Chapter 3
Towards an artificial ribosome

In this chapter two approaches based on a modular approach are investigated for the creation of an artificial ribosome. The two approaches make use of amino acid-oligonucleotide conjugates and follow different deprotection strategies, i.e., metal-catalysis and photoirradiation. The synthesis of amino acid-modified oligonucleotides is described and each of the deprotection techniques is studied. The performance of the system as an artificial ribosome following the photoirradiation approach is investigated.
3. 1. Introduction

3.1.1. Polypeptide synthesis in nature

One of the most fascinating processes taking place in living organisms, arguably, is the synthesis of proteins by the ribosome (Figure 1). The mechanism of action of the ribosome involves the use of messenger RNA (mRNA), which codes for the sequence of amino acids in the resulting protein, and the transfer RNA (tRNA), which contains the three nucleobase sequence (anticodon) complementary to the codons in the mRNA. Each tRNA molecule possesses a specific linked amino acid prior to polymerization into polypeptides. Thus, every anticodon corresponds specifically for one amino acid. By the attachment at their carboxylic ends, the amino acids are activated for peptide bond formation. Once the ribosome is assembled onto the mRNA, the amino acid-tRNA pairs complementary to the sequence are accommodated sequentially. The order in which the amino acid-tRNA pairs are incorporated is dictated by the codon-anticodon complementarity and assisted by the templating nature of the ribosome. The polypeptide chain elongation process takes place through an N to C stepwise chain transfer mechanism involving peptide bond formation between the carboxyl group at the end of a growing polypeptide chain and the amino group of an incoming amino acid-tRNA pair. The free tRNAs generated during the coupling steps are disassociated from the translation machinery by a translocation process. This results in a vacant position for a new incoming amino acid-tRNA pair and the coupling cycle starts once again. Following this mechanism, the growing peptidic chain is transferred consecutively until the stop codon is reached. Key aspects of the protein synthesis process are the ribosome, which acts as both catalyst and scaffold, and the templating effect of the mRNA that maintains the high effective molarity of the reactants.

![Figure 1](image-url)  
**Figure 1.** Mechanism followed by the natural ribosome in the synthesis of proteins.

Another category of polypeptides is the product of nonribosomal biosynthesis. This alternative synthetic means is carried out by the so-called nonribosomal peptide synthetases (NRPSs). NRPSs are modular multi-enzyme complexes which act as
templatting and translating machinery in the nucleic acid-independent synthesis of polypeptides. A key aspect in the NRPSs is the way the amino acids are attached to the protein template; they appear covalently bound as thioesters. Thioesters are functionalities labile enough to undergo a nucleophilic attack while stable enough to not, or only slowly, hydrolyze in aqueous solutions. The nonribosomal pathway of peptide synthesis follows a thiotemplate-directed mechanism (Figure 2). According to this mechanism, the peptide bond formation takes place between the thioester-activated carboxyl group of one residue and the amino group of the adjacent amino acid. The synthesis of the polypeptide is possible through a step-by-step elongation process which involves the transfer of the growing peptide chain. Termination of the process takes place by release of the polypeptide once the last module is reached or after macrocyclization by an intramolecular reaction.

![Figure 2. Schematic representation of the mechanism followed in the nonribosomal peptide synthesis.](image)

### 3.1.2. Solid phase peptide synthesis (SPPS)

The development of a method to not only synthesize proteins efficiently but also to incorporate non-proteinogenic amino acids for protein engineering has been always of major interest. In 1963 the solid phase peptide synthesis (SPPS) was introduced. Although the working mechanism of SPPS is different to the one followed by the natural ribosome, polypeptides can be efficiently synthesized and represents the technique most broadly used nowadays for this purpose. In SPPS, protected amino acids are attached to the peptidic chain via coupling of carbodiimide, or related activated esters, followed by deprotection steps. The automated synthesis makes use of a solid support to which the growing polypeptide chain is attached following deprotection and washing cycles. This method allows for the incorporation of non-proteinogenic amino acids; this represents a
major advantage to peptide synthesis. Unfortunately, the yield of the synthesis is generally inversely proportional to the length of the polypeptide. The way to circumvent this problem is by using an excess of the amino acids. This is in contrast with the mode of action of the natural ribosome which efficiently synthesizes polypeptides by maintaining the high effective molarity during the process provided by the templating effect of the mRNA and the ribosome itself.

3.1.3. DNA-templated organic synthesis

The increase in the effective molarity of the reactants by hybridization with a DNA template has been applied in templated organic synthesis (Figure 3). This approach makes use of DNA to bring reacting species in close proximity and promoting in this way the reaction between them. Although initially it was only applied to the ligation of DNA or analogues, this synthetic method showed to be applicable to a broad variety of chemical reactions. Furthermore, the design of the templated system has turned out to be very versatile.

![Figure 3. General scheme of DNA-templated organic synthesis.](image-url)

By using this approach, multistep coupling reactions were performed successfully. An interesting example involving the synthesis of a non-natural tripeptide was published by Liu and co-workers. The approach involved the use of amino acids linked to unique biotinylated oligonucleotides through a sulfone linker. The tripeptide synthesis was directed via an amine terminated template and comprised three rounds of DNA-templated amide formation, purification and linker cleavage steps. Although the annealing of the reagents was possible at widely varying distances from the growing molecule, the number of possible steps appeared to be limited. With every added amino acid-oligonucleotide module the distance between the reacting groups was increased. This resulted in a decrease in the reaction efficiency as the number of steps increased. Attempts to overcome this limitation involved the use of loop-forming structures. This design presented the additional limitation of possible formation of undesired secondary structures that could affect the reactivity of the system. Very recently, and posterior to the work discussed in this chapter, an alternative approach that successfully prevents these limitations has been published. This approach makes use of functionalized
oligonucleotides possessing additional non-complementary nucleobases to the DNA template (Figure 4).

![Diagram](image)

**Figure 4.** a) General scheme of the DNA-templated multi-step synthesis based on a strand displacement mechanism. b) Mechanism of the chemical group transfer in the DNA-templated oligomer synthesis.

By hybridization with the template the reactive groups are brought in close proximity. Once the coupling has taken place, the removal of the module is possible by complete hybridization with a “remover” DNA strand. After removal of the spent module, a new module can be accommodated, starting the cycle once again. In this way, the same reaction environment is maintained in every coupling step. This is a key step in the approach; analogous to the ribosomal peptide synthesis, a strand displacement approach is followed. Following this approach, a series of olefin oligomers were synthesized by sequential DNA-templated Wittig reactions.

### 3.2. Aim of the research

The goal of the research described in this chapter is the creation of a mimic of the ribosome using a DNA-based modular approach. The versatility of the DNA molecule in the creation of functional materials have been already discussed in Chapter 1; here, that important feature is further investigated by the construction of a system that resembles the mode of action of, arguably, one of the most fascinating machineries in nature, the ribosome. On one hand, this research will bring new insights in the field of DNA-templated synthesis due to the synthetic and modular nature of the approaches.
Furthermore, it will represent a step further towards the creation of such an attractive assembled machinery that has supposed a major pursuit for several years.

