Nucleotides

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Expeditious, Potentially Primordial, Aminoacylation of Nucleotides**

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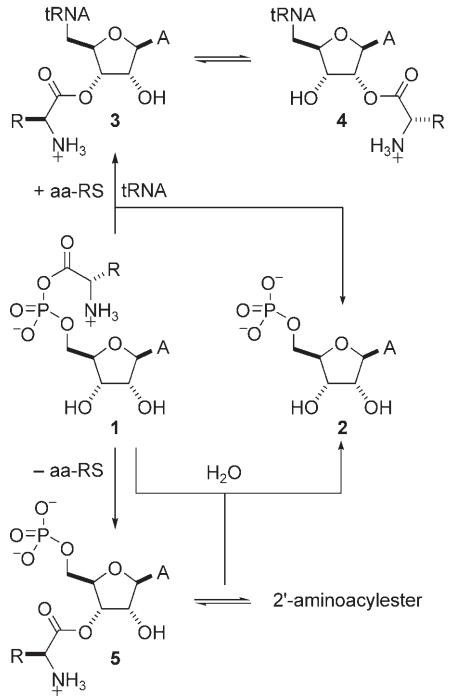
In contemporary biochemistry, enzymatically synthesized, activated aminoacyl-tRNA esters (aa-tRNA) serve as substrates for coded peptide synthesis by ribosomes,[1] and a major goal is to understand the evolutionary path through which this process arose. As a first step towards this goal, we have been looking to find a prebiotically plausible means for the aminoacylation of ribonucleotide 2′-/3′-hydroxy groups. A number of simple, potentially prebiotic, activated amino acid derivatives have been reported, most notably *N*-carboxyanhydrides (NCAs).[2] The case for NCAs in prebiotic evolution has recently been strengthened by the finding that they can be produced from amino acids through the action of the simple volcanic gas carbonyl sulfide.[3]

We therefore decided to investigate the aminoacylation of nucleotides by NCAs. Initially we were concerned that the nucleobase amino groups of adenine and cytosine would prove more nucleophilic than the 2′-/3′-hydroxy groups; however, recent findings show that NCAs react with inorganic phosphate to give aminoacyl phosphates.[4] This suggests that it might be possible to generate nucleotide aminoacyl esters by initial aminoacylation of the phosphate monoester, followed by intramolecular aminoacyl transfer. Nucleotide aminoacylation by way of intermediate carboxylic phosphoric anhydrides would be analogous to the chemistry of aminoacyl-tRNA synthetase (aa-RS) enzymes. These enzymes generate 5′-aminoacyladenylates (5′-aa-AMP) **1** (A = adenine) through an initial nucleophilic attack of an amino acid carboxylate on ATP. The aa-RS then catalyzes the intermolecular aminoacyl transfer from **1** to the 2′/3′-terminus of a cognate tRNA to give adenosine-5′-monophosphate (5′-AMP) (**2**) and an equilibrating mixture of 3′- and 2′-aa-tRNAs **3** and **4** (Scheme 1).



Scheme 1. The biochemistry and chemistry of 5′-aa-AMP **1**: biochemically (upper), **1** is used by aa-RS enzymes for the aminoacylation of cognate tRNA. In the absence of enzymes (lower), **1** can undergo intramolecular aminoacyl transfer and hydrolysis reactions.

When separated from the protective environment of an aa-RS, 5′-aa-AMPs are highly unstable and undergo hydrolysis and isomerization in aqueous solution.[5,6] The isomerization involves a slow initial intramolecular aminoacyl transfer from the 5′-phosphate to the 3′-hydroxy group via an eight-membered transition state to give the 3′-aminoacyl ester **5**, followed by rapid equilibration of the latter with a 2′-aminoacyl ester. If it were possible to generate 5′-aa-AMP **1** from 5′-AMP **2** by reaction with an NCA, then this intramolecular transfer would hopefully result in the sought-after formation of aminoacyl esters.

We first investigated whether the aminoacylation of a simple model phosphate monoester with an NCA was possible. We chose methyl phosphate **6** as the model phosphate monoester and the valyl derivative **7** to allow comparison with the previous work on inorganic phosphate[4] and because **7** can be easily prepared and stored (Scheme 2).[7] In a general sense, the reaction of a phosphate monoester with any electrophile is faster at pH values above 7 when the phosphate is in its dianionic state, (pK_a for the monoanionic state ≈ 6–7 (Supporting Information)); however, NCA hydrolysis/polymerization is also favored at high pH values.

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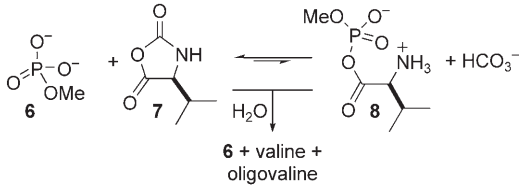
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

