are too low. This is observed for both the coupling of the reference compounds, as well as for the UPy functionalized substrates. Further work should therefore be focussed on identifying these side-products and optimizing the reaction conditions.
3.7 Experimental section

Materials

All chemicals were purchased from Sigma Aldrich and used as received, unless stated otherwise. All solvents were obtained from BioSolve. Deuterated solvents were purchased from Cambridge Isotope Laboratories. Triethylamine was dried and stored over KOH pellets.

Methods

$^1$H-NMR spectra were recorded on a Varian Mercury 400 MHz. Proton chemical shifts are reported in ppm downfield from TMS and carbon chemical shifts in ppm downfield of TMS using the resonance of the deuterated solvent as internal standard. The used abbreviations are s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, bs: broad singlet. MALDI-ToF MS spectra were obtained using a Perspective Biosystem Voyager-DE PRO spectrometer. Elemental analysis was performed on a Perkin-Elmer 2400 series II CHNS/O analyser.

7-Amino-1,8-naphthyridin-2-ol (6)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} & \quad \text{H}_2\text{N} \\
\text{HO} & \quad \text{OH} & \quad \text{HO} \\
\text{H}_2\text{N} & \quad \text{N} & \quad \text{OH}
\end{align*}
\]

Malic acid (9.6 g, 70.4 mmol) and pyridine-2,6-diamine (7.0 g, 64.0 mmol) were ground to a fine powder and cooled in an ice bath. Subsequently, concentrated sulfuric acid (32 mL) was dropwise added. The solution was heated to 110 °C for 3 hours, poured over ice and made alkaline with concentrated ammonium hydroxide until a pH of 8 was reached. Subsequently, the precipitate was filtrated and washed with H$_2$O. Removal of the solvent in vacuo yielded the product (11.9 g, quantitative). $^1$H-NMR (DMSO-d$_6$): $\delta = 10.60$ (bs, 1H, OH), 7.61 (d, 2H, Ar), 6.80 (s, 2H, NH$_2$), 6.33 (d, 1H, Ar), 6.10 (d, 1H, Ar). $^{13}$C-NMR (DMSO-d$_6$): $\delta = 168.69, 165.58, 155.61, 144.70, 142.34, 120.13, 110.11, 110.09$.

N-(7-Oxo-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide (7)

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{N} & \quad \text{OH} \\
& \quad \text{C}_{12}\text{H}_{22}\text{O} & \quad \text{CO}
\end{align*}
\]

A mixture of 2-amino-7-hydroxy-1,8-naphthyridine (14.0 g, 87.0 mmol) and dodecanoyl chloride (34.4, 147.9 mmol) in dry pyridine (36 mL) was stirred overnight at 110 °C under argon atmosphere and subsequently allowed to cool to room temperature. The precipitate was filtered off and recrystallized from toluene. The product was obtained as a brown solid (18.2 g, 61 %). $^1$H-NMR (CDCl$_3$): $\delta = 12.87$ (bs, 1H, NH), 11.84 (bs, 1H, NH), 8.43 (d, 1H, Ar), 7.99 (d, 1H, Ar), 7.72 (d, 1H, Ar), 6.63 (d, 1H, Ar), 2.67 (t, 2H, O=C-CH$_2$), 1.76 (m, 2H, O=C-CH$_2$-CH$_2$), 1.25 (m, 16H, alkyl-CH$_2$), 0.87 (t, 3H, alkyl-CH$_3$). $^{13}$C-NMR (CDCl$_3$): $\delta = 174.52, 165.27, 154.29, 148.62, 139.79, 139.05, 119.79, 111.17, 110.75, 37.10, 31.92, 29.66, 29.25, 25.38, 22.69, 14.13.$
N-(7-Chloro-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide (8)

A mixture of N-(7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide 7 (18.2 g, 50.1 mmol) in POCl₃ (150 mL) was stirred at 95 °C for 4 hours under argon atmosphere and subsequently allowed to cool to room temperature. The remaining solution was slowly poured into vigorously stirred iced water (2 L), followed by neutralization with concentrated aqueous NH₃ solution to pH = 8. The product was obtained as a white powder by filtration of the precipitate (17.2 g, 89 %).

1H-NMR (CDCl₃): δ = 8.58 (d, 1H, Ar), 8.32 (bs, 1H, NH), 8.18 (d, 1H, Ar), 8.09 (d, 1H, Ar), 7.41 (d, 1H, Ar), 2.47 (t, 2H, O=C-CH₂), 1.76 (m, 2H, O=C-CH₂-CH₂), 1.26 (m, 16H, alkyl-CH₂), 0.88 (t, 3H, alkyl-CH₃).

13C-NMR (CDCl₃): δ = 174.42, 154.12, 139.14, 138.74, 122.05, 115.18, 76.67, 38.07, 31.89, 29.58, 29.42, 29.31, 29.11, 25.21, 22.67, 14.11. MALDI-ToF MS: calculated 361.91, observed m/z 362.25 [M+H⁺].

N,N'-(1,8-Naphthyridine-2,7-diyl)didodecanamide (1)

A flask was charged with N-(7-chloro-7,8-dihydro-1,8-naphthyridin-2-yl)dodecanamide 8 (15.6 g, 42.8 mmol), dodecanamide (10.3 g, 51.3 mmol), Pd(OAc)₂ (593 mg, 2.6 mmol), Xantphos (3.3 g, 5.8 mmol), K₂CO₃ (5.9 g, 42.8 mmol) and dry dioxane (250 mL). Molecular sieves were added and the mixture was stirred overnight at room temperature under argon atmosphere. Subsequently, the mixture was heated to 80 °C and stirred overnight under argon atmosphere. After cooling to room temperature, the mixture was filtered and the residue was rinsed with CHCl₃. Recrystallization in ethanol and purification by flash chromatography (80 % CHCl₃ / 20 % EtOAc) yielded the product as a white solid (16.5 g, 73 %).