3.3. Design of an artificial ribosome

The design of the artificial ribosome described in this chapter comprises three different oligonucleotide modules, i.e. an oligonucleotide functionalized with the starting amino acid of the polypeptide sequence (Oligo 1), an oligonucleotide functionalized with the subsequent amino acid (Oligo 2) and a template complementary to both of them (Figure 5).

![Image of General design of a proposed artificial ribosome.

The mode of operation of the proposed system follows a thiotemplate-directed mechanism, similarly to the nonribosomal peptide synthesis described above. In this case, however, the DNA functions as both the templating and translating machinery. Thus, the templating action of the DNA brings the reactive species in close proximity assisting the peptide bond formation between the amino group of the amino acid attached to Oligo 2 and the thioester-activated carboxyl group on Oligo 1. It is important to notice that the first amino acid in the sequence should remain protected. This is necessary to maintain the unidirectionality in the peptide synthesis allowing for only one possible coupling reaction at a time. Analogous to the polypeptide synthesis in nature, the stepwise elongation process involves the consecutive transfer of the growing peptide chain to the adjacent amino acids. This minimizes the chance to incorporate mistakes in the sequence since the order in which the amino acids are annealed is dictated by their proximity to the growing peptide after each chain transfer step. Thus, once the chain has been transferred, the contiguous amino acid will be incorporated. Additionally, this design provides other advantageous features; the length of the oligonucleotides that code for each amino acid is not restricted. This is in contrast to the natural tRNAs which only require three nucleobases (anticodon) to codify for each amino acid. By increasing the number of nucleobases in the oligonucleotides specifically attached to an amino acid, the number of available anticodons is enlarged exponentially. This is interesting for the incorporation of non-proteinogenic amino acids in the polypeptide since a new anticodon...
can be easily assigned. Furthermore, this approach allows for, in principle, the incorporation of any possible amino acid. Contrary to the natural ribosome, the mechanism followed in the present approach is not assisted by proteins. The incorporation of unnatural amino acids in nature involves selective protein recognition steps thus, not every amino acid can be incorporated. The present approach overcomes this limitation since the amino acid-oligonucleotide linkage is performed synthetically.

Two approaches that follow different mechanisms of action are investigated. The first approach involves the use of protected amino acid-Oligo 2 modules assembled on a DNA template (Figure 6). The deprotection of each amino acid takes place stepwise by the action of a metal complex attached to the starting amino acid (on Oligo 1). This deprotection should lead to the liberation of the amine group, resulting in the peptide bond formation between the deprotected amino acid and the thioester-activated carboxyl group on Oligo 1 accompanied by chain transfer. The metal complex is then placed in close proximity to the next amino acid in the sequence thus, the deprotection, attack and chain transfer cycle can take place again. The design of the system is very important since the distance between amino acids will most probably influence the performance of the artificial ribosome.

![Figure 6. General scheme of the mechanism of action of the artificial ribosome based on metal catalyzed deprotection. The annealing of only a single amino acid is represented for simplification.](image)

The second approach also makes use of protected amino acids. However, in this case, the deprotection takes place via photoirradiation (Figure 7). The first amino acid in the sequence is unaffected by irradiation and will remain protected to maintain the unidirectionality of the synthesis. The adjacent amino acid can be deprotected via irradiation resulting in a free amine that can attack the thioester-activated carboxyl group on Oligo 1 and start in this way, the polypeptide synthesis. The first amino acid in the sequence will be subsequently transferred to Oligo 2 and the unfunctionalized oligonucleotide (Oligo 1) can be then displaced by the next protected amino acid-oligonucleotide module.
Figure 7. General scheme of the mechanism of action of the artificial ribosome based on photoinduced deprotection and thermal cycling. The annealing of only a single amino acid is represented for simplification.

The sequential addition of each of the protected modules is required after every coupling step since the same photolabile protecting group is used in each of the modules to be incorporated. In the presence of all the modules, multiple products would be obtained by nucleophilic attack between neighboring amino acids once they have been deprotected by irradiation.

The module displacement can be achieved by the addition of new modules with increasing number of nucleobases and thermal cycling of the system. The dissociation from the DNA template can be enforced by thermal denaturation and subsequent annealing at low temperature to continue the ligation process. This would provide a strong thermodynamic force for the hybridization of the new module and the removal of the now empty original module. This mode of action has been previously reported in DNA-templated synthesis in order to achieve turnover.¹⁴,¹⁵

The major difference between the two approaches lays in the way the amino acids are coupled to the polypeptide chain. In the first approach, the peptide sequence is determined by the DNA template analogous to the natural ribosome. The amino acids to be incorporated are hybridized from the beginning to the DNA template and their deprotection takes place consecutively. In contrast, the second approach incorporates the amino acids in the order in which each amino acid-oligonucleotide module is added. In this case, the deprotection should be done once the incoming module is annealed to the system. Thus, two different mechanisms of action are followed: approach 1 involves
the continuous transfer of the growing peptide chain along the DNA whereas approach 2 involves thermal cycling and strand displacement steps.

Another important point in the designs is the deprotection of the amino acids. While in the first approach the metal placed on the first amino acid catalyzes the deprotection of the adjacent one, the second approach does not need the intervention of any functionality on the neighboring module. The latter deprotection mechanism can result in a more advantageous design since the deprotection should be equally efficient in all the cases. Moreover, following the first approach, the metal catalyst is placed at increasingly longer distances from the following amino acid as the polypeptide chain grows, resulting in a decrease in the effective molarity of the reactive groups.

3.4. Approach 1: metal catalyzed deprotection

The construction of a system that follows the first approach requires a suitable metal catalyzed deprotection method. For this purpose, the palladium catalyzed (allyloxy)carbonyl (alloc) deprotection of amines was selected. This reaction has shown application in different fields like dipeptide synthesis, solid-phase peptide synthesis, total synthesis of complex natural products and postsynthetic modification of DNA. Moreover, it has proven to work effectively in aqueous solutions (Scheme 1).

![Scheme 1](image1)

**Scheme 1.** Alloc deprotection using a water soluble palladium catalyst. TPPT corresponds to triphenylphosphine trisulphonate.

This artificial ribosome design will require, thus, the placement of a palladium catalyst on the first amino acid of the polypeptide sequence attached to Oligo 1 and alloc protected amino acids linked to different Oligo 2 modules. A schematic representation of the action of the artificial ribosomal system is depicted in Scheme 2.
For the construction of such an artificial ribosomal system different protected amino acid-oligonucleotide conjugates are required: a module functionalized with a palladium catalyst and different modules containing alloc protected amino acids (Figure 8).

Each of the modules can be obtained by synthesis of the activated esters of the protected amino acids with subsequent attachment to thiol functionalized oligonucleotides. An initial study of the system involved the exclusive use of alloc-amino acid-oligonucleotide modules and the exploration of the optimum deprotection conditions. Therefore, that module was synthesized in the first place.

### 3.4.1. Synthesis of the activated ester of alloc-alanine

In the synthesis of the modified amino acids it is important to consider which are the protecting group on the amine and the activating group on the carboxylic acid. The first one depends on the approach followed; the latter, on the coupling strategy followed to link them to the oligonucleotides.

