

Bioorganic Chemistry | Very Important Paper |

VIP

# Synthesis of Peptido RNAs from Unprotected Peptides and Oligoribonucleotides via Coupling in Aqueous Solution

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**Abstract:** Peptido RNAs are hybrid molecules with a phosphoramidate link between the *N*-terminus of a peptide and the 5'-phosphate of an oligoribonucleotide. Such species are formed in spontaneous co-oligomerizations of amino acids and ribonucleotides in aqueous condensation buffer. To shed light on the properties of these fascinating molecules, a synthetic method for their preparation in monodisperse form is needed. Herein, we report how peptido RNAs can be prepared via solution-phase coupling of unprotected peptides and oligoribo-

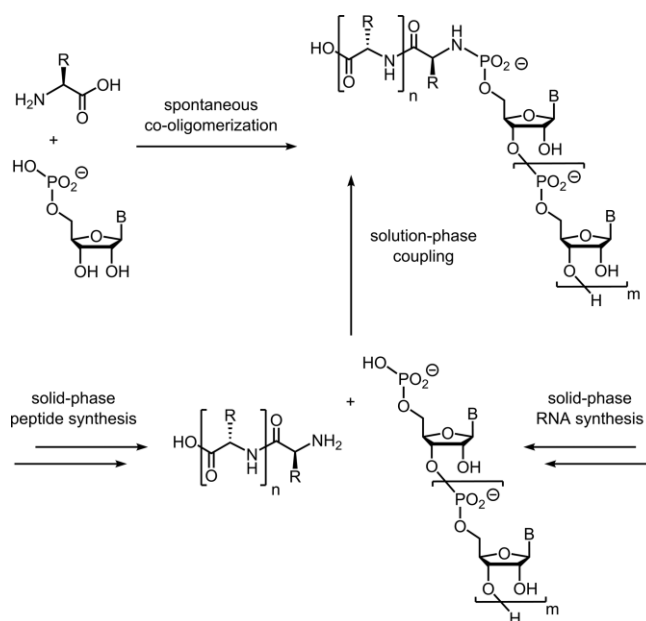
nucleotides in aqueous solution. The preferred protocol uses pre-activation of the 5'-phosphate of the RNA as an imidazolidine at pH 6.5, followed by precipitation and coupling to the peptide at pH 8 with an organocatalyst. The procedure gave peptido RNAs from water-soluble peptides and synthetic oligoribonucleotides in up to 68 % yield. The method is convenient and inexpensive and can produce NMR quantities, opening the door to the systematic exploration of the chemistry of peptido RNAs.

## Introduction

There are several classes of covalently linked hybrids containing a peptide and an RNA chain. The best-known class is that of peptidyl tRNAs, the intermediates of translation. They contain an ester bond between the C-terminus of the peptide and the 2'/3'-terminus of the RNA.<sup>[1]</sup> Full-size peptidyl tRNA is difficult to prepare synthetically, but analogs with more stable linkages have been synthesized.<sup>[2–6]</sup> Another class of covalently linked species is that of nucleopeptides, with a phosphodiester link between the side chain of an amino acid and a short oligoribonucleotide.<sup>[7,8]</sup> They are models for structures found in some viruses. A third class of such covalently linked molecular hybrids are peptido RNAs with a phosphoramidate link between the *N*-terminus of the peptide and the 5'-terminus of the oligoribonucleotides.

Peptido RNA was recently found to form spontaneously from free amino acids and ribonucleotides in aqueous condensation buffer (Scheme 1). Initially, the term 'peptidyl RNA' was used,<sup>[9]</sup> but then changed to 'peptido RNA'<sup>[10]</sup> to indicate more clearly that a different type of linkage is found in these species than in peptidyl tRNAs. Peptido RNAs form by efficient peptide chain growth, once the first amino acid is covalently captured by a

ribonucleotide phosphate, as well as simultaneous, slower oligomerization of the ribonucleotide. The capture event linking the amino acid to the nucleotide and peptide chain growth are more than one order of magnitude faster than the background oligomerization of the amino acid alone.<sup>[10]</sup> Chain growth in the peptido and/or RNA channel is observed for all of the 20 proteinogenic amino acids and all four canonical ribonucleotides.<sup>[11]</sup> Peptido RNA formation can be induced by different condensation agents,<sup>[10]</sup> and the reactivity in the different reaction channels of the reaction system can be tuned by small organocatalysts, such as 1-ethylimidazole and 1- or 3-methyladenine.<sup>[12]</sup>



Scheme 1. Formation or synthesis of peptido RNA.

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Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under <https://doi.org/10.1002/ejoc.202000929>.

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Hybrid molecules accessing peptide and nucleic acid structure space have a rich molecular recognition chemistry, making them interesting for both functional and structural studies.<sup>[13,14]</sup> Because peptido RNAs are reminiscent of the peptidyl tRNAs of translation but more stable, it is also interesting to ask whether they may have played a role in the molecular evolution leading to encoded protein synthesis. Experimental work to address this question has begun.<sup>[15]</sup>

The spontaneous condensation reactions producing peptido RNAs in condensation buffer lead to statistical chain length distributions. The products of oligomerization mixtures are therefore difficult to employ in studies on the properties of peptido RNAs. Chemical, biochemical and structural studies are much easier to perform with homogeneous, pure compounds. Therefore, there is a need for synthetic methodologies that produce monodisperse peptido RNAs in sufficient quantities and high purity.

Several methods for the preparation of peptide-oligonucleotide conjugates are known, most of them employing xenobiotic linkers between the peptide and the nucleic acid

portion.<sup>[16–21]</sup> Further there are methods for directly phosphoramidate-linked peptide DNA hybrids,<sup>[22,23]</sup> and there are literature protocols for the coupling of peptides with several reactive amino groups in homogeneous solution.<sup>[24,25]</sup> The latter contain estimated yields, based on the relative intensity of bands in gels. Exploratory experiments under these conditions<sup>[25]</sup> that had been optimized for long, cationic peptides and RNA, did not produce detectable quantities of product for our target molecules, prompting us to establish reaction conditions independently. Here we report the resulting syntheses of a range of peptido RNAs in pure form, using a convenient, protecting group-free synthesis in aqueous medium.

## Results and Discussion

Figure 1 shows the target molecules of our study. They consist of oligoribonucleotide strands 3–12 nucleotides in length. The very shortest sequence, trimer **1**, was used when rapid assignment of NMR spectra was required, whereas the longer sequen-