1H-NMR (CDCl₃): δ = 8.42 (d, 2H, Ar), 8.14 (d, 2H, Ar), 8.12 (bs, 2H, NH), 2.47 (t, 4H, O=C-CH₂), 1.56 (m, 4H, O=C-CH₂-CH₂), 1.26 (m, 32H, alkyl-CH₂), 0.88 (t, 6H, alkyl-CH₃).

13C-NMR (CDCl₃): δ = 172.2, 153.74, 138.98, 113.34, 110.01, 76.75, 38.13, 31.90, 29.60, 29.44, 29.33, 29.15, 25.29, 22.68, 14.12. MALDI-ToF MS: calculated 524.78, observed m/z 525.43 [M+H⁺], 1087.79 [dimer+K⁺]. Elemental analysis: calculated 73.24 %, H 9.99 %, N 10.68 %, O 6.10 %. Found C 73.35 %, H 9.59 %, N 10.26 %.

N-(6-(Heptan-3-yl)-4-oxo-1,4-dihydropyrimidin-2-yl)-1H-imidazole-1-carboxamide (10)

2-Amino-6-(pentan-2-yl)pyrimidin-4(1H)-one 9 (1.0 g, 4.8 mmol) and 1,1'-carbonyldiimidazole (1.3 g, 6.2 mmol) were dissolved in dry CHCl₃ (25 mL) and the mixture was stirred overnight at room temperature under argon atmosphere. After removal of the solvent in vacuo, the remaining solids were transferred to a separation funnel with CHCl₃ (80 mL) and extracted with H₂O (3x 40 mL) and brine (2x 40 mL). The organic layer was dried over MgSO₄. Removal of the solvent in vacuo yielded the product as a white solid (1.4 g, 96 %).

1H-NMR (CDCl₃): δ = 8.76 (s, 1H, N=CH-N), 7.63 (s, 1H, O=C(N-CH=CH), 7.07 (s, 1H, (O=C)N-CH=CH), 5.82 (s, 1H, O=C-CH=C), 2.51 (m, 1H, CH₂-CH=CH₂), 1.69 (m, 4H, alkyl-CH₂), 1.31 (m, 4H, alkyl-CH₂), 0.96 (t, 3H, CH₃), 0.89 (t, 3H, CH₃).
8-(3-(6-(Heptan-3-yl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)octanoic acid (11)

\[
\begin{align*}
\text{N-(4-Oxo-6-(pentan-2-yl)-1,4-dihydropyrimidin-2-yl)-1H-imidazole-1-carboxamide (10) (104 mg, 0.34 mmol) and 8-aminooctanoic acid (66.4 mg, 0.42 mmol) were dissolved in dry CHCl}_3 (10 mL) and triethylamine (71.8 μl, 0.51 mmol) was added. The reaction mixture was stirred overnight at 50 °C under argon atmosphere. After removal of the solvent and triethylamine \textit{in vacuo}, the resulting solid was transferred to a separation funnel with CHCl}_3 (10 mL) and extracted with 1M HCl (4 mL) and saturated aqueous NaHCO}_3 (4 mL). The organic layer was dried over MgSO}_4 and the solvent was \textit{removed in vacuo}. Purification by column chromatography (93 % CHCl}_3 / 7 % methanol) yielded the product as a white solid (108 mg, 80 %).

\text{1H-NMR (CDCl}_3): \delta = 13.20 (bs, 1H, NH), 11.85 (bs, 1H, NH), 10.02 (bs, 1H, NH), 5.87 (s, 1H, O=C-CH=CH), 3.27 (q, 2H, NH-(C=O)-NH-CH}_2), 2.32 (t, 2H, CH}_2-COOH), 1.61 (m, 6H, alkyl-CH}_2), 1.36 (m, 12H, alkyl-CH}_2), 0.87 (t, 6H, CH}_3).

\text{13C-NMR (CDCl}_3): \delta = 173.40, 156.68, 155.65, 154.85, 106.08, 45.30, 39.47, 33.88, 32.80, 29.28, 28.87, 28.25, 28.15, 26.53, 25.85, 24.24, 22.46, 13.86, 11.67.

MALDI-ToF MS: calculated 394.51, observed m/z 395.32 [M+H]^+.

4-(Benzyloxy)butan-1-ol (13)

\[
\begin{align*}
\text{Dry DMF (400 mL) was added to NaH (60 % dispersion in oil) (2.0 g, 48.8 mmol) and cooled to 0 °C. Butane-1,4-diol (4.0 g, 44.1 mmol) in dry DMF (20 mL) was slowly added and the reaction mixture was stirred for 10 minutes under argon atmosphere. Benzyl bromide (5.3 mL, 44.4 mmol) was cautiously added to the mixture. The mixture was allowed to acclimate to room temperature and stirred for 18 hours. Subsequently, the reaction was quenched by the addition of H}_2O (90 mL) and extracted with EtOAc (5x 100 mL). The organic layer was dried over MgSO}_4. Removal of the solvent \textit{in vacuo} yielded the product as a colorless oil (6.9 g, 88 %). \text{1H-NMR (CDCl}_3): \delta = 7.32 (m, 5H, Ar), 4.50 (s, 2H, Ar-CH}_2-O), 3.60 (t, 2H, CH}_2-CH}_2-OH), 3.50 (t, 2H, O-CH}_2-CH}_2), 1.66 (m, 4H, alkyl-CH}_2).

\text{13C-NMR (CDCl}_3): \delta = 138.14, 128.40, 127.70, 127.64, 73.03, 70.32, 62.61, 30.07, 26.64.