For the synthesis of alloc protected amino acids the acid group of alloc protected alanine was activated by reaction with N-hydroxysuccinimide (NHS) via a dicyclohexylcarbodiimide (DCC) coupling (Scheme 3).
3.4.2. Synthesis of amino acid-oligonucleotide conjugates

The modification of oligonucleotides was done using commercially available 3’ thiol modified oligonucleotides. After a deprotection step involving disulfide bond cleavage, the thiol was reacted with the activated ester of the alloc modified amino acid (1) (Scheme 4). The resulting thioester is crucial for the performance of the artificial ribosome with the present design.

![Scheme 4](image)

**Scheme 4.** Coupling between thiol functionalized oligonucleotides and alloc protected alanine.

The coupling reaction was monitored by reversed phase HPLC (rp-HPLC) and the coupled product was purified by size exclusion chromatography.

3.4.3. Study of the artificial ribosome based on approach 1

The testing of the artificial ribosome following the first approach implies the consecutive deprotection of the alloc protecting group of the amino acid attached to the oligonucleotide adjacent to the palladium complex (Scheme 2).

The palladium-catalyzed alloc deprotection has been reported in the postsynthetic modification of DNA.\(^{19}\) In this case, DNA attached to a solid support and organic solvents were used. However, the deprotection of alloc protected molecules attached to DNA has not been studied in aqueous solutions. Hence, initial tests were performed in the deprotection of the alloc-modified amino acid-oligonucleotide conjugate (2) using a water soluble palladium catalyst following published procedures\(^{20}\) and characterizing the products by MALDI-TOF (Scheme 5).

![Scheme 5](image)

**Scheme 5.** Reaction scheme of the alloc deprotection of the protected alanine-oligonucleotide conjugate.
The presence of organic solvents can lead to DNA precipitation. Therefore the experiments were performed using exclusively water as solvent. Unfortunately, using the reported conditions no deprotection of the amino acid was observed. Different reaction conditions were investigated where the palladium source, the nucleophilic scavenger and the solvent where varied (Table 1).

**Table 1.** Different conditions studied for the alloc deprotection. DEA stands for diethylamine and Nu for nucleophilic scavenger.

<table>
<thead>
<tr>
<th>Pd(OAc)$_2$ mol%</th>
<th>TPPTS mol%</th>
<th>Nu.</th>
<th>Eq. Nu.</th>
<th>Solvent</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>DEA</td>
<td>4</td>
<td>H$_2$O</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>DEA</td>
<td>4</td>
<td>H$_2$O</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>DEA</td>
<td>4000</td>
<td>H$_2$O</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>TolSO$_2$Na</td>
<td>2</td>
<td>H$_2$O</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>TolSO$_2$Na</td>
<td>4</td>
<td>H$_2$O</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>DEA</td>
<td>5</td>
<td>Citric acid buffer pH</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>DEA</td>
<td>4</td>
<td>CH$_3$CN : H$_2$O 1:1</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>DEA</td>
<td>4</td>
<td>CH$_3$CN : H$_2$O 3:2</td>
<td>2</td>
</tr>
<tr>
<td>50 eq</td>
<td>100 eq</td>
<td>DEA</td>
<td>4</td>
<td>H$_2$O</td>
<td>2</td>
</tr>
<tr>
<td>5 mol% Pd(PPh$_3$)$_4$*</td>
<td>-</td>
<td>TolSO$_2$Na</td>
<td>4</td>
<td>H$_2$O</td>
<td>3</td>
</tr>
</tbody>
</table>

* The catalyst was dissolved in the minimal amount of methanol.

However, 0% conversion in the deprotection of the alloc group was observed for all tested conditions. Possibly, the presence of multiple coordination sites on the DNA resulted in the blocking of the catalytic site of the metal, preventing the palladium to coordinate to the alloc group and consequently, the reaction to proceed. Since the proposed method did not provide successful results in the deprotection catalyzed by palladium, this approach was abandoned.

3.5. **Approach 2: photoinduced deprotection**

The deprotection step in the second approach will be affected by photoirradiation. This deprotection technique represents a very clean methodology since no additional chemicals are required and, taking into account the numerous possible reactive groups in the modified oligonucleotides, it would minimize the possible side reactions. The photocleavable protecting group chosen for this approach is an o-nitrobenzylcarbamate derivative (NBz) which is known to be effectively cleaved by irradiation at 365 nm (Scheme 6).\textsuperscript{[21, 22]} The selected wavelength of irradiation is of importance since DNA can be damaged when exposed to UV light in the range of 200-315 nm.\textsuperscript{[23, 24]}
Scheme 6. Photocleavage of o-nitrobenzylcarbamate protected amines.

The design requires a protected amino acid linked to Oligo 1. This protecting group should remain unaltered after UV irradiation to prevent the formation of undesired coupled products. For this purpose, the amino group of the first amino acid on the sequence was acetylated. The subsequent amino acid-oligonucleotide modules are protected by o-nitrobenzylcarbamate and they will be added sequentially after every coupling step.

The mechanism of action of the proposed artificial ribosomal system is depicted in Scheme 7.

Scheme 7. Proposed mechanism of action for the artificial ribosome based on photoinduced deprotection.

The construction of this artificial ribosomal system implies the synthesis of several protected amino acid-oligonucleotide conjugates, i.e., acetylated amino acid-Oligo 1 and o-nitrobenzylcarbamate-amino acid-Oligo 2 modules. Due to the chemical diversity that the modules can present, acetylated alanine derivatives were linked to the oligonucleotides via two different functionalities and linker lengths to investigate the difference in their reactivity. Different photolabile protected amino acids were also linked to oligonucleotides with variable spacer lengths. Below, a summary of all the synthesized conjugates can be found (Figure 10). A detailed description of the synthetic steps followed is provided thereafter.
3.5.1. Synthesis of amino acid derivatives

The acetylated amino acid was synthesized by reaction of alanine with acetic anhydride to obtain the N-acyl amino acid (Scheme 8). The synthesis of the activated ester of the acetylated amino acid was achieved via a DCC coupling with trichlorophenol (TCP). This activating group was selected since the N-hydroxysuccinimide ester proved to give problems in purification due to degradation.

![Scheme 8](image)

**Scheme 8.** Acetylation of alanine and synthesis of its activated ester.

The o-nitrobenzylcarbamate functionality was coupled to the amino acid by reaction of o-nitrobenzyl alcohol with disuccinimidylcarbonate followed by reaction with alanine (Scheme 9). The same procedure was followed for the synthesis of a phenylalanine derivative.

![Scheme 9](image)

**Scheme 9.** Synthesis of carbamate o-nitrobenzyl protected amino acids.

Two different activated esters of both amino acid derivatives were synthesized by reaction with N-hydroxysuccinimide and trichlorophenol following similar procedures to...
the ones described in Schemes 3 and 8. Since the phenylalanine derivative precipitated when trying to couple it to the oligonucleotide (vide infra), an alternative protecting group containing two methoxy groups on the aromatic ring was used (Scheme 10). In this case, the product proved to be completely soluble under the conditions used.

Scheme 10. Synthesis of 4,5-dimethoxy o-nitrophenyl protected phenyl alanine.