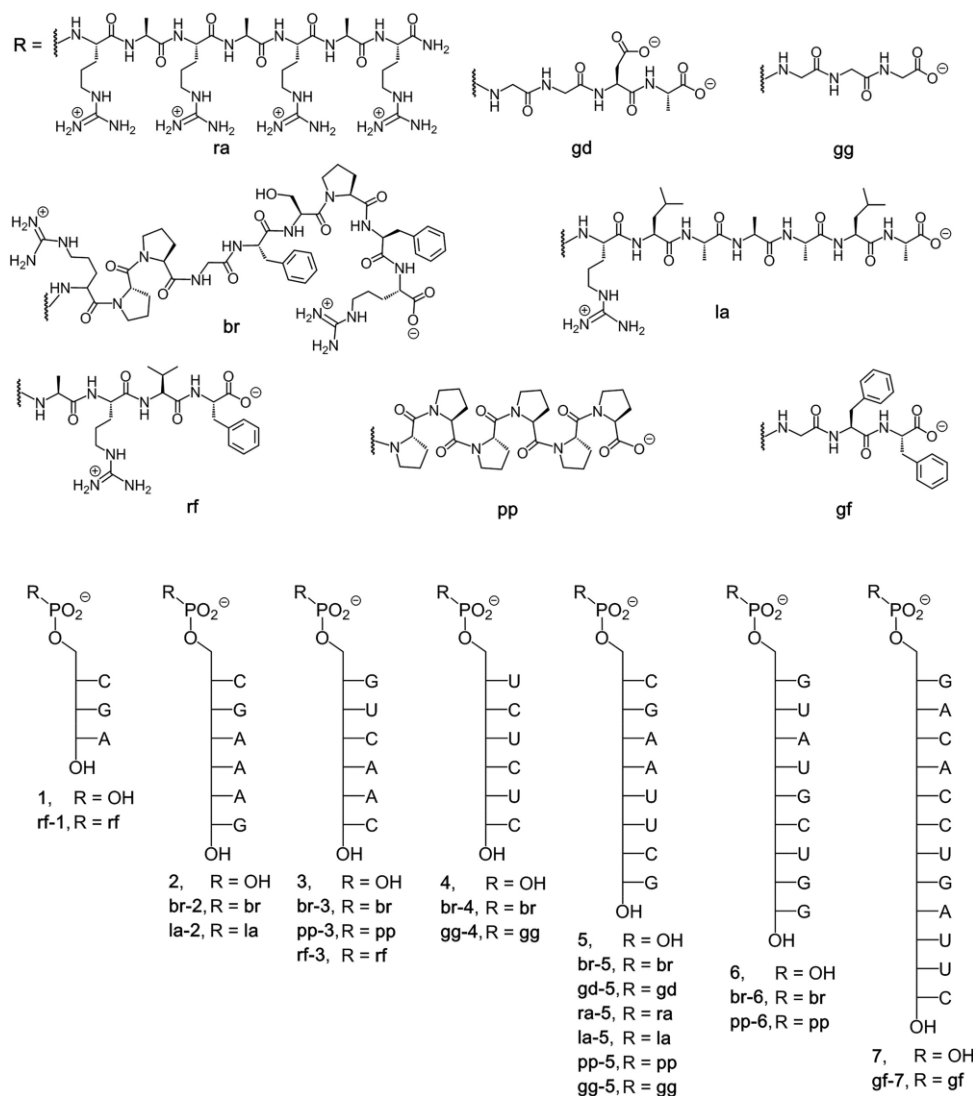


Figure 1. Target peptido RNAs; in the products, peptides are phosphoramidate-linked to the 5'-phosphate of the oligoribonucleotide.

ces (2–7) were employed when the ability to form stable duplexes was desirable. The latter included a self-complementary sequence (5) derived from the Dickerson dodecamer,<sup>[26]</sup> and sequences that allow the formation of heteroduplexes (2–4, 6, 7). Oligonucleotide 1 is a shorter version of 5, for which interesting structural details were expected upon formation of a (partially paired) duplex. The upper part of Figure 1 shows the structures of the peptide chains that were coupled to the oligoribonucleotides.

Among them are sequences with sterically demanding residues (pp), aromatic residues (e.g. gf), cationic residues (ra, rf), and a lipophilic sequence (la). Further, we included bradykinin (br) in the test group, a vasoactive nonapeptide hormone with a range of different amino acid residues.<sup>[27]</sup> Sequences with lysine or ornithine residues were not included to avoid product mixtures resulting from reactions of their side chain amino groups. It is currently unclear whether thioesters are formed in coupling reactions in aqueous solution,<sup>[11]</sup> and disulfide formation is difficult to suppress fully, so cysteine residues were also excluded. All RNA strands and proline hexamer pp were assembled by conventional solid-phase syntheses, as reported in the Experimental Part. The 5'-phosphate of the oligoribonucleotides was introduced by using a known ethylsulfonylethyl phosphoramidite<sup>[28]</sup> as 'chemical phosphorylation reagent' in the final coupling cycle of the RNA synthesis. Other phosphorylation agents exist.<sup>[29]</sup>

Several routes to the target molecules of Figure 1 were considered. Conventional solid-phase peptide synthesis is performed from C- to N-terminus, so generating the peptide on the preassembled RNA chain, as in the synthesis of aminoacyl oligonucleotides<sup>[30,31]</sup> would require an unconventional approach, with carboxy-protected building blocks. There are methods for such 'reversed' solid-phase peptide syntheses with mild cleavage of the protecting groups, but partial racemization via oxazolone intermediates during activation for the next coupling cycle, remains a problem.<sup>[32]</sup> The underlying coupling and deprotection chemistry would also have to be adjusted to avoid side reactions in the oligonucleotide segment.<sup>[33]</sup>

Next, a 'block condensation' of preassembled peptides to a solid support-bound oligoribonucleotide was considered. This requires coupling chemistry that produces the desired phosphoramidate link, e.g. via redox coupling, starting from a 5'-H-phosphonate.<sup>[34,35]</sup> Exploratory experiments under conditions similar to those used for coupling 3'-aminonucleosides,<sup>[36]</sup> were successful with primary amines, but produced unsatisfactory results with polar peptides, in part because of solubility issues in organic solvents. When DMSO was used in combination with bases that keep the amine in the unprotonated state,<sup>[22,23]</sup> strand cleavage and isomerization reactions led us to abandon this approach.

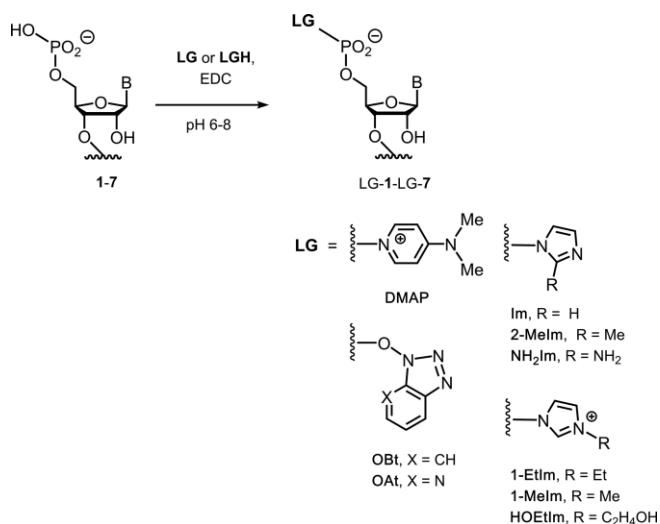
Finally, we settled on coupling peptide and oligoribonucleotide in aqueous solution. Oligoribonucleotides are well soluble in water, and so are most peptides. When unprotected strands are employed, such a coupling can take place late in the overall route, avoiding incompatibilities of protecting groups or deprotection conditions. Further the solvent is non-toxic and inexpensive. Finally, extensive experience with the in situ activation

of 5'-phosphates in recent years<sup>[37,9,10,12]</sup> helped to find conditions that lead to the necessary chemoselectivity.

Experiments involving a one-pot activation/coupling reaction with a large excess of the water-soluble carbodiimide EDC gave discouraging results. Crude reaction mixtures contained side products that were difficult to remove during purification. For example, a reaction mixture containing 10 mM pGUUGAC, 225 mM Gly<sub>3</sub> and 500 mM EDC at pH 6.5 showed peptide oligomers coupled to the RNA and EDC adducts as side products when analyzed by MALDI-TOF mass spectrometry. This led us to abandon this, the most simple approach to forging the covalent link between peptide and oligoribonucleotide.

Instead, we focused on a two-step approach, with separate activation of the 5'-phosphate and coupling of the activated species to the peptide. These two steps, when performed in aqueous solution, have different pH optima, as previously observed in the context of copying,<sup>[38]</sup> ligation,<sup>[39]</sup> or coupling to other nucleophiles.<sup>[40]</sup> The activation requires the introduction of a leaving group to turn the phosphate into an electrophile. Leaving groups known from peptide chemistry, such as benzotriazole or pyridine derivatives may be introduced in activation reactions driven by carbodiimides.<sup>[41,42]</sup> For the activation of the 5'-phosphate of nucleotides in aqueous media, imidazole or imidazole derivatives are frequently used as leaving groups,<sup>[43–45]</sup> making them attractive candidates for the two-step protocol sought.

Scheme 2 gives an overview of the activation methods tested. The progress of the activation reaction was monitored by MALDI-TOF mass spectrometry. Table 1 lists the results thus obtained, based on uncorrected peak intensities, assuming that the introduction of the leaving group has a negligible effect on the desorption and ionization efficiency of the oligonucleotide and that the hydrolysis of the activated species is minimal during sample preparation.



Scheme 2. Activation of the 5'-phosphate of oligonucleotides in EDC-driven reactions introducing a leaving group.