((4-Bromobutoxy)methyl)benzene (14)

\[
\begin{align*}
\text{CBr}_4 (3.8 g, 11.3 mmol) was added to a solution of 4-(benzyloxy)butan-1-ol (1.7 g, 9.4 mmol) in dry CH}_2Cl}_2 (30 mL) and cooled to 0 °C. Subsequently, PPh}_3 (4.9 g, 18.8 mmol) was added in portions. The mixture was stirred overnight at room temperature under argon atmosphere and dried \textit{in vacuo}. The remaining solids were washed with ether (5x 20 mL) and filtered, followed by concentration of the collective ether extracts \textit{in vacuo}. Purification using column chromatography (96 % heptane / 4 % EtOAc) yielded the product (2.0 g, 87 %). \text{1H-NMR (CDCl}_3): \delta = 7.33 (m, 5H, Ar), 4.50 (s, 2H, Ar-CH}_2-
O), 3.50 (t, 2H, O-CH<sub>2</sub>CH<sub>2</sub>), 3.43 (t, 2H, CH<sub>2</sub>-CH<sub>2</sub>-Br), 1.98 (m, 2H, alkyl-CH<sub>2</sub>), 1.76 (m, 2H, alkyl-CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ = 138.68, 128.62, 127.83, 127.81, 73.15, 69.47, 33.99, 29.96, 28.60.

9-(Benzyl oxy)nonane-2,4-dione (15)

![Chemical Structure](image)

2,4-Pentanedione was slowly added to a suspension of NaH (60 % dispersion in oil) (522 mg, 13.1 mmol) in THF (25 mL) and cooled to 0 °C. The mixture was stirred for 10 minutes under argon atmosphere. n-BuLi (1.6 M in hexanes) (6.6 mL) was dropwise added over a period of 15 minutes and the mixture was stirred for 20 minutes at 0 °C. ((4-Bromobutoxy)methyl)benzene 14 (2.8 g, 11.5 mmol) in THF (1.5 mL) was dropwise added and the mixture was stirred for 2 hours at room temperature. The reaction was quenched with a mixture of concentrated HCl (2 mL) in H<sub>2</sub>O (2.5 mL) and Et<sub>2</sub>O (10 mL). Subsequently, the organic layer was washed with brine (3x 5 mL), dried over MgSO<sub>4</sub> and the solvent was removed in vacuo. Purification using column chromatography (93 % heptane / 7 % EtOAc) yielded the product (2.1 g, 78 %).

1H-NMR (CDCl<sub>3</sub>): δ = 7.32 (m, 5H, Ar), 5.47 (s, 1H, ((C=O)-CH=COH), 4.48 (s, 2H, Ar-CH<sub>2</sub>), 3.53 (s, 2H, (C=O)-CH<sub>2</sub>-(C=O)), 3.46 (t, 2H, O-CH<sub>2</sub>-CH<sub>2</sub>-OH), 2.26 (t, 2H, CH<sub>2</sub>-CH<sub>2</sub>-C=O), 2.20 (s, 3H, CH<sub>2</sub>-(C=O)-CH<sub>3</sub>), 2.03 (s, 3H, CH=COH-CH<sub>3</sub>), 1.62 (m, 4H, alkyl-CH<sub>2</sub>), 1.40 (m, 2H, alkyl-CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ = 204.06, 202.11, 194.02, 191.42, 138.56, 128.33, 127.60, 127.49, 127.48, 99.77, 72.89, 70.11, 70.05, 43.70, 38.15, 31.87, 30.88, 29.69, 29.47, 25.86, 25.65, 25.49, 24.97, 24.96, 23.15, 22.68, 14.11. MALDI-ToF MS: calculated 262.34, observed 262.26 [M+H<sup>+</sup>], 285.20 [M+Na<sup>+</sup>], 301.16 [M+K<sup>+</sup>].

9-Hydroxynonane-2,4-dione (16)

A solution of 9-(benzyl oxy)nonane-2,4-dione 15 (2.1 g, 8.1 mmol) in EtOAc (30 mL) was bubbled through with N<sub>2</sub> for 10 minutes. A spatula tip Pd/C was added to the mixture and the reaction vessel was placed under H<sub>2</sub>-atmosphere in a Parr-reactor for 18 hours at 50 psi. Filtration over Celite and subsequent removal of the solvent in vacuo, yielded the product (1.4 g, 99 %). 1H-NMR (CDCl<sub>3</sub>): δ = 5.49 (s, 1H, ((C=O)-CH=COH), 3.66 (t, 2H, CH<sub>2</sub>-CH<sub>2</sub>-OH), 3.57 (s, 2H, (C=O)-CH<sub>2</sub>-(C=O)), 2.29 (t, 2H, CH<sub>2</sub>-CH<sub>2</sub>-C=O), 2.24 (s, 3H, CH<sub>2</sub>-(C=O)-CH<sub>3</sub>), 2.05 (s, 3H, CH=COH-CH<sub>3</sub>), 1.59 (m, 4H, alkyl-CH<sub>2</sub>), 1.41 (m, 2H, alkyl-CH<sub>2</sub>). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ = 204.25, 202.35, 194.17, 191.35, 138.56, 128.33, 127.60, 127.49, 127.48, 99.77, 72.89, 70.11, 70.05, 43.70, 38.15, 31.87, 30.88, 29.69, 29.47, 25.86, 25.65, 25.49, 24.97, 24.96, 23.15, 22.68, 14.11. MALDI-ToF MS: calculated 172.11, observed m/z 173.24 [M+H<sup>+</sup>], 195.19 [M+Na<sup>+</sup>].