3.5.2. Synthesis of amino acid-oligonucleotide conjugates

In the synthesis of modified oligonucleotides for this approach, several considerations were taken into account. To demonstrate the proof-of-principle of the design, the initial objective was to achieve a single peptidic coupling. For this reason, the modified oligonucleotide containing the amino acid protected with the photolabile group did not require a thioester moiety. Therefore, an amide group was chosen as the linkage between the amino acid and the oligonucleotide. This results in more stable conjugates that are easier to synthesize and purify. The modified oligonucleotide containing the acetylated amino acid (first amino acid in the sequence) required the presence of a thioester moiety, which is susceptible to the nucleophilic attack from the neighboring amino acid.

Moreover, the distance between the amino acid and the oligonucleotide can have an influence on the spatial orientation of the amino acids and, subsequently, on the efficiency of peptidic coupling reaction. Therefore, amino acid-oligonucleotide conjugates containing aliphatic spacers of three and six carbons were synthesized.

The synthesis of the acetylated amino acid-oligonucleotide conjugate (7) was achieved by reaction of the 5' thiol modified oligonucleotide and the activated ester of acetylated alanine (3). In this case, the TCP activated amino acid was used (Scheme 11).

Scheme 11. Coupling between thiol-functionalized oligonucleotides and the activated ester of acetylated alanine.
When the same procedure was followed for the coupling of trichlorophenyl alanine derivatives to 3’ amino modified oligonucleotides, conversions of 30% were obtained (Scheme 12). The lower reactivity can be associated to the lower nucleophilicity of the amine compared to the thiol group.

Scheme 12. Coupling between amino functionalized oligonucleotides and trichlorophenyl activated amino acid derivatives.

Full conversions were obtained, however, when the NHS esters of the alanine derivatives were used. This is due to the higher reactivity of the NHS activated esters compared to the trichlorophenyl (TCP) equivalents (Scheme 13). Similarly, the oligonucleotide-amino acid conjugate containing a spacer of six carbons was synthesized.

Scheme 13. Coupling between amino functionalized oligonucleotides and NHS activated amino acid derivatives.

A precipitate appeared when the DMF solution of the trichlorophenyl ester of the protected phenyl alanine was added to the aqueous solutions of amino-functionalized oligonucleotides and no conversion was obtained. This could be attributed to the apolar nature of the molecule. In contrast, when the NHS ester of the protected amino acid containing two methoxy groups on the aromatic ring was used, no precipitate was observed and full conversion was obtained (Scheme 14).
Towards an artificial ribosome

Scheme 14. Coupling between amino-functionalized oligonucleotides and different activated esters of protected phenyl alanine.

The performance of the artificial ribosome may be dependent on both the spatial orientation of the amino acids and the reactivity of the thioester moieties. In order to study the influence in its performance, an amino acid-oligonucleotide conjugate containing a more labile thioester was synthesized. The thioester was positioned on the α position of a carbonyl group resulting in a more reactive thioester.\(^{25}\) 5’ Amino functionalized oligonucleotides were reacted with the NHS ester of dithioglycolic acid followed by reduction of the disulfide and subsequent reaction with the TCP activated ester of the acetylated alanine (Scheme 15).

Scheme 15. Synthesis of a more reactive acetylated alanine-oligonucleotide conjugate.

In summary, diverse amino acid-oligonucleotide conjugates have been synthesized. This variety of conjugates enables the construction and testing of different artificial ribosomes and will allow for the optimization of the proposed system.
3.5.3. Study of the artificial ribosome based on approach 2

In order to test if the UV irradiation would lead to the deprotection of the amino acid and the effect of the irradiation on the DNA, an initial test was performed using only the protected amino acid-oligonucleotide conjugate (8) (Scheme 16).

![Scheme 16. Reaction scheme of the deprotection of alanine-oligonucleotide conjugate by photoirradiation.](image)

The procedure followed was based on published results in which the same protecting group was photocleaved by irradiation at 365 nm for 1 h. When the same light source was applied to a solution of 8 in phosphate buffer the fully deprotected compound was obtained as sole product. The results were confirmed by rp-HPLC and MALDI-TOF (before irradiation: m/z 5341 (calcd. 5333), after irradiation: m/z 5157 (calcd. 5154)), which proved the presence of a single compound corresponding to the deprotected product. These results not only showed the effectiveness of the method but also proved that irradiation at 365 nm did not give rise to any damage in the DNA. Thus, this method is suitable for application in the present project.

Encouraged by these results, the system containing the modified modules Oligo 1, Oligo 2 and the template was tested in its performance as an artificial ribosome. Equimolar amounts of the acetylated amino acid-oligonucleotide conjugate (7), protected amino acid-oligonucleotide conjugate (9) and of the template complementary to both of them were mixed to obtain a 5 μM solution in 100 mM phosphate buffer pH 8.5. The system was subjected to thermal hybridization and transferred to a quartz cuvette where it was irradiated at 365 nm for 1 hour. After irradiation, the sample was allowed to stand for an additional hour at room temperature to allow the peptide coupling to occur.

The system was analyzed by RP-HPLC and MALDI-TOF. The results obtained via both techniques before irradiation showed clear peaks for each of the species present (Figures 11a and 12a). However, the interpretation of the results after irradiation was not trivial. The RP-HPLC chromatogram showed peaks corresponding to new species while others, present before the irradiation, disappeared. Nevertheless, due to the overlap of peaks no unequivocal evidence for the successful performance of the system could be obtained.
Towards an artificial ribosome

Figure 11. rp-HPLC traces of the artificial ribosome based on the second approach: a) before and b) after irradiation at 365 nm. Retention time (r. t.) at 34 min corresponds to the template, r. t. at 37 min corresponds to the acetylated amino acid-oligonucleotide conjugate (7) and r. t. at 41 min corresponds to the protected amino acid-oligonucleotide conjugate (9).

MALDI-TOF results did not provide a clear conclusion either. Interestingly, the peaks appeared considerably broadened after irradiation at 365 nm (Figure 12b).

Figure 12. MALDI-TOF spectra of the artificial ribosome based on the second approach a) before and b) after irradiation at 365 nm. m/z 4861 corresponds to the double charged template (calcd. m/z 4860), m/z 5231 corresponds to the acetylated amino acid-oligonucleotide conjugate (7) (calcd. m/z 5231) and m/z 5357 corresponds to the protected amino acid-oligonucleotide conjugate (9) (calcd. m/z 5373). The peak at m/z 5083 corresponds to a fragmentation as a result of the laser intensity observed when measured oligonucleotide samples.

Electron Spray Ionization (ESI) measurements revealed the presence of phosphate ions from the reaction buffer in the sample remaining after the purification. This could possibly explain the broadening of the peaks in the MALDI-TOF spectrum. The presence of the ions can alter the capability of the species in desorbing from the matrix what would result in a decrease in the sensitivity of the measurement. To overcome the problem, the system was tested using triethylammonium acetate buffer (TEAA) 50 mM, pH 7 which is a
volatile buffer and can easily be removed by lyophilization. The system was tested in this case using the more activated conjugate 10, containing a thioester on the α position of a carbonyl group, instead of 7 in an attempt to promote the coupling (Scheme 17).