Figure 2 shows mass spectra for active species obtained with octamer 5 and dimethylaminopyridine (DMAP) or imidazole as leaving group. Figure 2a shows a typical result from a reaction

Table 1. Results of activation reactions with oligoribonucleotides **1–7** using different methods, as determined by mass spectrometry.<sup>[a]</sup>

| RNA                     | LG/LGH             | Conc. LG/<br>LHG [M] | EDC [M] | pH  | t [min] | conversion [%] |
|-------------------------|--------------------|----------------------|---------|-----|---------|----------------|
| <b>1</b>                | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 83             |
| <b>2</b>                | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 75             |
| <b>2</b> <sup>[b]</sup> | OAt                | 0.33                 | 0.33    | 6.0 | 100     | 70             |
| <b>3</b>                | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 87             |
| <b>4</b>                | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 76             |
| <b>5</b>                | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 89             |
|                         | 1-EtIm             | 0.29                 | 0.2     | 6.5 | 50      | 61             |
|                         | 1-Melm             | 0.29                 | 0.2     | 6.5 | 60      | 65             |
|                         | 2-Melm             | 0.29                 | 0.2     | 6.5 | 120     | 83             |
|                         | HOEtIm             | 0.29                 | 0.2     | 6.5 | 60      | 49             |
|                         | NH <sub>2</sub> Im | 0.29                 | 0.2     | 6.5 | 140     | 76             |
|                         | OAt                | 0.33                 | 0.5     | 6.0 | 270     | 70             |
|                         | OBt                | 0.3                  | 0.5     | 6.0 | 80      | 85             |
| <b>5</b> <sup>[c]</sup> | DMAP               | 0.15                 | 0.3     | 8.0 | 330     | 72             |
|                         |                    | 0.15                 | 0.5     | 8.0 | 80      | 76             |
|                         |                    | 0.1                  | 0.5     | 8.0 | 180     | 64             |
| <b>5</b> <sup>[d]</sup> |                    | 0.3                  | 0.5     | 8.0 | 210     | 60             |
|                         |                    | 0.15                 | 0.5     | 8.0 | 120     | 87             |
|                         |                    | 0.15                 | 0.5     | 7.0 | 60      | 81             |
| <b>6</b>                | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 86             |
| <b>6</b> <sup>[e]</sup> | OAt                | 0.3                  | 0.5     | 6.0 | 60      | 50             |
|                         |                    | 0.5                  | 0.3     | 6.0 | 90      | 65             |
|                         |                    | 5                    | 3       | 6.0 | 90      | 65             |
|                         |                    | 1                    | 0.6     | 6.0 | 90      | 75             |
| <b>7</b> <sup>[f]</sup> | Im                 | 0.29                 | 0.2     | 6.5 | 60      | 84             |
|                         | OAt                | 0.33                 | 0.5     | 6.0 | 90      | 70             |
| <b>7</b> <sup>[c]</sup> | DMAP               | 0.1                  | 0.5     | 8.0 | 110     | 72             |

[a] Conditions: Aqueous solution, 4 mM oligoribonucleotide, except for. [b] 2 mM. [c] 2.5 mM. [d] Here, 4 mM was used for **5**. [e] 10 mM. [f] 3 mM.

carried out under slightly basic conditions (pH 8.0). Peaks for two types of side products can be discerned. Those at higher molecular weight were assigned to side products from reactions with EDC, such as guanidines or *N*-acylureas. Lowering the pH was found to reduce these side reactions, probably because the most reactive position at the nucleobases, *N1* of uracil,<sup>[46]</sup> was no longer deprotonated to a kinetically significant extent. A minor side product detected at lower mass was most probably caused by cyclization of activated **5**, as the mass of the peak was 18 Da lower than the one of the parent compound. Cyclization reactions of short oligonucleotides of peptidyl oligodeoxynucleotides have been observed previously.<sup>[39,47]</sup>

Among the imidazole derivatives tested, the *N*-alkylated species give imidazolium phosphates when condensed with the 5'-terminus, a species which is much more reactive than the imidazolides obtained with their unalkylated counterparts. For imidazolium bisphosphates, the increase in reactivity has been determined to be approx. 600-fold.<sup>[48]</sup> At the same time, the increase in reactivity makes the imidazolium phosphates more prone to hydrolyze and harder to handle than imidazolides or oxybenzotriazoles. On the other hand, imidazoles with imidazole itself (Im) as leaving group proved easy to prepare and handle. When the pH was increased to above pH 7 at the end of the activation reaction for imidazolides, the active amide was reasonably stable.

We then sought a protocol for removing the excess of activating agent and leaving group prior to coupling to the peptide. For this, precipitation of the activated oligonucleotide was

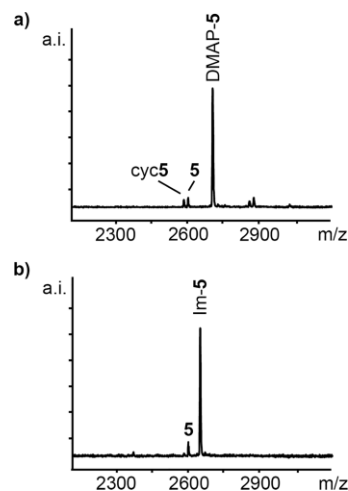
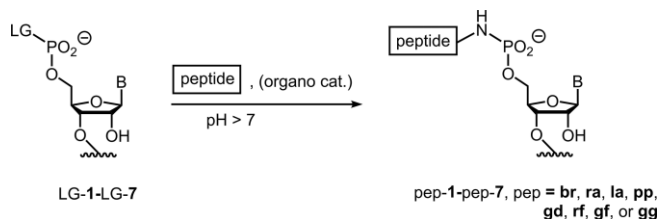


Figure 2. MALDI-TOF spectra from the activation of oligonucleotide **5** with different leaving groups in EDC-driven reactions. a) Reaction with DMAP at pH 8.0 after 2 h. The peak labeled “cyc5” has a mass 18 Da lower than that of **5**, indicating that it is caused by a dehydration reaction, most likely a cyclization. b) Reaction with imidazole at pH 6.5 after 1 h.

induced by adding the reaction mixture to a cold 0.1 M solution of NaClO<sub>4</sub> in acetone/diethyl ether. The precipitate was allowed to settle for 20 min, and the supernatant was removed after centrifugation. The resulting solid was washed with cold acetone and dried in a stream of nitrogen. The activated oligonucleotide thus isolated was then taken up in a basic aqueous solution of the peptide to induce the coupling to the phosphoramidate.

Scheme 3 shows the coupling reaction in a general form. Again, the progress of the transformation was monitored by MALDI-TOF mass spectrometry. Here, the change in structure is more significant than for the activation reaction, making quantitative or semiquantitative analysis without correction for differences in desorption and ionization more questionable. We therefore opted for determining a correction factor<sup>[49]</sup> for representative cases. Surprisingly, calibration plots from spectra of mixtures of RNA **5** and peptido RNA **br-5** showed that the latter gives more intense signals when present at the same concentration as the former, even though the bradykinin chain increases the molecular weight by more than 1000 Da. In the event, the correction factor for detection of peptido RNA **br-5** was found to be 0.62. Perhaps the cationic peptide makes it easier to obtain the singly charged pseudomolecular ion that typically dominates the spectrum than the naked oligoribonucleotides with its eight negative charges.



Scheme 3. General reaction scheme for coupling of a peptide to the activated oligonucleotide; LG = leaving group. See Figure 1 and Scheme 2 for structures.

Figure 3 shows mass spectra from the coupling of bradykinin to different pre-activated forms of oligonucleotide **5**, and Table 2 lists the yields obtained after HPLC or cartridge purification. The best results were obtained when imidazolides were reacted in the presence of 1-ethylimidazole as organocatalyst. The role that this alkylated heterocycle can play in condensation reactions has recently been highlighted in our quantitative studies on co-oligomerization of amino acids<sup>[12]</sup> and nucleotides and ribonucleotides by themselves.<sup>[50]</sup> The reactivity of the imidazolide is boosted by adding the catalyst, and clean and rapid conversion to the peptido RNA results. In favorable cases, the yield of isolated peptido RNA approached or exceeded 50 % after HPLC purification (Table 2) despite the unavoidable loss through partial hydrolysis.

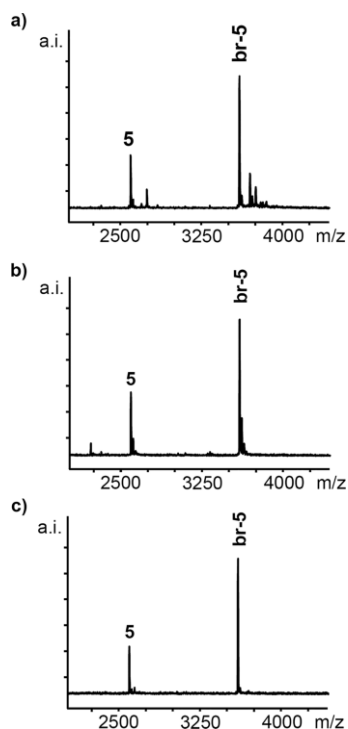


Figure 3. MALDI-TOF spectra from the coupling of activated **5** with bradykinin (**br**) at pH 8 in water. Coupling of a) 4 mM DMAP-**5** to **br** (38 mM) after 16 h, b) 3 mM Im-**5** to **br** (67 mM) after 9 d, and c) 3 mM Im-**5** to **br** (63 mM) with 100 mM 1-EtIm after 16 h.