6,8-Dioxononyl 8-(3-(6-(Heptan-3-yl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)octanoate (2)

![Chemical Structure](image)

8-(3-(6-(Heptan-3-yl)-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)octanoic acid 11 (138 mg, 0.35 mmol) and 9-hydroxynonane-2,4-dione 16 (64.9 mg, 0.38 mmol) were dissolved in toluene (40 mL). Subsequently, Novozym 435 (81 mg) was added to the solution and the mixture was slowly stirred at the rotovap (65 °C, 250 mbar) for 8 hours. The mixture was filtered and dried in vacuo. Purification
by column chromatography (98 % CHCl\textsubscript{3} / 2 % methanol) yielded the product as a yellow oil (146 mg, 76 %). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}): \(\delta = 13.25\) (bs, 1H, NH), 11.91 (bs, 1H, NH), 10.21 (bs, 1H, NH), 5.82 (s, 1H, O=CH=C), 5.49 (s, 1H, ((C=O)-CH=COH), 4.06 (t, 2H, O=C-O-CH\textsubscript{2}), 3.57 (s, 2H, (C=O)-CH\textsubscript{2}-(C=O)), 3.22 (q, 2H, NH-(C=O)-NH-CH\textsubscript{2}), 2.28 (t, 2H, CH\textsubscript{2}-CH\textsubscript{2}-C=O), 2.28 (t, 2H, O-(C=O)-CH\textsubscript{2}), 2.24 (s, 3H, CH\textsubscript{2}-(C=O)-CH\textsubscript{3}), 2.05 (s, 3H, CH=COH-CH\textsubscript{3}), 1.61-1.25 (m, 24H, alkyl-CH\textsubscript{2}), 0.87 (t, 6H, alkyl-CH\textsubscript{3}). \textsuperscript{13}C-NMR (CDCl\textsubscript{3}): \(\delta = 194.10, 193.83, 191.37, 173.86, 156.67, 155.45, 154.87, 106.19, 99.81, 99.77, 67.95, 63.99, 63.95, 62.68, 57.89, 38.05, 34.30, 32.87, 29.30, 29.08, 28.93, 28.39, 26.50, 25.57, 25.24, 24.92, 22.46, 13.87, 11.69. MALDI-ToF MS: calculated 548.71, observed m/z 549.38 [M+H\textsuperscript{+}], 571.35 [M+Na\textsuperscript{+}], 587.33 [M+K\textsuperscript{+}].

Ethyl 3-(2-amino-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (18)

A mixture of diethyl 2-acetylpentanedioate (1.9 mL, 8.7 mmol) and guanidine carbonate (784 mg, 8.7 mmol) in ethanol (20 mL) was refluxed for 18 hours under argon atmosphere and subsequently allowed to cool to room temperature. Precipitation at 0\textdegree C yielded the product as a white solid (690 mg, 35 %). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}): \(\delta = 4.09\) (q, 2H, O-CH\textsubscript{2}-CH\textsubscript{3}), 2.70 (t, 2H, O=C-CH\textsubscript{2}-CH\textsubscript{2}), 2.51 (t, 2H, O=C-CH\textsubscript{2}-CH\textsubscript{2}), 2.21 (s, 3H, C-CH\textsubscript{3}), 1.26 (t, 3H, CH\textsubscript{2}-CH\textsubscript{3}). MALDI-ToF MS: calculated 225.24, observed m/z 226.29 [M+H\textsuperscript{+}], 248.24 [M+Na\textsuperscript{+}].

Ethyl 3-(2-(3-butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (19)

Ethyl 3-(2-amino-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (18) (1.0 g, 4.6 mmol) in dry DMF (25 mL) was heated to 70\textdegree C. 1-Isocyanatobutane (770 \mu L, 6.9 mmol) was added and the mixture was stirred for 6 hours at 70\textdegree C under argon atmosphere. After removal of the solvent and excess 1-isocyanatobutane in vacuo, the product was obtained as a white solid (1.4 g, 95 %). \textsuperscript{1}H-NMR (CDCl\textsubscript{3}): \(\delta = 12.88\) (bs, 1H, NH), 11.89 (bs, 1H, NH), 10.11 (bs, 1H, NH), 4.08 (q, 2H, O-CH\textsubscript{2}-CH\textsubscript{3}), 3.24 (q, 2H, NH-(C=O)-NH-CH\textsubscript{2}), 2.68 (t, 2H, O-(C=O)-CH\textsubscript{2}-CH\textsubscript{2}), 2.60 (t, 2H, O-(C=O)-CH\textsubscript{2}-CH\textsubscript{2}), 2.30 (s, 3H, C-CH\textsubscript{3}), 1.57 (t, 2H, alkyl-CH\textsubscript{2}), 1.41 (t, 2H, CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{3}), 1.24 (t, 3H, O-CH\textsubscript{2}-CH\textsubscript{3}), 0.94 (t, 3H, CH\textsubscript{3}). \textsuperscript{13}C-NMR (CDCl\textsubscript{3}): \(\delta = 173.32, 156.64, 153.31, 143.97, 116.09, 104.99, 60.34, 39.66, 32.12, 31.35, 21.15, 20.12, 17.17, 14.23, 13.75. MALDI-ToF MS: calculated 324.38, observed m/z 325.30 [M+H\textsuperscript{+}], 347.27 [M+Na\textsuperscript{+}].

3-(2-(3-Butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoic acid (20)

KOH (106 mg, 1.63 mmol) was dissolved in H\textsubscript{2}O (20 mL) and ethyl 3-(2-(3-butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (99.7 mg, 0.31 mmol) was added. The reaction mixture was stirred overnight at 80\textdegree C under argon atmosphere. The product was precipitated by adding
concentrated hydrochloric acid and subsequently filtered. The product was obtained as a white solid (505 mg, 88 %). $^1$H-NMR (DMF): $\delta = 3.22$ (q, 2H, NH-(C=O)-NH-CH$_2$), 2.68 (t, 2H, HOOC-CH$_2$), 2.47 (t, 2H, HOOC-CH$_2$-CH$_2$), 2.21 (s, 3H, C-CH$_3$), 1.51 (m, 2H, alkyl-CH$_2$), 1.34 (m, 2H, alkyl-CH$_2$), 0.91 (t, 3H, C-CH$_3$). $^{13}$C-NMR (DMF): $\delta = 174.37$, 156.52, 41.91, 39.88, 39.10, 35.18, 35.01, 32.36, 31.71, 31.57, 30.94, 30.01, 21.28, 19.86, 19.74, 19.58, 13.47, 13.35, 13.27.

$$(E)$-4-(2-Nitrovinyl)phenyl-3-(2-(3-butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (3)