![Scheme 17. Proposed mechanism of the artificial ribosome based on photoinduced deprotection when using a more activated conjugate.](image)

The MALDI-TOF spectra of the system before and after irradiation were conclusive this time (Figure 13). After irradiation, a new species appeared which corresponded to the deprotected amino acid attached to Oligo 2 (m/z 5194). Unfortunately, the mass corresponding to Oligo 2 attached to both amino acids (m/z 5309) was not observed.

![Figure 13. MALDI-TOF spectra of the artificial ribosome using the more activated conjugate (10) and TEAA buffer a) before and b) after irradiation at 365 nm. m/z 4861 corresponds to the double charged template (calcd. m/z 4860), m/z 5240 corresponds to the more activated acetylated amino acid-oligonucleotide conjugate (10) (calcd. m/z 5231), m/z 5370 corresponds to the protected amino acid-oligonucleotide conjugate (9) (calcd. m/z 5373), m/z 5194 corresponds to the deprotected amino acid-oligonucleotide conjugate (calcd. m/z 5196).](image)

Although the deprotection takes place while the whole ribosomal system is assembled, the results obtained by MALDI indicate that the coupling reaction does not occur. Different factors can be considered to explain the obtained results. The change to TEAA buffer allowed for the characterization of the system after irradiation; on the other hand, it represented not only a change in the buffer composition but also a lowering of the pH. Considering that the pKa of a free amine is around 9-10, this decrease in pH could affect
the nucleophilicity of the free amine after photodeprotection, reducing its reactivity. Working with different buffers could lead to difficulties in the characterization of the system since several purification steps would be required to effectively remove the remaining ions.

Parameters that can be changed for the optimization of the system include: the reaction time and the orientation and distance between the amino acids. The studied systems were allowed to react for 1 hour. Although the coupling step is not expected to require long periods of time, the design and complexity of the system under study can affect the kinetics of the reaction. Therefore, longer standing periods after irradiation could result in the desired coupled product. Furthermore, the distance between the amino acids and the oligonucleotides can be modified by changing the length of the aliphatic spacer. The distance between amino acids can be increased by the introduction of additional nucleobases in the middle of the template. This could also influence the hindrance between neighboring chains and therefore, the effectiveness of the coupling. In order to find the optimal design of the system, modeling studies could be necessary. Unfortunately, difficulties can be foreseen in the modeling of such a complex system.

3.6. Summary and conclusions

The construction of an artificial ribosome has been studied following two different approaches that involved the use of amino acid- oligonucleotide conjugates in a DNA template organic synthesis reaction. The first approach was based on the consecutive alloc deprotection of amino acids catalyzed by a water soluble palladium catalyst. Unfortunately, the deprotection could not be achieved probably due to the poisoning of the Pd catalyst by the multiple functionalities present on the oligonucleotide.

The second approach made use of photolabile protecting groups derivatives of o-nitrophenol. The deprotection of the protected amino acid- oligonucleotide conjugate by irradiation at 365 nm was achieved successfully, without damaging the DNA. The results obtained when the deprotection was performed on the ribosomal system did not show the coupled product although the deprotection took place in this case also. The fact that the deprotection takes place when the artificial ribosome is assembled but no coupling reaction occurs indicates that the design and the reaction conditions need to be optimized.

Despite the fact that no peptide bond formation was obtained yet, the second approach appears as a very promising system towards the construction of an artificial ribosome. It has been demonstrated that the two reactive species necessary for the coupling are present in the assembly. However, further optimization would be necessary.
3.7. Experimental section

General remarks

Chemicals were purchased from Sigma-Aldrich or Acros and were used without further purification. Column chromatography was performed on silica gel (Aldrich, 230-400 mesh). Oligonucleotides were purchased from BioTez Berlin-Buch GMbH. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian Mercury Plus 200, Varian VXR 300 or Varian Mercury Plus 400 spectrometer at room temperature. ESI mass spectra of small organic molecules were recorded on a Thermo Fisher Scientific Orbitrap XL HRMS with JEOL MS route JMS-600H. ESI spectra of oligonucleotides were recorded on a Triple Quadrupole LC/MS/MS Mass spectrometer (API 3000, Perkin-Elmer Sciei Instruments). MALDI-TOF spectra were recorded on a Voyager-DE Pro apparatus (Matrix: 20 μL of 2,4,6-trihydroxyacetophenone 0.5 M solution in ethanol + 10 μL of ammonium citrate dibasic solution 0.1 M in Milli Q water + 2 μL of sample solution in Milli Q water) in negative mode. Reversed phase – HPLC analysis were performed on a Shimadzu LC-10AD VP, Waters Xterra MS C18 column (3.0 x 150 mm, particle size 3.5 μm) using a gradient of CH$_3$CN/TEAA buffer 50 mM pH 7; gradient: 05/95 0 to 10 min, to 35/65 at 60 min, to 70/30 at 65 min. Flow: 0.5 mL/min. The concentrations of oligonucleotide solutions were estimated by absorption measurements at 260 nm using a Thermo Scientific Nanodrop 1000 spectrometer. The molar absorptivities used for 5' and 3' modified oligonucleotides were 171.06 mM$^{-1}$.cm$^{-1}$ and 179.21 mM$^{-1}$.cm$^{-1}$, respectively. UV irradiation experiments were done using a Vilber Lourmat VL-8.LC with 8W 365 nm tube UV lamp.

$^{2,5}$-dioxopyrrolidin-1-yl 2-(((allyloxy)carbonyl)amino)propanoate (1)

![Chemical structure]

N-allyloxy carbonyl-L-alanine dicyclohexylammonium (1.5 g, 4.2 mmol), N-hydroxysuccinimide (548 mg, 4.7 mmol) and dicyclohexylcarbodiimide (DCC) (983 mg, 4.7 mmol) were dissolved in 20 mL of THF. The solution was stirred overnight at room temperature and then cooled to 0 ºC. The precipitate was filtered off and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (SiO$_2$, pentane/ethyl acetate 1:3, R$_f$ = 0.7) to give 1 as a white solid (760 mg, 78%). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 5.91 (m, 1H), 5.28 (d, $J$ = 17.1, 1H), 5.23 (d, $J$ = 10.5 Hz, 1H), 4.76 (q, $J$ = 7.3 Hz, 1H), 4.59 (m, 1H), 2.83 (s, 4H), 1.57 (d, $J$ = 7.3 Hz, 3H) ppm; $^{13}$C-NMR (CDCl$_3$, 50 MHz) δ 168.79, 168.72, 155.83, 132.38, 118.08, 66.10, 48.02, 25.58, 18.60 ppm; MS (ESI) m/z 293.0745 ([M+Na]$^+$, calcd: 293.0750), 571.0926 ([M+H]$^+$, calcd: 271.0930); mp = 79.5-80.7 ºC.
2,4,6-trichlorophenyl 2-acetamidopropanoate (3)