The reaction times required to obtain the results of Table 2 were quite different. So, although the final yields do not differ much for some products with and without organocatalyst, the reaction times do. For example, the coupling of **Im-5** to bradykinin took over 8 d before the activated species was consumed. The same reaction with 1-EtIm as organocatalyst went to completion in less than one day. We monitored phosphoramidate formation by <sup>31</sup>P-NMR, in model reactions with Gly<sub>3</sub> and activated adenosine 5'-monophosphate (AMP). At pH 8, 40 mM Im-AMP, 400 mM Gly<sub>3</sub>, and 100 mM 1-ethylimidazole (or no organocatalyst), the kinetics of Figure 4 were obtained. A fit to the data gave a second order rate constant (*k*) of 0.44 ± 0.006 mM<sup>-1</sup> h<sup>-1</sup> for the reaction without 1-EtIm and an apparent second order rate constant of *k* = 1.48 ± 0.03 mM<sup>-1</sup> h<sup>-1</sup> for the reaction in the presence of the alkylated imidazole. Also,

Table 2. Results from coupling of peptides to activated oligoribonucleotides.

| LG-ON                        | pep       | equiv. | pH  | org. cat.             | yield [%] |
|------------------------------|-----------|--------|-----|-----------------------|-----------|
| OAt- <b>2</b>                | <b>br</b> | 6.3    | 8.9 | –                     | 10        |
| Im- <b>5</b>                 | <b>br</b> | 20     | 8   | –                     | 41        |
| 1-EtIm- <b>5</b>             | <b>br</b> | 9      | 8   | –                     | 35        |
| 1-Melm- <b>5</b>             | <b>br</b> | 9      | 8   | –                     | 34        |
| 2-Melm- <b>5</b>             | <b>br</b> | 20     | 8.2 | –                     | 26        |
| HOEtIm- <b>5</b>             | <b>br</b> | 9      | 8   | –                     | 16        |
| NH <sub>2</sub> Im- <b>5</b> | <b>br</b> | 9      | 8   | –                     | –         |
| DMAP- <b>5</b>               | <b>br</b> | 9      | 8   | –                     | 38        |
| OAt- <b>5</b>                | <b>br</b> | 8      | 9.2 | –                     | 33        |
| Obt- <b>5</b>                | <b>br</b> | 20     | 8.3 | –                     | 36        |
| OAt- <b>6</b>                | <b>br</b> | 4      | 8   | –                     | 13        |
| Im- <b>2</b>                 | <b>br</b> | 17     | 8   | 1-EtIm                | 42        |
| Im- <b>3</b>                 | <b>br</b> | 22     | 8   | 1-EtIm                | 60        |
| Im- <b>4</b>                 | <b>br</b> | 22     | 8   | 1-EtIm                | 31        |
| Im- <b>5</b>                 | <b>br</b> | 20     | 8   | 1-EtIm <sup>[b]</sup> | 39        |
|                              |           | 19     | 8   | 1-EtIm                | 40        |
|                              |           | 20     | 8   | 1-EtIm <sup>[c]</sup> | 44        |
|                              |           | 20     | 8   | 1-EtIm <sup>[d]</sup> | 41        |
|                              |           | 9      | 8   | 2-NH <sub>2</sub> Im  | 27        |
|                              |           | 12     | 8   | HOEtIm                | 43        |
|                              |           | 23     | 8   | 1-Melm                | 47        |
|                              |           | 20     | 8.2 | 2-Melm                | 36        |
|                              |           | 11     | 8   | Py                    | 20        |
|                              |           | 23     | 8   | tetrazole             | –         |
| Im- <b>6</b>                 | <b>br</b> | 22     | 8   | 1-EtIm                | 40        |
| Im- <b>1</b>                 | <b>rf</b> | 10     | 8   | 1-EtIm                | 42        |
| Im- <b>2</b>                 | <b>la</b> | 20     | 8.9 | 1-EtIm                | 16        |
| Im- <b>3</b>                 | <b>pp</b> | 10     | 8.2 | 1-EtIm                | 42        |
|                              | <b>rf</b> | 36     | 8   | 1-EtIm                | 31        |
| Im- <b>4</b>                 | <b>gg</b> | 35     | 8   | 1-EtIm                | 68        |
| Im- <b>5</b>                 | <b>gd</b> | 37     | 8   | 1-EtIm                | 43        |
|                              | <b>ra</b> | 11     | 8   | 1-EtIm                | 20        |
|                              | <b>la</b> | 20     | 8.9 | 1-EtIm                | 13        |
|                              | <b>pp</b> | 10     | 8.2 | 1-EtIm                | 42        |
|                              | <b>gg</b> | 59     | 8   | 1-EtIm                | 35        |
| Im- <b>6</b>                 | <b>pp</b> | 10     | 8.2 | 1-EtIm                | 25        |
| Im- <b>7</b>                 | <b>gf</b> | 35     | 8.9 | 1-EtIm                | 53        |

[a] Conditions: 2–10 mM of activated oligoribonucleotide in aqueous solution, 100 mM of organocatalyst. [b] 84 mM. [c] 168 mM. [d] 336 mM. Yields were determined by UV/Vis spectroscopy after chromatographic purification.

at these concentrations of nucleotide and peptide, the hydrolysis was found to be negligible. This and the acceleration in the presence of the organocatalyst confirm that the method performs well in concentrated solution.

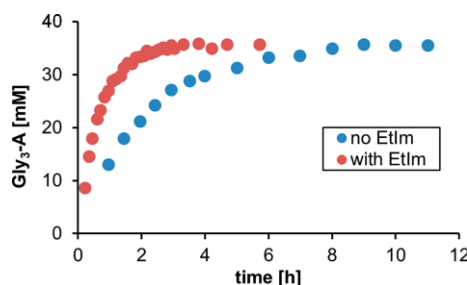
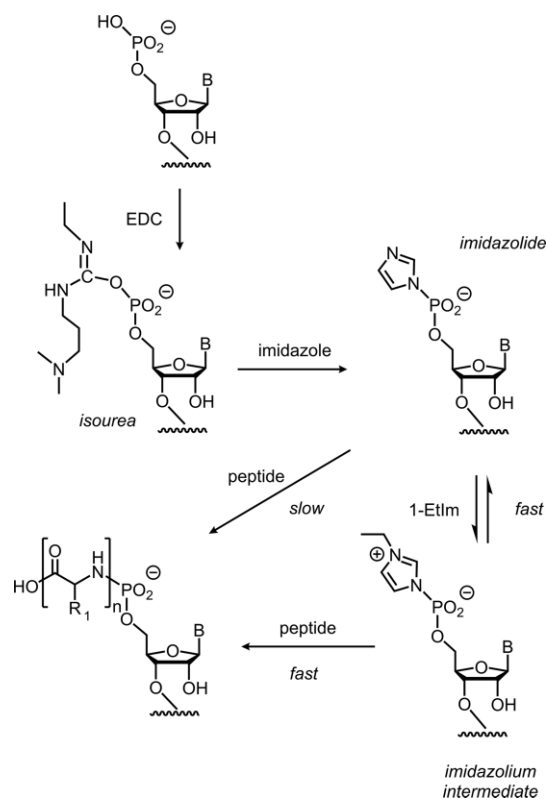


Figure 4. Kinetics of the coupling of Gly<sub>3</sub> to the imidazolide of adenosine 5'-monophosphates with and without 1-EtIm as organocatalyst (100 mM), as monitored by <sup>31</sup>P-NMR 161 MHz spectroscopy. Conditions: 40 mM AMP, 400 mM Gly<sub>3</sub>, H<sub>2</sub>O/D<sub>2</sub>O (9:1, v/v), pH 8. See Figure S1–S2 (SI) for representative spectra and further details.



Overall, our scheme for the synthesis of peptido RNAs involves the steps shown in Scheme 4. The 5'-phosphate group reacts with EDC to give the isourea, which then reacts with imidazole to give the imidazolide in the activation mixture. When the imidazolide is exposed to the organocatalyst in the coupling mixture, some of the more reactive imidazolium phosphate is formed in what is probably an equilibrium reaction. The imidazolium intermediate then reacts with the *N*-terminus of the peptide. Other organocatalysts, such as 1-methylimidazole or 1-(2-hydroxyethyl)imidazole gave similar results as 1-ethylimidazole (Table 2), but tetrazole or 2-aminoimidazole gave no or much less peptido RNA product, indicating that the electronic and steric properties of the heterocycle are important to achieve efficient organocatalytic transamidation and subsequent coupling in the reaction mixture.