3-(2-(3-Butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoic acid 20 (275 mg, 0.93 mmol) was dissolved in DMF (100 mL) and cooled to 0 °C. DMAP (11.3 mg, 0.09 mmol) and EDC (178 mg, 1.02 mmol) were added and the mixture was stirred at 0 °C for 0.5 hour under argon atmosphere. Subsequently $(E)$-4-(2-nitrovinyl)phenol (153 mg, 0.93 mmol) was added and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the remaining solids were dissolved in CHCl$_3$ (10 mL) and extracted with H$_2$O (3x 10 mL). The organic phase was dried over MgSO$_4$ and the solvent was removed in vacuo. The crude product was purified by column chromatography (95 % CHCl$_3$ / 5 % methanol) and obtained as a yellow solid (110 mg, 27 %). $^1$H-NMR (CDCl$_3$): $\delta = 12.97$ (bs, 1H, NH), 11.90 (bs, 1H, NH), 10.08 (bs, 1H, NH), 8.02 (d, 1H, CH=CH), 7.56 (d, 2H, Ar), 7.53 (d, 1H, CH=CH), 7.18 (d, 1H, Ar), 6.91 (d, 1H, Ar), 3.27 (q, 2H, NH-(C=O)-NH-CH$_2$), 2.93 (t, 2H, O=C-O-CH$_2$-CH$_2$), 2.82 (t, 2H, O=C-O-CH$_2$-CH$_2$), 2.33 (s, 3H, C-CH$_3$), 1.57 (m, 2H, alkyl-CH$_2$), 1.38 (m, 2H, alkyl-CH$_2$), 0.93 (t, 3H, alkyl-CH$_3$). MALDI-ToF MS: calculated 443.45, observed m/z 444.20 [M+H$^+$].

$(E)$-4-(2-Nitrovinyl)phenyl(6-(3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)carbamate (4)

$(E)$-4-(2-Nitrovinyl)phenol (180.6 mg, 1.09 mol) and 1-(6-isocyanatohexyl)-3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)urea 21 (384.7 mg, 1.31 mmol) were dissolved in CHCl$_3$ (10 mL). Two drops of dibutyltin dilaurate were added and the mixture was stirred overnight at 40 °C under argon atmosphere. Subsequently, silica powder was added and the mixture was stirred for 2 hours, filtered and dried in vacuo. The product was obtained as a yellow solid (123 mg, 25 %). $^1$H-NMR (CDCl$_3$): $\delta = 13.16$ (bs, 1H, NH), 11.86 (bs, 1H, NH), 10.12 (bs, 1H, NH), 8.02 (d, 1H, CH=CH), 7.57 (d, 1H, CH=CH), 7.52 (m, 4H, Ar), 5.87 (s, 1H, O=C-CH=), 5.55 (b, 1H, NH), 3.29 (q, 2H, NH-(C=O)-NH-CH$_2$), 2.36 (t, 2H, O-(C=O)-NH-CH$_2$), 2.21 (s, 3H, CH$_3$), 1.65 (m, 4H, alkyl-CH$_2$), 1.12 (m, 4H, alkyl-CH$_2$). MALDI-ToF MS: calculated 458.47, observed m/z 459.23 [M+H$^+$].
6,8-Dioxononyl 3-(2-(3-butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoate (30)

3-(2-(3-Butylureido)-6-methyl-4-oxo-1,4-dihydropyrimidin-5-yl)propanoic acid 20 (516 mg, 1.74 mmol) was dissolved in DMF (12 mL) and cooled to 0 °C. EDC (502 mg, 2.62 mmol) and DMAP (43.3 mg, 0.35 mmol) were added and the mixture was stirred for 0.5 hour at 0 °C under argon atmosphere. The mixture was allowed to cool to room temperature and 9-hydroxydecane-2,4-dione 16 (334 mg, 1.80 mmol) was added. Subsequently, the mixture was stirred overnight at room temperature and dried in vacuo. The remaining solids were dissolved in CHCl₃ (10 mL) and extracted with H₂O (3x 5 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo.

Purification by column chromatography (5 % methanol / 95 % CHCl₃) yielded the product as a white solid (662 mg, 84 %). ¹H-NMR (CDCl₃): δ = 12.89 (bs, 1H, NH), 11.89 (bs, 1H, NH), 10.11 (bs, 1H, NH), 5.47 (s, 1H, ((C=O)-CH=COH), 4.04 (t, 2H, O=C-O-CH₂), 3.56 (s, 2H, (C=O)-CH₂-(C=O)), 3.26 (q, 2H, NH-(C=O)-NH-CH₂), 2.70 (t, 2H, O-(C=O)-CH₂-CH₂), 2.59 (t, 2H, O-(C=O)-CH₂-CH₂), 2.30 (s, 3H, C-CH₃), 2.27 (t, 2H, CH₂-(C=O)-CO), 2.26 (s, 3H, CH₂-(C=O)-CH₂), 2.05 (s, 3H, CH=COH-CH₃), 1.39 (m, 6H, alkyl-CH₂), 1.39 (m, 4H, alkyl-CH₂), 0.94 (t, 3H, alkyl-CH₃). ¹³C-NMR (CDCl₃): δ = 193.86, 191.30, 173.37, 172.29, 156.66, 153.35, 143.99, 116.07, 99.77, 64.20, 64.16, 57.86, 39.64, 38.05, 32.05, 31.72, 31.34, 30.93, 28.40, 28.36, 25.55, 25.34, 25.21, 24.93, 22.87, 21.14, 20.15, 20.11, 17.19, 13.77, 13.71. MALDI-ToF MS: calculated 450.53, observed m/z 451.24 [M+H⁺].