2-Acetamidopropanoic acid (200 mg, 1.5 mmol) and 2, 4, 6-trichlorophenol (300 mg, 1.5 mmol) were dissolved in 20 mL of dichloromethane. N, N'- dicyclohexylcarbodiimide (DCC) (320 mg, 1.5 mmol) was added and the solution was stirred overnight at room temperature. After cooling to 0 °C the precipitate was filtered off and the solvent was evaporated. The product was purified by column chromatography (SiO₂, pentane/ethyl acetate 1:1, Rf = 0.55) to give 2 as a light brown solid (200 mg, 40%). ^1H-NMR (CDCl₃, 400 MHz) δ 7.37 (s, 2H), 6.25 (d, 1H), 4.99 (q, J = 7.5 Hz, 1H), 2.04 (s, 3H), 1.63 (d, J = 7.3 Hz, 3H) ppm; ^13C-NMR (CDCl₃, 75 MHz) δ 169.93, 169.85, 132.65, 129.63, 128.94, 128.88, 48.15, 23.31, 18.71 ppm; Elem. anal. calcd. for C₁₁H₁₀Cl₃NO₂N: 4.51, C: 42.54, H: 3.25, found N: 4.59, C: 43.49, H: 3.55; MS (ESI) m/z 331.9616 ([M+Na]^+, calcd: 331.9624), 309.9799 ([M+H]^+, calcd: 309.9805).

Methyl 2-aminopropanoate hydrochloride

Synthesized following published procedures.[²⁶] (Yield: 99%) ^1H-NMR (D₂O, 400 MHz) δ 4.05 (q, J = 7.3 Hz, 1H), 3.68 (s, 3H), 1.38 (d, J = 7.4 Hz, 3H) ppm; ^13C-NMR (D₂O, 100 MHz) δ 170.79, 53.50, 49.57, 16.30 ppm; MS (ESI) m/z 104.0704 ([M+H]^+, calcd: 104.0712).

Methyl 2-((2-mitrobenzyl)amino)propanoate

O-nitrobenzylobromide (324 mg, 1.5 mmol) was added to a solution of methyl 2-aminopropanoate hydrochloride (314 mg, 2.25 mmol) and diisopropylethylamine (0.7 mL, 4.5 mmol) in 10 mL of DMF. The solution was stirred at room temperature for 2 h and 100 mL of ethyl acetate was added. The mixture was washed with 3 x 50 mL of H₂O and dried over Na₂SO₄. The solvent was evaporated and the product was purified by column chromatography (SiO₂, hexane/ethyl acetate 1:9, Rf = 0.1) to yield the product as a brown oil (200 mg, 56%). ^1H-NMR (CDCl₃, 300 MHz) δ 7.92 (d, J = 8.1, 1H), 7.56 (m, 2H), 7.38 (t, J = 8.0 Hz, 1H), 4.10-3.93 (m, 2H), 3.69 (s, 3H), 3.39 (q, J = 7.3 Hz, 1H), 1.95 (s, 1H), 1.31 (d, J = 7.1 Hz, 3H) ppm; ^13C-NMR (CDCl₃, 75 MHz) δ 176.02, 135.45, 133.33, 131.21, 128.25, 124.95, 118.70, 56.59, 52.09, 49.15, 19.39 ppm; MS (ESI) m/z 239.1027 ([M+H]^+, calcd: 239.1110).

2-((2-nitrobenzyl)amino)propanoic acid

To a solution of methyl 2-((2-nitrobenzyl)amino)propanoate in 15 mL of THF/H₂O 1:1, 7.5 mL of a concentrated sodium hydroxide solution was added. The solution was stirred overnight at room temperature and acidified with 1 M aq. HCl solution to pH 5. The solvent was evaporated and the crude product recrystallized from H₂O to give the product as a grey solid (60 mg, 33%).
H-NMR (D$_2$O, 400 MHz) $\delta$ 8.01 (d, $J = 8.4$ Hz, 1H), 7.55 (m, 2H), 7.45 (m, 1H), 4.30 (q, $J = 6.7$ Hz, 2H), 4.06 (q, $J = 7.7$ Hz, 1H), 1.42 (d, $J = 7.7$ Hz, 3H) ppm; mp = 217-218 °C.

2, 5-dioxopyrrolidin-1-yl 2-nitrobenzyl carbonate

N, N’-disuccinimidyl carbonate (628 mg, 2.45 mmol) was added to a solution of o-nitrobenzyl alcohol (250 mg, 1.6 mmol) and triethylamine (0.45 mL, 3.3 mmol) in 25 mL of dry acetonitrile. The mixture was stirred under nitrogen at room temperature for 1 hour. After evaporation of the solvent, the crude product was dissolved in 30 mL of chloroform. The organic solution was washed with 3 x 20 mL of saturated NaHCO$_3$ solution, 3 x 20 mL of brine and dried over Na$_2$SO$_4$. Evaporation of the solvent gave the product as a light yellow solid (485 mg, 99%).

H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.23 (d, $J = 7.7$ Hz, 1H), 7.73 (m, 2H), 7.56 (m, 1H), 5.80 (s, 2H), 2.86 (s, 4H) ppm; $^{13}$C-NMR (CDCl$_3$, 50 MHz) $\delta$ 168.51, 146.62, 151.36, 134.46, 130.19, 129.25, 128.22, 125.37, 68.88, 25.45 ppm; Elem. anal. calcd. for C$_{12}$H$_{10}$N$_2$O$_7$: N: 9.52, C: 48.99, H: 3.43, found N: 9.42, C: 48.64, H: 3.65; MS (CI) m/z 295.06 ([M+H]$^+$, calcd. 295.06); mp = 113.9-114.6 °C.

2-((((2-nitrobenzyl)oxy)carbonyl)amino)propanoic acid (4)

Synthesized following published procedures.$^{[21]}$ (Yield: 70%) H-NMR (CDCl$_3$, 300 MHz) $\delta$ 8.12 (d, $J = 8.2$ Hz, 1H), 7.73 (m, 2H), 7.72 (m, 1H), 5.47 (s, 2H), 4.18 (q, $J = 7.6$ Hz, 1H), 1.41 (d, $J = 8.2$ Hz, 3H) ppm; $^{13}$C-NMR (DMSO, 50 MHz) $\delta$ 179.42, 160.63, 152.26, 139.28, 138.28, 133.92, 129.97, 67.97, 54.47, 22.25 ppm; Elem. anal. calcd. for C$_{11}$H$_{12}$N$_2$O$_6$: N: 10.44, C: 49.26, H: 4.51, found N: 10.18, C: 49.16, H: 4.57; MS (ESI) m/z 291.05773 ([M+Na]$^+$, calcd. 291.0593); mp = 132-135 °C.

2-((((2-nitrobenzyl)oxy)carbonyl)amino)-3-phenylpropanoic acid (5)

Synthesized following the same procedure as for 2-((((2-nitrobenzyl)oxy)carbonyl)amino)propanoic acid (3) using phenylalanine instead of alanine. (51%) H-NMR (CDCl$_3$, 200 MHz) $\delta$ 8.08 (d, $J = 8.13$ Hz, 1H), 7.0-7.7 (m, 8H), 5.34 (d, $J = 8.4$ Hz, 1H), 4.71 (q, $J = 7.3$ Hz, 1H), 3.18 (dd, $J = 13.3$ Hz, 2H) ppm; $^{13}$C-NMR (CDCl$_3$, 50 MHz) $\delta$ 175.94, 155.27, 147.24, 135.37, 133.79, 132.79, 129.32, 128.72, 128.56, 127.32, 125.00, 63.71, 54.59, 37.67 ppm; MS (Cl) m/z 367.09 ([M+Na]$^+$, calcd. 367.09); mp = 111-113 °C.