Scheme 4. Steps of the activation and coupling leading to the formation of peptido RNA with and without organocatalyst.

Finally, we performed a synthesis of Phe-Val-Arg-Ala-CGA-3' (**rf-1**), starting from 2  $\mu\text{mol}$  of **1** to obtain sufficient material for NMR spectroscopy. The peptido trinucleotide was obtained in 42 % yield from peptide and oligonucleotide after cartridge purification. Figure 5 shows the  $^1\text{H}$ -NMR spectrum in phosphate buffer at pD 7.4. This peptido RNA is short enough to give little peak overlap at the field strength chosen.

The assignment of all resonances in the low field region was readily achieved using a data set with TOCSY, COSY, ROESY, HSQC, and two HMBC spectra ( $^3\text{P}$ - $^1\text{H}$  and  $^{13}\text{C}$ - $^1\text{H}$ ). Spectra are shown in Figures S3–S10 of the Supporting Information. This confirmed that our methodology produces pure material and is suitable for the preparation of samples for structural studies.

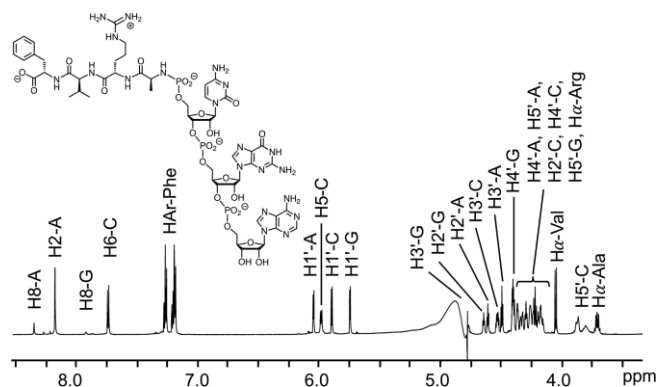


Figure 5.  $^1\text{H}$ -NMR spectrum of Phe-Val-Arg-Ala-CGA-3' (**rf-1**) in  $\text{D}_2\text{O}$  at 700 MHz. Conditions: 200 mM phosphate buffer, pD 7.4, 400 mM NaCl, 25  $^\circ\text{C}$ . Assignments are given above each resonance.

We note that in several instances, cartridge purification was sufficient to produce pure peptido RNAs.

## Conclusions

In here we report the results of a study on the activation and coupling of 5'-phosphorylated oligoribonucleotides to unprotected peptides in aqueous solution. A screen of leaving groups, introduced in the activation reaction, led to imidazolides as the preferred active amides. The imidazolides can be isolated by precipitation and then coupled to the peptide under basic conditions. A 1-alkylated imidazole acts as organocatalyst in the coupling reactions, shortening the reaction times to less than one day. The pH shift between the activation reaction and coupling, as well as the removal of remaining reagents from the activation through a mild precipitation step that does no damage the labile intermediate, allows for clean and efficient transformations. The method thus paves the way for the routine preparation of peptido RNAs of moderate size from inexpensive starting materials, using a rapid and convenient protocol.

## Experimental Section

Phosphoramidite building blocks for the synthesis of RNA strands were from Future Synthesis (Poznań, Poland). The controlled-pore glass loaded with the first nucleoside was from Sigma/Aldrich (Taufkirchen, Germany) and so were all reagents used for solid-phase synthesis. The phosphorylating agent 2-[2-(4,4'-dimethoxytrityloxy)ethylsulfonyl]ethyl-(2-cyanoethyl)-(*N,N*-diisopropyl)phosphoramidite (**8**) was synthesized in house, following a literature protocol.<sup>[28]</sup> Peptides were purchased from Bachem (Bubendorf, Switzerland) or Genscript Biotech (Piscataway Township, USA). The Fmoc-protected and chlorotrityl resin-bound proline as well as the coupling reagents for solid-phase peptide synthesis were from Iris Biotech (Martredwitz, Germany). The activating agent EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) was from Carbo-synth (Compton, Berkshire Great Britain). Other chemicals or solvents were from Sigma Aldrich (Taufkirchen, Germany), Acros (Geel, Belgium), or Merck (Darmstadt, Germany). The NMR spectra were measured on a Bruker 700, 500 or 400 MHz spectrometer (Bruker, Rheinstetten, Germany) in  $\text{D}_2\text{O}$  or  $\text{H}_2\text{O}/\text{D}_2\text{O}$  (9:1, v/v).  $^1\text{H}$ -NMR spectra were acquired with 300, 400 and 700 MHz,  $^{31}\text{P}$ -NMR spectra at

284 or 161 MHz and  $^{13}\text{C}$ -NMR spectra at 176 MHz. Chemical shifts are given in  $\delta$  values (ppm) and coupling constants (J) are given in Hertz. Multiplicities are given as: *s* (singlet), *d* (doublet), *dd* (doublet of doublets), *t* (triplet), *q* (quartet) and *m* (multiplet). Concentrations of oligonucleotides were determined with a NanoDrop UV/Vis microspectrometer (1000 NanoDrop Technologies, Wilmington, USA). The absorption of aqueous solutions was determined at 260 nm with 1 mm path length, as the average of three measurements per sample.

**MALDI-TOF MS.** The MALDI-TOF spectra were acquired on a Bruker MICROFLEX spectrometer (Billerica, MA) equipped with a  $\text{N}_2$  laser (337 nm). Monitoring of reactions involved drawing 0.1  $\mu\text{L}$  of the reaction solution, diluting with distilled water (4  $\mu\text{L}$ ), and treating with approx. three beads of cation exchange resin (Dowex 50 WX8–200, ammonium form). An aliquot of the supernatant (0.5  $\mu\text{L}$ ) was mixed with 0.5  $\mu\text{L}$  of a mixture of matrix (0.3 M trihydroxyacetophenone in EtOH) and co-matrix (0.1 M aqueous triammonium citrate) (2:1, v/v) on the MALDI target. Measurements were taken in linear negative mode, using the Flex Control software. Calculated masses are average masses, masses found are maxima of the unresolved isotope pattern.

**HPLC purification.** Purification of peptido RNAs was by RP HPLC with an UltiMate 3000 system from Thermo Fischer (Karlsruhe, Germany). A C18 column EC 250 mm/4.6 mm Nucleosil 120–5 (Macherey-Nagel, Düren, Germany) or a DNA-Pac RP 4  $\mu\text{m}$  2.1  $\times$  100 mm (Thermo Fischer, Karlsruhe, Germany) was used with either a 0.05 M ammonium bicarbonate (pH 8) or a 0.1 M triethylammonium acetate buffer (pH 7) and a gradient of acetonitrile. The column was held at 55  $^\circ\text{C}$  and a flow of 0.6–0.65 mL/min was used. Detection was at 260 nm. Fractions were analyzed by MALDI-TOF MS.

**Cartridge purification.** Peptido RNAs and oligonucleotides were purified by using either a Sep-Pak Vac C18 3cc RP cartridge or a Sep-Pak Vac QMA 3cc IEX cartridge. The RP cartridges were rinsed with acetonitrile and equilibrated with either 0.1 M triethylammonium bicarbonate buffer (TEAB, pH 8) or 0.05 M ammonium bicarbonate buffer (pH 8). The crude product was dissolved in one of these buffers, applied to the cartridge, and the target compound was eluted with a gradient of acetonitrile. The IEX cartridges were first rinsed with water, and then equilibrated with ammonium carbonate buffer (5 mM, pH 8). The crude was dissolved in the same buffer, followed by eluting the product with a gradient of increasing ammonium carbonate concentration (5–500 mM).

**Oligoribonucleotide synthesis.** The 5'-phosphorylated oligonucleotides were synthesized from 2'-TBDMS-protected phosphoramidites with acyl base protection, using a PE 8909 Expedite DNA synthesizer and the protocol recommended by the manufacturer. The final coupling cycle used phosphorylating agent (**8**). The removal of the protecting groups and the release from the support was induced by incubating the cpg with AMA (methylamine/ $\text{NH}_4\text{OH}$ , 1:1, v/v, 2 mL) at 55  $^\circ\text{C}$  for 10 min. The 2'-TBDMS groups were removed using  $\text{NET}_3\cdot 3\text{HF}$  for 1 h per nucleotide (e.g. 8 h for an octamer), followed by quenching with TMS-OMe (5 mL per mL of  $\text{NET}_3\cdot 3\text{HF}$ ). The oligonucleotides precipitated from the quenched solution, and were isolated by centrifugation. The crudes were then purified by RP cartridge, using 0.1 M TEAB buffer/ $\text{CH}_3\text{CN}$ , or IEX, eluting with increasing ammonium carbonate, as described above.