6-(3-(6-Methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl 4-vinylbenzoate (33)

4-Vinylbenzoic acid (557 mg, 3.76 mmol) was dissolved in DMF (20 mL) and cooled to 0 °C. Subsequently, DMAP (106 mg, 0.87 mmol) and EDC (1.4 g, 7.2 mmol) were added and the mixture was stirred at 0 °C for 0.5 hour under argon atmosphere. 1-(6-Hydroxyhexyl)-3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)urea 32 (776 mg, 2.89 mmol) was added and the mixture was stirred overnight at 50 °C. Subsequently, the solvent was removed in vacuo and the remaining solids were dissolved in CHCl₃ (10 mL) and extracted with H₂O (3x 5 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo. Purification by column chromatography (96 % CHCl₃ / 4 % methanol) yielded the product as a white solid (860 mg, 75 %). ¹H-NMR (CDCl₃): δ = 13.10 (bs, 1H, NH), 11.84 (bs, 1H, NH), 10.17 (bs, 1H, NH), 7.97 (d, 2H, Ar), 7.46 (d, 2H, Ar), 6.71 (q, 1H, Ar-CH=CH₂), 5.83 (d, 1H, Ar-CH=CH₂), 5.89 (s, 1H, O=C-CH=C), 5.36 (d, 1H, Ar-CH=CH₂), 4.30 (t, O=C-O-CH₃), 3.26 (q, 2H, NH-(C=O)-NH-CH₂), 2.21 (s, 3H, CH₃), 1.78-1.46 (m, 8H, alkyl-CH₂). ¹³C-NMR (CDCl₃): δ = 173.03, 166.39, 156.56, 154.65, 148.19, 141.77, 136.02, 129.81, 129.59, 126.04, 116.34, 106.67, 65.00, 39.85, 29.38, 28.61, 26.58, 25.77, 18.91. MALDI-ToF MS: calculated 450.53, observed m/z 451.24 [M+H⁺].
(E)-6-(3-(6-Methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl 4-(2-nitrovinyl)benzoate (31)

A flask was charged with 6-(3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl 4-vinylbenzoate 33 (549 mg, 1.38 mmol) and dried in a vacuum oven at 40 °C. The flask was placed in a pre-heated oil-bath at 60 °C and CHCl₃ (18 mL) was added. AgNO₂ (264 mg, 1.71 mmol), Ag₂CO₃ (190 mg, 0.69 mmol), TEMPO (86 mg, 0.55 mmol) and oven-dried molecular sieves (4 Å) were added and the mixture was stirred for 36 hours at 60 °C under argon atmosphere. The mixture was filtered over Celite and washed with ethyl acetate (3x 20 mL). Purification by column chromatography (95 % CHCl₃/5 % methanol) yielded the product (438 mg, 72 %).

1H-NMR (CDCl₃): δ = 13.09 (bs, 1H, NH), 11.82 (bs, 1H, NH), 10.17 (bs, 1H, NH), 8.10 (d, 2H, Ar), 7.99 (d, 1H, CH=CH-NO₂), 7.62 (d, 1H, CH=CH-NO₂), 7.60 (d, 2H, Ar), 5.81 (s, 1H, O=C-CH=C), 4.34 (t, O=C-O-CH₂), 3.26 (q, 2H, NH-(C=O)-NH-CH₂), 2.22 (s, 3H, CH₃), 1.79-1.47 (m, 8H, alkyl-CH₂).

13C-NMR (CDCl₃): δ = 166.39, 156.57, 154.65, 149.19, 141.77, 136.03, 129.81, 129.59, 126.04, 116.34, 106.67, 65.00, 39.85, 29.39, 28.62, 25.77, 18.91. MALDI-ToF MS: calculated 443.45, observed m/z 444.22 [M+H⁺].

6,8-Dioxononyl hexanoate (36)

9-Hydroxynonane-2,4-dione 16 (159 mg, 0.92 mmol) and hexanoic acid (116 μl, 0.92 mmol) were dissolved in toluene (150 mL). Novozym 435 (64 mg) was added and the mixture was slowly stirred at the rotovap (65°, 250 mbar) for 8 hours, followed by filtration and removal of the solvent in vacuo. Purification by column chromatography (88 % CHCl₃/12 % methanol) yielded the product (169 mg, 68 %). ¹H-NMR (CDCl₃): δ = 5.48 (s, 1H, ((C=O)-CH=COH), 4.06 (t, 2H, O=C-O-CH₂), 3.57 (s, 2H, (C=O)-CH₂-(C=O)), 2.29 (t, 2H, CH₂-CH₂-C=O), 2.27 (s, 3H, CH₂-(C=O)-CH₃), 2.23 (s, 3H, CH₃), 1.64-1.31 (m, 12H, alkyl-CH₂), 0.89 (t, 3H, alkyl-CH₂). ¹³C-NMR (CDCl₃): δ = 193.85, 191.36, 173.93, 99.76, 63.98, 38.06, 34.32, 31.30, 28.40, 25.58, 25.24, 24.94, 24.67, 22.30, 13.90. MALDI-ToF MS: calculated 270.36, observed m/z 293.26 [M+Na⁺].

6,8-Dioxononyl (6-(3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)carbamate (37)

1-(6-Isocyanatohexyl)-3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)urea 21 (127 mg, 0.43 mmol) and 9-hydroxynonane-2,4-dione 16 (49.7 mg, 0.29 mmol) were dissolved in CHCl₃ (10 mL). Two drops of dibutyltin dilaurate were added and the mixture was stirred overnight at 50 °C under argon atmosphere and subsequently dried in vacuo. Purification by column chromatography (90 % CHCl₃/10 % THF) yielded the product (118 mg, 88 %). ¹H-NMR (CDCl₃): δ = 13.13 (bs, 1H, NH), 11.86 (bs, 1H, NH), 10.13 (bs, 1H, NH), 5.84 (s, 1H, O=C=CH=C), 5.48 (s, 1H, ((C=O)-CH=COH), 4.03 (t, 2H, NH-(C=O)-O-CH₃), 3.56 (s, 2H, (C=O)-CH₂-(C=O)), 3.24 (q, 2H, NH-(C=O)-NH-CH₂), 3.14 (q, 2H, CH₂-NH-(C=O)-O), 2.27 (t, 2H, CH₂-CH₂-C=O), 2.27 (s, 3H, CH₂-(C=O)-CH₃), 2.23 (s, 3H, CH₃), 2.05 (s, 3H, CH=COH-CH₃), 1.64-1.31 (m, 12H, alkyl-CH₂), 0.89 (t, 3H, alkyl-CH₂). ¹³C-NMR (CDCl₃): δ = 193.85, 191.36, 173.93, 173.93, 99.76, 63.98, 38.06, 34.32, 31.30, 28.40, 25.58, 25.24, 24.94, 24.67, 22.30, 13.90. MALDI-ToF MS: calculated 270.36, observed m/z 293.26 [M+Na⁺].
6-(3-(6-Methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl 4-(3-acetyl-9-(((6-(3-(6-methyl-4-oxo-1,4-dihydropyrimidin-2-yl)ureido)hexyl)carbamoyloxy)-1-nitro-4-oxononan-2-yl)benzoate (39)