2, 5-dioxopyrrolidin-1-yl 2-((((2-nitrobenzyl)oxy)carbonyl)amino)propanoate

N-hydroxysuccinimide (56 mg, 0.49 mmol) was added to a stirring solution of 2-((((2-nitrobenzyl)oxy)carbonyl)amino)propanoic acid and 1-ethyl-3-(3-dimethylaminopropyl)
carbodiimide hydrochloride (EDC) (283 mg, 1.47 mmol) in 200 mL of dichloromethane. The mixture was stirred overnight at room temperature and washed with 3 x 20 mL of saturated NaHCO₃ solution, 3 x 20 mL of water and dried over Na₂SO₄. The solvent was evaporated under reduced pressure and the product purified by column chromatography (SiO₂, pentane/ethyl acetate 1:2, Rf = 0.3) to yield the product as a white solid (100 mg, 30%). ¹H-NMR (CDCl₃, 200 MHz) δ 8.10 (d, J = 9.1 Hz, 1H), 7.61 (m, 2H), 7.48 (m, 1H), 5.56 (s, 2H), 5.42 (d, J = 7.8 Hz, 1H), 4.78 (q, J = 7.6 Hz, 1H), 2.85 (s, 4H), 1.63 (d, J = 7.3 Hz, 3H) ppm; ¹³C-NMR (CDCl₃, 50 MHz) δ 168.61, 168.53, 154.78, 147.30, 132.59, 128.06, 128.63, 124.99, 63.84, 48.16, 18.66 ppm; Elem. anal. calcd. for C₁₅H₁₅N₃O₈: N: 11.50, C: 49.32, H: 4.14, found: N: 11.16, C: 49.05, H: 4.20; MS (ESI) m/z 388.07465 ([M+Na]⁺, calcd. 388.0757); mp = 48-50 °C.

2, 4, 6-trichlorophenyl 2-((((2-nitrobenzyl)oxy)carbonyl)amino)propanoate

2, 4, 6-trichlorophenol (148 mg, 0.75 mmol) was added to a stirred solution of 2-(((2-nitrobenzyl)oxy)carbonyl)amino)propanoic acid and EDC (429 mg, 2.24 mmol) in 20 mL of dichloromethane. The mixture was stirred overnight at room temperature and washed with 3 x 20 mL of saturated NaHCO₃ solution, 3 x 20 mL of water and dried over Na₂SO₄. The solvent was evaporated under reduced pressure and the product was purified by column chromatography (SiO₂, heptane/ethyl acetate 2:1, Rf = 0.35) to give the product as a white solid (75 mg, 25%). ¹H-NMR (CDCl₃, 400 MHz) δ 8.09 (d, J = 8.2 Hz, 1H), 7.63 (m, 2H), 7.50 (m, 1H), 7.38 (s, 2H), 5.57 (s, 2H), 5.46 (d, J = 7.4 Hz, 1H), 4.78 (q, J = 7.7 Hz, 1H), 1.69 (d, J = 7.4 Hz, 3H) ppm; ¹³C-NMR (CDCl₃, 50 MHz) δ 169.36, 154.95, 147.28, 142.32, 133.79, 132.77, 132.46, 129.36, 128.73, 128.69, 128.65, 125.00, 63.38, 49.70, 18.48 ppm; Elem. anal. calcd. for C₁₇H₁₃Cl₃N₂O₆: N: 6.26, C: 45.61, H: 2.93, found: N: 6.02, C: 45.25, H: 2.79; MS (ESI) m/z 468.97 ([M+H]⁺, calcd. 468.97); mp = 96-97.3 °C.

2,4,6-trichlorophenyl 2-(((2-nitrobenzyl)oxy)carbonyl)amino-3-phenylpropanoate

The product was obtained from 2-(((2-nitrobenzyl)oxy)carbonyl)amino)-3-phenylpropanoic acid as a white solid following the procedure described for 2, 4, 6-trichlorophenyl 2-(((2-nitrobenzyl)oxy)carbonyl)amino)propanoate. Rf = 0.55, ¹H-NMR (CDCl₃, 400 MHz) δ 8.09 (d, J = 7.7, 1H) 7.27-7.62 (m, 10H), 5.51 (s, 2H), 5.29 (d, J = 9.6 Hz, 1H), 5.04 (q, J = 7.8 Hz, 1H), 3.51 (dd, J₁ = 6.6 Hz, J₂ = 14.6 Hz, 1H), 3.19 (dd, J₁ = 8.2 Hz, J₂ = 15.6 Hz, 1H) ppm; ¹³C-NMR (CDCl₃, 50 MHz) δ 168.31, 155.05, 147.16, 135.09, 133.76, 132.82, 132.50, 129.36, 128.80, 128.73, 128.50, 128.42, 127.43, 124.97, 63.72, 54.62, 37.82 ppm; Elem. anal. calcd. for C₂₃H₁₅Cl₃N₂O₆: N: 5.35, C: 52.74, H: 3.27, found: N: 5.21, C: 52.24, H: 3.18; MS (ESI) m/z 545.00 ([M+Na]⁺, calcd. 545.01); mp = 142 °C.
2-((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)amino)-3-phenylpropanoic acid (6)

Synthesized following published procedures.\[27\] (20%) $^1$H-NMR (CDCl$_3$, 200 MHz) $\delta$ 7.70 (s, 1H), 7.28 (m, 3H), 7.17 (m, 2H), 6.91 (s, 1H), 5.52 (s, 2H), 5.27 (d, $J = 9.0$ Hz, 1H), 4.70 (q, $J = 7.2$ Hz, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.20 (dd, $J_1 = 5.2$ Hz, $J_2 = 12.4$ Hz, 2H) ppm; $^{13}$C-NMR (CDCl$_3$, 50 MHz) $\delta$ 155.40, 153.60, 148.12, 139.64, 135.33, 128.78, 127.84, 127.39, 109.82, 108.17, 63.98, 60.62, 56.40, 37.55 ppm; Elem. anal. calcd. for C$_{19}$H$_{20}$N$_2$O$_8$: N: 6.93, C: 56.43, H: 4.99, found: N: 6.85, C: 56.35, H: 5.03; MS (ESI) m/z 427.1104 ([M+Na]$^+$, calcd. 427.1117); mp = 170.9-171.1 °C.