**CGA (1).** Yield: 75 %; RP cartridge (TEAB), eluted at 5 %  $\text{CH}_3\text{CN}$ ; MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{29}\text{H}_{37}\text{N}_{13}\text{O}_{21}\text{P}_3$  [ $\text{M} - \text{H}$ ] $^-$ : 997, found 997.

**CGAAAG (2).** Yield: 44 %; RP cartridge (TEAB), eluted with 5 %  $\text{CH}_3\text{CN}$ ; MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{59}\text{H}_{73}\text{N}_{28}\text{O}_{40}\text{P}_6$  [ $\text{M} - \text{H}$ ] $^-$ : 2000, found 1998.

**GUCAAC (3).** Yield: 70 %; RP cartridge (TEAB), eluted at 5 %  $\text{CH}_3\text{CN}$ ; MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{57}\text{H}_{72}\text{N}_{23}\text{O}_{42}\text{P}_6$  [ $\text{M} - \text{H}$ ] $^-$ : 1937, found 1936.

**UCUCUC (4).** Yield: 50 %; RP cartridge (TEAB), eluted at 6 %  $\text{CH}_3\text{CN}$ ; MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{54}\text{H}_{70}\text{N}_{15}\text{O}_{46}\text{P}_6$  [ $\text{M} - \text{H}$ ] $^-$ : 1851, found 1851.

**CGAAUUCG (5).** Yield: 66 %; RP cartridge (TEAB), eluted at 9 %  $\text{CH}_3\text{CN}$ ; MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{76}\text{H}_{95}\text{N}_{30}\text{O}_{57}\text{P}_8$  [ $\text{M} - \text{H}$ ] $^-$ : 2589, found 2589.

**GUAUGCUGG (6).** Yield: 66 %; RP cartridge (TEAB), eluted at 11 %  $\text{CH}_3\text{CN}$ ; MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{86}\text{H}_{106}\text{N}_{34}\text{O}_{66}\text{P}_9$  [ $\text{M} - \text{H}$ ] $^-$ : 2951, found 2951.

**GACACCUGAUUC (7).** Yield: 38 %; IEX cartridge: eluted at 400 mM ( $\text{NH}_4$ ) $_2\text{CO}_3$ , MALDI-TOF MS: *m/z* calcd. for  $\text{C}_{113}\text{H}_{142}\text{N}_{43}\text{O}_{85}\text{P}_{12}$  [ $\text{M} - \text{H}$ ] $^-$ : 3834, found 3836.

**H-(Pro) $_6$ -OH (pp).** The 2-chlorotrityl resin (500 mg) loaded with Fmoc-protected proline (0.6 mmol/g) was treated with DCM/DMF (5 mL, 1:1, v/v). Deprotection was performed with piperidine/DMF (5 mL, 1:4, v/v) for 2  $\times$  10 min. Activation and coupling were carried out by treating Fmoc-Pro-OH (320 mg, 900  $\mu\text{mol}$ , 3 equiv.) with *N,N*-diisopropylethylamine (DIPEA, 306  $\mu\text{L}$ , 1800  $\mu\text{mol}$ , 6 equiv.) in DMF/DCM (5 mL, 1:1, v/v), and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium tetrafluoroborate (DMTMMT, 295 mg, 900  $\mu\text{mol}$ , 3 equiv.). After stirring for 3 min, the solution was added to the resin and incubated for 2 h at 50  $^\circ\text{C}$ . The resin was then washed with DMF/DCM (20 mL, 1:1, v/v), and the coupling step was repeated. The peptide was cleaved from the support with a solution of TFA/ $\text{H}_2\text{O}$ /DCM (10 mL per gram of resin, 1:1:8, v/v/v) with stirring for 2 h. Then, the resin was filtered off, and the filtrate was added to ice cold diethyl ether (10 mL per mL of TFA solution) to obtain a white precipitate. This solid was isolated, washed with diethyl ether (10 mL per mL of TFA solution), dissolved in water and lyophilized. A white solid (190 mg, 266  $\mu\text{mol}$ , 89 %) of H-(Pro) $_6$ -OH was obtained that was used without further purification.  $^1\text{H}$ -NMR (700 MHz,  $\text{D}_2\text{O}$ , 25  $^\circ\text{C}$ ):  $\delta$  = 4.78–4.41 (m, 6 H, CH $\alpha$ ), 3.85–3.34 (m, 12 H,  $\delta$ (CH $_2$ )), 2.60–2.30 (m, 6 H,  $\beta$ (CH $_2$ )), 2.09–1.99 (m, 14 H,  $\gamma$ (CH $_2$ ),  $\beta$ (CH $_2$ )), 1.94–1.88 (m, 4 H,  $\beta$ (CH $_2$ )).  $^{13}\text{C}$ -NMR (176 MHz,  $\text{D}_2\text{O}$ , 25  $^\circ\text{C}$ ):  $\delta$  = 175.9, 172.0, 171.5, 171.4, 171.0, 167.6, 59.3, 59.0, 58.9, 58.6, 58.5, 58.7, 47.6, 47.5, 47.3, 46.6, 28.7, 28.2, 27.9, 27.8, 27.7, 24.5, 24.5, 24.5, 23.8. MS (ESI $^-$ ): *m/z* calcd. for  $\text{C}_{30}\text{H}_{44}\text{N}_6\text{O}_7$  [ $\text{M} - \text{H}$ ] $^-$ : 599.320, found 599.320.

**Anion-exchange for peptides.** Peptides obtained as trifluoroacetate or acetate salts were subjected to anion exchange to the chloride form to avoid side reactions. The following protocol is for bradykinin and is representative. The TFA salt (30 mg, 25.4  $\mu\text{mol}$ ) was dissolved in aqueous HCl (1 M, 0.3 mL), and the trifluoroacetic acid was removed by extracting five times with diethyl ether (3 mL each). The solution was then neutralized with NaOH solution (10 mM), and the peptide was precipitated with acetone (30 mL). Removal of the trifluoroacetate was verified by  $^{19}\text{F}$ -NMR, and the concentration of the peptide was determined by  $^1\text{H}$ -NMR spectroscopy, using signal intensities and 3-(trimethylsilyl)-1-propanesulfonic acid as internal standard. The solution was then lyophilized, and the peptide was dissolved in water to give a stock solution.

**Activation of 5'-phosphate as imidazolide (General Protocol A).** The following protocol for Im-5 and is representative. An aqueous solution (1 mL) of imidazole (20 mg, 290  $\mu\text{mol}$ , 290 mM) and EDC

hydrochloride (38 mg, 290  $\mu\text{mol}$ , 200 mM) was adjusted to pH 6.5 with a 6 M HCl solution. An aliquot of this solution was added to the lyophilized oligoribonucleotide (**5**, 100 nmol) to give a final concentration of 3 mM (30  $\mu\text{L}$ ). The mixture was allowed to react at 25  $^{\circ}\text{C}$ , and the activation of the 5'-phosphate was monitored by MALDI-TOF MS. After 1 h, the pH was adjusted to a value of 8.0 with a 10 M NaOH solution, and the oligonucleotide Im-**5** was precipitated by adding a solution of 100 mM  $\text{NaClO}_4$  in acetone/diethyl ether (1.5:1, v/v, 3 mL) at  $-20^{\circ}\text{C}$ . The mixture was centrifuged and the supernatant discarded. The precipitate was washed with acetone (5 mL at  $-20^{\circ}\text{C}$ ), again isolated by centrifugation, and blown dry with nitrogen. MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{79}\text{H}_{97}\text{N}_{32}\text{O}_{56}\text{P}_8$  [M - H] $^-$ : 2639, found 2640.