A mixture of UPy functionalized nitrostyrene 31 (88.9 mg, 0.20 mmol), UPy functionalized 2,4-pentanedione 37 (93.3 mg, 0.20 mmol) and NaPy 1 (263 mg, 0.50 mmol) were dissolved in CHCl₃ (10 mL). Subsequently the mixture was added to a flask with K₂CO₃ (5.5 mg, 0.04 mmol). The reaction mixture was stirred vigorously for 18 hours under argon atmosphere. UPy functionalized nitrostyrene (17.8 mg, 0.04 mmol) was added extra in order to approach 100 % conversion of UPy functionalized 2,4-pentanedione 37 and the mixture was stirred for 3 days. UPy functionalized nitrostyrene (17.8 mg, 0.04 mmol), NaPy (80.0 mg, 0.15 mmol) and K₂CO₃ (16.5 mg, 0.12 mmol) were added extra and the mixture was stirred for 18 hours. The mixture was extracted with H₂O (3x 5 mL). The organic layer was dried over MgSO₄ and the solvent was removed in vacuo. Purification by column chromatography (98 % CHCl₃ / 2 % propanol) yielded the product (130 mg, 72 %).¹H-NMR (CDCl₃): δ = 13.12 (bs, 1H, NH), 11.85 (bs, 1H, NH), 10.18 (bs, 1H, NH), 7.98 (d, 2H, Ar), 7.26 (d, 2H, Ar), 5.83 (s, 2H, O=C-CH=C), 4.99 (bs, 1H, NH-(C=O)-O), 4.64 (m, 2H, CH₂-NO₂), 4.30 (t, 2H, Ar-(C=O)-O-CH₂), 4.30 (d, 2H, Ar-(C=O)-O-CH₂), 3.25 (q, 4H, NH-(C=O)-NH-CH₂), 3.14 (q, 2H, CH₂NH-(C=O)-O), 2.28 (s, 2H, (O=C)-CH₂-(C=O)-CH₂), 2.22, (s, 6H, CH₃), 1.94 (s, 2H, CH₃-(C=O)-CH₂-(C=O)), 1.80-1.20 (m, 22H, alkyl-CH₂).

Isopentyl 4-vinylbenzoate (41)

4-Vinylbenzoic acid 40 (397 mg, 2.68 mmol) was dissolved in CHCl₃ (8 mL) and cooled to 0 °C. DMAP (277 mg, 2.26 mmol) and EDC (434 mg, 2.26 mmol) were added and the mixture was stirred at 0 °C for 0.5 hour. 3-methylbutan-1-ol (200 mg, 2.29 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was extracted with H₂O (3x 5 mL). The organic phase was dried over MgSO₄ and the solvent was removed in vacuo. Purification by column chromatography (70 % heptane / 30 % CHCl₃) yielded the product (314 mg, 64 %).¹H-NMR (CDCl₃): δ = 7.98 (d, 2H, Ar), 7.45 (d, 2H, Ar), 6.76 (q, 1H, Ar-CH=CH₂), 5.88 (d, 1H, Ar-CH=CH₂), 5.36 (d, 1H, Ar-CH=CH₂), 4.35 (t, 2H, O=C-O-CH₂), 1.80 (m, 1H, (CH₃)₂-CH), 1.67 (q, 2H, O=C-CH₂-CH₂), 0.97 (d, 6H, 1.7-1.2 (m, 14H, alkyl-CH₂).¹C-NMR (CDCl₃): δ = 193.92, 191.40, 173.14, 156.55, 154.68, 148.26, 106.67, 99.79, 64.45, 40.67, 39.62, 38.08, 29.74, 29.34, 28.82, 26.26, 26.11, 25.55, 25.34, 25.31, 24.97, 18.95. MALDI-ToF MS: calculated 465.54, observed m/z 466.29 [M+H⁺], 488.28 [M+Na⁺], 504.25 [M+K⁺].
CH$_3$). $^{13}$C-NMR (CDCl$_3$): $\delta =$ 141.78, 136.02, 129.81, 129.62, 126.04, 116.34, 63.58, 37.41, 25.21, 22.51.

(\(E\))-Isopentyl 4-(2-nitrovinyl)benzoate (42)

A round bottom flask was charged with isopentyl 4-vinylbenzoate 41 (310 mg, 1.42 mmol) and dried in a vacuum oven at 40 °C. The flask was placed in a pre-heated oil-bath at 60 °C and CHCl$_3$ (6 mL) was added. Subsequently, AgNO$_2$ (666 mg, 4.26 mmol), TEMPO (87.4 mg, 0.57 mmol) and oven-dried molecular sieves (4 Å) were added to the mixture and stirred overnight at 60 °C under argon atmosphere. The reaction mixture was filtered over Celite and washed with ethyl acetate (3x 10 mL). Purification by column chromatography (80 % heptane / 20 % CHCl$_3$) yielded the product (341 mg, 91 %).