2,5-dioxopyrrolidin-1-yl 2-((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)amino)-3-phenylpropanoate

The product was obtained from 2-((((4,5-dimethoxy-2-nitrobenzyl)oxy)carbonyl)amino)-3-phenylpropanoic acid as a light yellow solid following the procedure described for 2,5-dioxopyrrolidin-1-yl 2-((((2-nitrobenzyl)oxy)carbonyl)amino)propanoate. $R_f = 0.45$; $^1$H-NMR (CDCl$_3$, 200 MHz) $\delta$ 7.70 (s, 1H), 7.30 (m, 5H), 6.90 (s, 1H), 5.51 (d, $J = 5.3$ Hz, 2H), 5.25 (d, $J = 8.2$ Hz, 1H), 5.08 (q, $J = 7.3$ Hz, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.31 (dd, $J_1 = 5.6$ Hz, $J_2 = 11.0$ Hz, 1H), 2.86 (s, 4H) ppm; $^{13}$C-NMR (CDCl$_3$, 50 MHz) $\delta$ 168.44, 167.50, 154.90, 153.60, 139.69, 134.22, 129.59, 128.84, 127.83, 127.61, 64.09, 60.62, 56.44, 56.38, 37.88, 25.58 ppm; Elem. anal. calcd. for C$_{23}$H$_{23}$N$_3$O$_{10}$: N: 8.38, C: 55.09, H: 4.62, found: N: 8.00, C: 54.76, H: 4.78; MS (ESI) m/z 524.1251 ([M+Na]$^+$, calcd. 524.1281); mp: 79-81 °C.

2-acetamidopropanoic acid

Acetic anhydride (2 mL, 20 mmol) was added to a suspension of L-alanine (1.5 g, 17 mmol) in 25 mL of acetic acid. The mixture was stirred at room temperature until all L-alanine was dissolved and the solvent was evaporated under reduced pressure. 5 mL of acetone were added and the mixture was stirred vigorously. The mixture was filtered and the residue was washed with 3 x 10 mL of acetone. After drying 2-acetamidopropanoic acid was obtained as a white solid (586 mg, 26%). $^1$H-NMR (DMSO, 400 MHz) $\delta$ 8.13 (d, $J = 6.8$ Hz, 1H), 4.14 (q, $J = 7.3$ Hz, 1H), 1.80 (s, 3H), 1.21 (d, $J = 7.3$ Hz, 3H) ppm; $^{13}$C-NMR (DMSO, 100 MHz) $\delta$ 174.96, 169.66, 48.11, 22.97, 17.85 ppm; Elem. anal. calcd. for C$_5$H$_9$NO$_2$: N: 10.68, C: 45.80, H: 6.92, found N: 10.72, C: 45.36, H: 6.94; FTMS + ESI: m/z 154.05 ([M+Na]$^+$, calcd: 154.05), 132.07 ([M+H]$^+$, calcd: 132.06); mp = 114.0-116.5 °C.
Bis(2,5-dioxopyrrolidin-1-yl)2,2'-disulfanediylidiacetate

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (946 mg, 4.94 mmol) was added to a stirring solution of dithioglycolic acid (300 mg, 1.64 mmol) and N-hydroxysuccinimide (378 mg, 3.29 mmol) in 20 mL of dichloromethane. The mixture was stirred overnight at room temperature, washed with 3 x 30 mL of water and dried over Na$_2$SO$_4$. The solvent was evaporated under reduced pressure and the product purified by column chromatography (SiO$_2$, hexane/ethyl acetate 1:5, R$_f$ = 0.5) to give the product as an orange solid (139 mg, 23%). $^1$H-NMR (CDCl$_3$, 300 MHz) δ 3.92 (s, 4H), 2.85 (s, 8H) ppm; $^{13}$C-NMR (CDCl$_3$, 50 MHz) δ 168.72, 165.07, 38.71, 25.59 ppm; Elem. anal. calcd. for C$_{12}$H$_{12}$N$_2$O$_8$S$_2$: N: 7.44, C: 38.30, H: 3.21, found: N: 7.05, C: 38.22, H: 3.28; MS (Cl) m/z: MS (Cl) m/z 377.01 ([M+H]$^+$, calcd. 377.01); mp = 42.9 °C.

General procedure for the deprotection of thio-modified oligonucleotides

500 μL of D,L-1,4-dithiothreitol (DTT) 0.2 M in phosphate buffer was added to a solution of disulfide protected thio-modified oligonucleotide (500 μL, 200 μL) in Milli Q water. The mixture was shaken and allowed to stand for 1 hour at room temperature. The deprotected product was purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7).

General procedure for the coupling of thiol-functionalized oligonucleotides to modified amino acids:

The activated ester of the corresponding amino acid (100 μL, 20 mg/mL in DMF) was added to a solution of the deprotected thio-modified oligonucleotide (200 μL, 75 μM) in
phosphate buffer 100 mM, pH 7.5). The mixture was shaken and allowed to stand overnight at room temperature. The coupled product was purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7) and lyophilized.

\[
\text{RP – HPLC: retention time = 44 min} \\
\text{MALDI-TOF m/z 5304 (calcd. 5298)}
\]

\[
\text{RP – HPLC: retention time = 37 min} \\
\text{MALDI-TOF m/z 5238 (calcd. 5231)} \\
\text{ESI m/z 5232}
\]

\[
\text{RP – HPLC: retention time = 33 min} \\
\text{MALDI-TOF m/z 5246 (calcd. 5246)}
\]

General procedure for the coupling of amino modified oligonucleotides to modified amino acids:

80 µL of phosphate buffer 100 mM pH 7.5 were added to a 20 µL solution 200µM of amino-modified oligonucleotide in Milli Q water. A solution of the corresponding amino acid (100 µL, 20 mg/mL in DMF) was added and mixed. The solution was allowed to stand overnight at room temperature. The coupled product was purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7) and dried by lyophilization.

\[
\text{RP – HPLC: retention time = 38 min} \\
\text{MALDI-TOF m/z 5341 (calcd. 5333)} \\
\text{ESI m/z 5334}
\]
Towards an artificial ribosome

**General procedure for the photocleavage experiments:**

A solution of nitrobenzyl protected amino acid modified oligonucleotide (50 μL, 20 μM) in Milli Q water was diluted with 50 μL of phosphate buffer 200 mM pH 8. The mixture was irradiated at 365 nm for 1 hour. The deprotected product was purified by size exclusion chromatography (Sephadex™ G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7) and lyophilized.

**RP – HPLC: retention time = 32 min**
MALDI-TOF m/z 5223 (calcd. 5223)

**RP – HPLC: retention time = 32 min**
MALDI-TOF m/z 5223 (calcd. 5223)

**RP – HPLC: retention time = 32 min**
MALDI-TOF m/z 5223 (calcd. 5223)
General procedure for the testing of the artificial ribosome based on photoinduced deprotection:

Equimolar amounts of thio-ester 5’ modified oligonucleotide, 3’ modified oligonucleotide and template in aqueous solution were mixed. The mixture was diluted with phosphate buffer (200 mM, pH 8.5) or TEAA buffer (50 mM, pH 7) to a final concentration of 5 μM. The system was assembled by thermal hybridization: warming to 80 °C and slowly cooling to room temperature. The sample was irradiated for 1 h at 365 nm and allowed to stand at room temperature for an additional hour. The products were purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM pH 7), lyophilized and characterized by reversed phase-HPLC, MALDI-TOF or ESI.

3.8. References

Towards an artificial ribosome