**Coupling of peptide to activated RNA (General Protocol B).** The following protocol is for **br-5** and is representative. An aqueous solution (32  $\mu\text{L}$ ) of bradykinin (2  $\mu\text{mol}$ , 63 mM) and 1-EtIm (3.2  $\mu\text{mol}$ , 100 mM) was adjusted to pH 8 and used to dissolve oligoribonucleotide Im-**5** (100 nmol, 3 mM). The mixture was allowed to react at 25  $^{\circ}\text{C}$  for 24 h until the activated oligoribonucleotide was fully consumed. The progress of the coupling was monitored by MALDI-TOF MS. The solution was diluted with water (200  $\mu\text{L}$ ), and peptido RNA **br-5** was purified by HPLC with a flow of 0.6 mL/min, 55  $^{\circ}\text{C}$ , 0.1 M TEAA/ $\text{CH}_3\text{CN}$  0% for 5 min, 0–10% in 15 min, 10–22% in 30 min,  $t_R$  = 39 min. Peptido RNA **br-5** was obtained in a yield of 40% (40 nmol) after lyophilization of product-containing fractions. MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{126}\text{H}_{166}\text{N}_{45}\text{O}_{67}\text{P}_8$  [M - H] $^-$ : 3631, found 3629.

**Phe-Val-Arg-Ala-CGA-3' (rf-1).** Activated RNA Im-**1** (2  $\mu\text{mol}$ ), prepared according to General Protocol A was treated with peptide **rf** (100 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 24 h to give a concentration of 10 mM oligoribonucleotide. RP cartridge (25  $^{\circ}\text{C}$ , 0.05 M  $\text{NH}_4\text{HCO}_3$ ) with product eluting at 9%  $\text{CH}_3\text{CN}$  was followed by IEX cartridge with product eluting at 200 mM  $(\text{NH}_4)_2\text{CO}_3$ . Yield: 840 nmol (42%).  $^1\text{H-NMR}$  (700 MHz,  $\text{D}_2\text{O}$ , 400 mM NaCl, 200 mM phosphate buffer, pD 7.4, 25  $^{\circ}\text{C}$ ):  $\delta$  = 8.33 (s, 1 H, AH8), 8.16 (s, 1 H, AH2), 7.90 (s, 1 H, GH8), 7.72 (d,  $^3J$  = 7.5 Hz, 1 H, CH6), 7.17–7.26 (m, 5 H, Phe(ArH)), 6.04 (d,  $^3J$  = 4.2 Hz, 1 H, AH1'), 5.98 (d,  $^3J$  = 7.5 Hz, 1 H, CH6), 5.89 (d,  $^3J$  = 5.5 Hz, 1 H, CH1'), 5.74 (d,  $^3J$  = 4.2 Hz, 1 H, GH1'), 4.82 (m, 1 H, GH3'), 4.65 (dd,  $^3J$  = 4.7 Hz, 1 H, GH2'), 4.62 (dd,  $^3J$  = 4.7 Hz, 1 H, AH2'), 4.53–4.55 (m, 1 H, CH3'), 4.50 (dd,  $^3J$  = 4.7 Hz, 1 H, AH3'), 4.40–4.42 (m, 2 H, PheH $\alpha$ , GH4'), 4.37–4.38 (m, 1 H, AH4'), 4.33–4.35 (m, 1 H, AH5'), 4.30 (dd,  $^3J$  = 5 Hz, 1 H, CH2'), 4.27–4.28 (m, CH4'), 4.24–4.26 (m, 1 H, GH5'), 4.23 (t, 3 H,  $^3J$  = 7 Hz, ArgH $\alpha$ ), 4.18–4.21 (m, 1 H, AH5'), 4.16–4.18 (m, 1 H, GH5'), 4.06 (d,  $^3J$  = 8.2 Hz, 1 H, ValH $\alpha$ ), 3.79–3.90 (m, 2 H, CH5'), 3.70–3.74 (m, 1 H, AlaH $\alpha$ ), 3.10 (dd,  $^2J$  = 14 Hz,  $^3J$  = 4.7 Hz, 1 H, PheH $\beta$ ), 3.06 (t,  $^3J$  = 7.3 Hz, 2 H, ArgH $\delta$ ), 2.90 (dd,  $^2J$  = 14 Hz,  $^3J$  = 8.2 Hz, 1 H, PheH $\beta$ ), 1.95–2.01 (m, 1 H, ValH $\beta$ ), 1.62–1.72 (m, 2 H, ArgH $\beta$ ), 1.36–1.52 (m, 2 H, ArgH $\gamma$ ), 1.28 (d,  $^3J$  = 7 Hz, 3 H, AlaH $\beta$ ), 0.86 (dd,  $^3J$  = 7,  $^2J$  = 11 Hz, 6 H, ValH $\gamma$ ).  $^{13}\text{C-NMR}$  (176 MHz,  $\text{D}_2\text{O}$ , 400 mM NaCl, 200 mM phosphate buffer, pD 7.4, 25  $^{\circ}\text{C}$ ):  $\delta$  = 180.5, 180.1, 175.9, 175.1, 168.7, 160.2, 159.3, 158.1, 156.4, 155.6, 153.7, 151.1, 143.5, 141.9, 140.3, 132.0, 131.2, 129.4, 121.3, 99.39, 91.6, 91.2, 90.6, 85.7, 84.7, 84.5, 77.1, 76.9, 76.2, 75.6, 72.3, 67.7, 66.6, 62.3, 59.3, 56.1, 53.7, 43.4, 40.6, 32.9, 31.1, 27.1, 23.0, 22.9, 21.3, 20.6. MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{52}\text{H}_{72}\text{N}_{20}\text{O}_{25}\text{P}_3$  [M - H] $^-$ : 1470, found 1468.

**Phe-Val-Arg-Ala-GUCAAC-3' (rf-3).** Activated RNA Im-**3** (100 nmol), prepared according to General Protocol A was treated with peptide **rf** (109 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 20 h with a concentration of 3 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55  $^{\circ}\text{C}$ , 0.1 M TEAA):

$\text{CH}_3\text{CN}$  0% for 5 min, 0–10% in 20 min, 10–18% in 40 min,  $t_R$  = 33 min. Yield: 31 nmol (31%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{80}\text{H}_{107}\text{N}_{30}\text{O}_{46}\text{P}_6$  [M - H] $^-$ : 2411, found 2410.

**Arg-Phe-Pro-Ser-Phe-Gly-Pro-Pro-Arg-CGAAAG-3' (br-2).** Activated RNA Im-**2** (100 nmol), prepared according to General Protocol A was treated with peptide **br** (68 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 24 h with a concentration of 4 mM oligoribonucleotide. HPLC (Flow 0.8 mL/min, 55  $^{\circ}\text{C}$ , 0.05 M  $\text{NH}_4\text{HCO}_3$ ):  $\text{CH}_3\text{CN}$  0% for 5 min, 0–10% in 50 min,  $t_R$  = 50 min. Yield: 42 nmol (42%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{109}\text{H}_{144}\text{N}_{43}\text{O}_{50}\text{P}_6$  [M - H] $^-$ : 3042, found 3041.

**Arg-Phe-Pro-Ser-Phe-Gly-Pro-Pro-Arg-GUCAAC-3' (br-3).** Activated RNA Im-**3** (100 nmol), prepared according to General Protocol A was treated with peptide **br** (74 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 18 h with a concentration of 4 mM oligoribonucleotide. HPLC (Flow 0.8 mL/min, 55  $^{\circ}\text{C}$ , 0.05 M  $\text{NH}_4\text{HCO}_3$ ):  $\text{CH}_3\text{CN}$  0% for 5 min, 0–15% in 50 min,  $t_R$  = 37 min. Yield: 60 nmol (60%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{107}\text{H}_{143}\text{N}_{38}\text{O}_{52}\text{P}_6$  [M - H] $^-$ : 2979, found 2981.

**Arg-Phe-Pro-Ser-Phe-Gly-Pro-Pro-Arg-UCUCUC-3' (br-4).** Activated RNA Im-**4** (100 nmol), prepared according to General Protocol A was treated with peptide **br** (63 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 16 h with a concentration of 3 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55  $^{\circ}\text{C}$ , 0.1 M TEAA):  $\text{CH}_3\text{CN}$  0% for 5 min, 0–15% in 10 min, 15–25% in 25 min,  $t_R$  = 26 min. Yield: 31 nmol (31%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{104}\text{H}_{141}\text{N}_{30}\text{O}_{56}\text{P}_6$  [M - H] $^-$ : 2893, found 2892.

**Arg-Phe-Pro-Ser-Phe-Gly-Pro-Pro-Arg-GUAUGCUGG-3' (br-6).** Activated RNA Im-**6** (100 nmol), prepared according to General Protocol A was treated with peptide **br** (67 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 24 h with a concentration of 4 mM oligoribonucleotide. IEX cartridge (25  $^{\circ}\text{C}$ , 0.05 M  $(\text{NH}_4)_2\text{CO}_3$ ): with product eluting at 300 mM  $(\text{NH}_4)_2\text{CO}_3$ . Yield: 40 nmol (40%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{136}\text{H}_{177}\text{N}_{49}\text{O}_{76}\text{P}_9$  [M - H] $^-$ : 3993, found 3992.