$^1$H-NMR (CDCl$_3$): $\delta =$ 8.12 (d, 2H, Ar), 8.01 (d, 1H, CH=CH-NO$_2$), 7.64 (d, 1H, CH=CH-NO$_2$), 7.62 (d, 2H, Ar), 4.38 (t, O=C-O-CH$_2$), 1.80 (m, 1H, (CH$_3$)$_2$CH), 1.68 (q, 2H, O=C-O-CH$_2$-CH$_2$), 0.98 (d, 6H, CH$_3$). $^{13}$C-NMR (CDCl$_3$): $\delta =$ 166.25, 138.59, 137.61, 134.05, 133.36, 130.38, 128.95, 64.15, 37.31, 25.19, 22.49.

6,8-Dioxononyl butylcarbamate (43)

9-Hydroxynonane-2,4-dione 16 (135 mg, 0.78 mmol) and 1-isocyanatobutane (389 mg, 3.92 mmol) were dissolved in CHCL$_3$ (10 mL) and 2 drops of DBTDL were added to the mixture. The reaction mixture was stirred overnight at 40 °C under argon atmosphere and subsequently dried in vacuo. Purification by column chromatography (84 % heptane / 16 % EtOAc) yielded the product as a white solid (197 mg, 94 %). $^1$H-NMR (CDCl$_3$): $\delta =$ 5.49 (s, 1H, ((C=O)-CH=COH), 4.63 (bs, 1H, NH-(C=O)-O), 4.04 (t, 2H, NH-(C=O)-O-CH$_3$), 3.57 (s, 2H, (C=O)-CH$_2$-(C=O)), 3.16 (q, 2H, CH$_2$-NH-(C=O)-O), 2.28 (t, 2H, CH$_2$-CH$_2$-C=O), 2.17 (s, 3H, CH$_3$-(C=O)-CH$_3$), 2.06 (s, 3H, CH=COH-CH$_3$), 1.40-1.70 (m, 10H, alkyl-CH$_2$), 0.92 (t, 3H, alkyl-CH$_3$). $^{13}$C-NMR (CDCl$_3$): $\delta =$ 193.92, 191.40, 156.68, 99.78, 64.48, 57.89, 43.57, 40.67, 38.08, 32.06, 30.93, 28.78, 25.55, 25.35, 35.30, 24.96, 22.96, 19.87, 13.72. MALDI-ToF MS: calculated 271.36, observed m/z 294.25 [M+Na$^+$].
3.8 References

1) Figures made by Tim Paffen.
Conclusions and outlook

In this research we set out to develop a new mechanism to obtain a positive feedback loop, based on the self-accelerated release of catalyst from a supramolecular scaffold. Here, the quadruple hydrogen bonding motifs NaPy and UPy are respectively used as catalyst and supramolecular scaffold. One of the key elements of the self-accelerating reaction is the reported ability of NaPy to catalyze the Michael addition. Therefore, we first tried to reproduce the catalytic studies reported in literature. However, no catalytic activity of NaPy towards the Michael addition between 2,4-pentanediol and trans-β-nitrostyrene was observed. Our studies have shown that the catalytic activity of NaPy towards the Michael addition was likely a result of a synergy between NaPy and K$_2$CO$_3$. Individually, NaPy and K$_2$CO$_3$ don’t catalyze the reaction efficiently, while high reaction rates are obtained when a combination of both is used. Incomplete removal of K$_2$CO$_3$ in the last step of the synthesis of NaPy, likely resulted in the reported conversions for the Michael addition. Based on our findings we propose a mechanism in which NaPy functions as a phase transfer catalyst for K$_2$CO$_3$.

The catalytic activity of NaPy in the presence of various carbonates has been tested. The Michael addition only took place efficiently using K$_2$CO$_3$ or Cs$_2$CO$_3$. There appears to be roughly a linear dependence of the reaction rate on the amount of K$_2$CO$_3$. The synergy between NaPy and K$_2$CO$_3$ is very selective towards the Michael substrates. Only four of the sixteen tested combinations showed product formation using NaPy and K$_2$CO$_3$ as catalysts, while only for two combinations it appeared to be a result of a strong synergistic relation between NaPy and K$_2$CO$_3$. Due to their high reactivity, 2,4-pentanediol and trans-β-nitrostyrene were chosen as substrates for the self-accelerating reaction. Since the catalytic activity of NaPy turned out to be a consequence of contamination with K$_2$CO$_3$, the influence of our findings on the previously reported buffering of the catalytic activity have been investigated. Our studies show that it is possible to improve the buffering strongly by controlling the amount of K$_2$CO$_3$ during the concentration dependent catalytic studies.

For the development of a self-accelerated reaction, we synthesized a UPy functionalized 2,4-pentanediol, UPy functionalized nitrostyrenes and NaPy as starting compounds. Attempts to couple the UPy functionalized substrates using NaPy and K$_2$CO$_3$ didn’t result in the formation of the bifunctional UPy. In order to have an indication of their reactivity, reference reactions have been performed. This reveals that the problems during the formation of the bifunctional UPy is caused by the low reactivity of the UPy functionalized nitrostyrene compounds. The presence of functional groups directly next to the nitrostyrene motif likely resulted in deactivation by mesomeric effects.

In order to increase the reactivity, a UPy functionalized nitrostyrene has been synthesized which has an activating group connected to the nitrostyrene motif. This resulted in a strong increase of the reactivity compared to the previously synthesized UPy functionalized nitrostyrenes. The NaPy/K$_2$CO$_3$ catalyzed Michael addition of the UPy functionalized substrates resulted in product formation, however the occurrence of side-reactions is observed. Therefore, modifications are made to the molecular structure of the UPy functionalized substrates, followed by formation of the corresponding bifunctional UPy using NaPy and K$_2$CO$_3$. Although all peaks can be assigned, the integrals corresponding to Michael adduct are too low. This is observed for both the coupling of the reference compounds, as well as for the UPy functionalized substrates and suggests that significant amounts of side-products are formed.
Nevertheless, coupling of the reference compounds at concentrations of 5 and 0.5 mM, showed that 90 % conversion is obtained in respectively 2 and 10 days respectively. These results show that the reaction can be performed on practical timescales at the required concentrations. Future work should be focussed on identifying the side-products and optimizing the reaction conditions.
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