**Ala-Asp-Gly-Gly-CGAAUUCG-3' (gd-5).** Activated RNA Im-**5** (50 nmol), prepared according to General Protocol A was treated with peptide **gd** (146 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 6 h with a concentration of 4 mM oligoribonucleotide. HPLC (DNA-Pac RP 4  $\mu\text{m}$  2.1  $\times$  100 mm, 55  $^{\circ}\text{C}$ , Flow: 0.35 mL/min, 0.1 M TEAA):  $\text{CH}_3\text{CN}$  0% for 5 min, 0–0.5% in 15 min, 0.5–1% in 30 min,  $t_R$  = 20 min. Yield 21.5 nmol (43%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{87}\text{H}_{111}\text{N}_{34}\text{O}_{63}\text{P}_8$  [M - H] $^-$ : 2889, found 2887.

**Arg-Ala-Arg-Ala-Arg-CGAAUUCG-3' (ra-5).** Activated RNA Im-**5** (800 nmol), prepared according to General Protocol A was treated with peptide **ra** (45 mM) and 1-EtIm (116 mM) at pH 8.3 according to General Protocol B for 90 h with a concentration of 4 mM oligoribonucleotide. HPLC (Flow 0.8 mL/min, 55  $^{\circ}\text{C}$ , 0.05 M  $\text{NH}_4\text{HCO}_3$ ):  $\text{CH}_3\text{CN}$  0% for 5 min, 0–10% in 50 min,  $t_R$  = 33 min. Yield: 160 nmol (20%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{109}\text{H}_{159}\text{N}_{50}\text{O}_{63}\text{P}_8$  [M - H] $^-$ : 3426, found 3424.

**Ala-Leu-Ala-Ala-Ala-Leu-Arg-CGAAUUCG-3' (la-5).** Activated RNA Im-**5** (100 nmol), prepared according to General Protocol A was treated with peptide **la** (40 mM) and 1-EtIm (100 mM) at pH 8.9 according to General Protocol B for 52 h with a concentration of 2 mM oligoribonucleotide. HPLC (Flow 0.8 mL/min, 55  $^{\circ}\text{C}$ , 0.05 M  $\text{NH}_4\text{HCO}_3$ ):  $\text{CH}_3\text{CN}$  0% for 5 min, 0–10% in 50 min,  $t_R$  = 35 min. Yield: 13 nmol (13%). MALDI-TOF MS:  $m/z$  calcd. for  $\text{C}_{106}\text{H}_{149}\text{N}_{40}\text{O}_{64}\text{P}_8$  [M - H] $^-$ : 3255, found 3253.

**(Pro) $_6$ -CGAAUUCG-3' (pp-5).** Activated RNA Im-**5** (100 nmol), prepared according to General Protocol A was treated with peptide **pp**



(50 mM) and 1-EtIm (100 mM) at pH 8.2 according to General Protocol B for 16 h with a concentration of 4 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55 °C, 0.05 M NH<sub>4</sub>HCO<sub>3</sub>): CH<sub>3</sub>CN 0 % for 5 min, 0–15 % in 50 min,  $t_R = 25$  min. Yield: 42 nmol (42 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>106</sub>H<sub>137</sub>N<sub>36</sub>O<sub>63</sub>P<sub>8</sub> [M – H]<sup>-</sup>: 3171, found 3172.

**(Gly)<sub>3</sub>-CGAAUUCG-3' (gg-5).** Activated RNA Im-5 (100 nmol), prepared according to General Protocol A was treated with peptide **gg** (177 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 3 h with a concentration of 3 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55 °C, 0.1 M TEAA): CH<sub>3</sub>CN 0 % for 5 min, 0–5 % in 15 min, 5–8 % in 45 min,  $t_R = 44$  min. Yield: 35 nmol (35 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>82</sub>H<sub>104</sub>N<sub>33</sub>O<sub>60</sub>P<sub>8</sub> [M – H]<sup>-</sup>: 2760, found 2760.

**Ala-Leu-Ala-Ala-Ala-Leu-Arg-CGAAAG-3' (la-2).** Activated RNA Im-2 (100 nmol), prepared according to General Protocol A was treated with peptide **la** (39 mM) and 1-EtIm (100 mM) at pH 8.9 according to General Protocol B for 52 h with a concentration of 2 mM oligoribonucleotide. HPLC (Flow 0.8 mL/min, 55 °C, 0.05 M NH<sub>4</sub>HCO<sub>3</sub>): CH<sub>3</sub>CN 0 % for 5 min, 0–10 % in 50 min,  $t_R = 40$  min. Yield: 16 nmol (16 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>89</sub>H<sub>127</sub>N<sub>38</sub>O<sub>47</sub>P<sub>6</sub> [M – H]<sup>-</sup>: 2667, found 2666.

**(Pro)<sub>6</sub>-GUCAAC-3' (pp-3).** Activated RNA Im-3 (100 nmol), prepared according to General Protocol A was treated with peptide **pp** (50 mM) and 1-EtIm (100 mM) at pH 8.2 according to General Protocol B for 16 h with a concentration of 5 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55 °C, 0.05 M NH<sub>4</sub>HCO<sub>3</sub>): CH<sub>3</sub>CN 0 % for 5 min, 0–15 % in 50 min,  $t_R = 27$  min. Yield: 42 nmol (42 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>87</sub>H<sub>114</sub>N<sub>29</sub>O<sub>48</sub>P<sub>6</sub> [M – H]<sup>-</sup>: 2520, found 2519.

**(Pro)<sub>6</sub>-GUAUGCUGG-3' (pp-6).** Activated RNA Im-6 (100 nmol), prepared according to General Protocol A was treated with peptide **pp** (50 mM) and 1-EtIm (100 mM) at pH 8.2 according to General Protocol B for 16 h with a concentration of 5 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55 °C, 0.05 M NH<sub>4</sub>HCO<sub>3</sub>): CH<sub>3</sub>CN 0 % for 5 min, 0–15 % in 50 min,  $t_R = 25$  min. Yield: 25 nmol (25 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>116</sub>H<sub>148</sub>N<sub>40</sub>O<sub>72</sub>P<sub>9</sub> [M – H]<sup>-</sup>: 3533, found 3536.

**(Gly)<sub>3</sub>-UCUCUC-3' (gg-4).** Activated RNA Im-4 (100 nmol), prepared according to General Protocol A was treated with peptide **gg** (177 mM) and 1-EtIm (100 mM) at pH 8 according to General Protocol B for 3 h with a concentration of 3 mM oligoribonucleotide. HPLC (Flow 0.6 mL/min, 55 °C, 0.05 M NH<sub>4</sub>HCO<sub>3</sub>): CH<sub>3</sub>CN 0 % for 5 min, 0–6 % in 20 min,  $t_R = 7.5$  min. Yield: 68 nmol (68 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>60</sub>H<sub>79</sub>N<sub>18</sub>O<sub>49</sub>P<sub>6</sub> [M – H]<sup>-</sup>: 2022, found 2023.

**Phe-Phe-Gly-GACACCUAGUUC-3' (gf-7).** Activated RNA Im-7 (100 nmol), prepared according to General Protocol A was treated with peptide **gf** (117 mM) and 1-EtIm (100 mM) at pH 8.6 according to General Protocol B with a concentration of 3 mM oligoribonucleotide. The reaction mixture was incubated for 1 h at 50 °C in an ultrasonic bath. HPLC (Flow 0.6 mL/min, 55 °C, 0.1 M TEAA): CH<sub>3</sub>CN 0 % for 5 min, 0–18 % in 40 min,  $t_R = 38$  min. Yield: 53 nmol (53 %). MALDI-TOF MS:  $m/z$  calcd. for C<sub>133</sub>H<sub>163</sub>N<sub>46</sub>O<sub>88</sub>P<sub>12</sub> [M – H]<sup>-</sup>: 4186, found 4186.

## Acknowledgments

The authors thank H. Griesser, D. Göhringer, S. Motsch, O. Bernhard and E. Kervio for discussions. This work was supported by Volkswagen Foundation (grant Az 92 768) and Deutsche Forschungsgemeinschaft (DFG) project ID 364653263 - TRR 235, as well as grant RI 1063/16-1. Open access funding enabled and organized by Projekt DEAL.

**Keywords:** Oligonucleotides · RNA · Peptides · Organocatalysis · Phosphoramidates

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Received: July 3, 2020