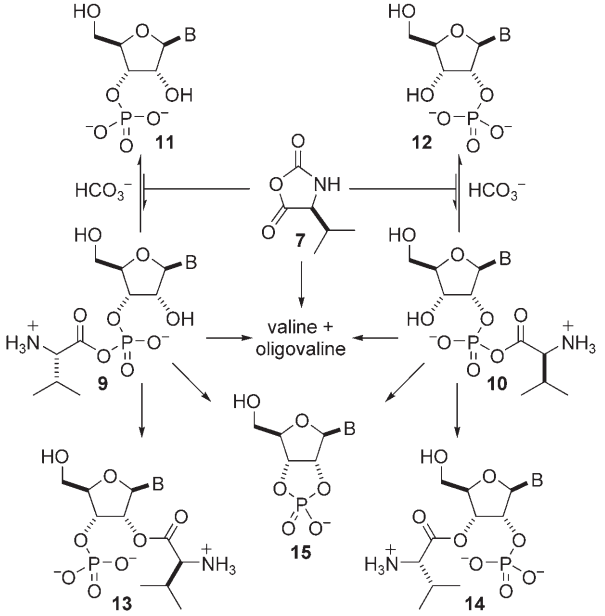


Scheme 2. Aminoacylation of model phosphate monoester by Val-NCA **7**.

To slow such degradative reactions while still allowing reaction of the NCA with the phosphate group of **6**, we conducted our experiments under very slightly acidic conditions. In this case, although **6** was predominantly monoanionic, some of the reactive dianionic form still existed. Using ^{1}H NMR spectroscopy for analysis, we observed a low-level conversion of **6** and **7** into L-valyl methyl phosphate **8** (not isolated, according to ^{1}H NMR integration $\approx 23\,\%$ based on **7**, see Supporting Information). The conversion was only transient, however, and **8** was gradually converted back into the starting methyl phosphate **6** along with valine and oligovaline.

These observations imply that the reaction of **6** and **7** to give **8** is an equilibrium that is rapidly established and lies in favor of **6** and **7**. The equilibrium is eventually dissipated through the hydrolysis of **7** and **8** to valine, and the subsequent reaction of the valine with further **7** and **8** giving oligovaline. The existence of the proposed equilibrium was proven when we found that **8**, as prepared by conventional synthesis, was almost completely converted into **6** and **7** in the presence of bicarbonate. Once again, hydrolysis/polymerization of the activated valine derivatives subsequently yielded the free amino acid and oligomers.

We next turned our attention to the reaction of nucleotides with Val-NCA **7**. 5′-AMP **2** showed similar behavior to methyl phosphate **6**, and we observed an equilibrium production of 5′-Val-AMP **1** (R = *i*Pr). However, the equilibrium was dissipated by NCA hydrolysis/polymerisation before the rearrangement of **1** into **5** occurred (see Supporting Information).[5,8] We next studied the reaction of other regioisomeric nucleotides with Val-NCA **7**. We recognized that if a nucleoside 3′/2′-valyl phosphate **9**/**10** could be transiently formed from the corresponding 3′/2′-nucleotide **11**/**12** and **7**, then intramolecular aminoacyl transfer to the 2′/3′-OH group, via a seven-membered transition state[9] might now be faster than hydrolysis/polymerization of the NCA or the intermediate mixed anhydrides (Scheme 3). We reasoned, therefore, that even an unfavorable equilibrium situation might lead to subsequent intramolecular aminoacyl transfer, and that this transfer should then displace the equilibrium according to Le Chatelier's principle. There was a major concern with the 3′/2′-nucleotides, however, as we foresaw cyclization of the intermediate 3′/2′-aminoacyl phosphates **9**/**10** to give the 2′,3′-cyclic phosphate **15** as a likely competing reaction (Scheme 3). Indeed, 2′/3′-phosphates can be cyclized with great ease, to 2′,3′-cyclic phosphates via five-membered transition states, by almost any form of phosphate activation.[10]



Scheme 3. Aminoacylation/cyclization of 3′- and 2′-nucleotides by Val-NCA **7**.

Undeterred by this concern, we initially studied the reaction of cytidine-3′-monophosphate (3′-CMP) **11** (base (B) = cytosine (C)) with Val-NCA **7**, again under very slightly acidic conditions. After 55 min, no new signals consistent with **9** (B = C) were observed in the ^{1}H NMR spectrum. However, new signals were observed which were tentatively assigned to the aminoacyl-transfer product **13** (B = C) (Figure 1 a)). In

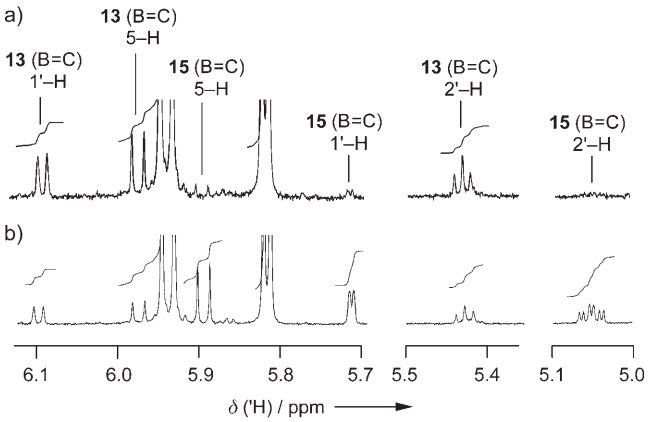


Figure 1. ^{1}H NMR spectra (500 MHz, D_2O) of the products of the reaction of 3′-CMP **11** (B = C) with Val-NCA **7**. a) Spectrum acquired after 1 h. b) Spectrum acquired after spiking the sample with authentic **15** (B = C) (0.5 mg).

particular, a diagnostic triplet between $\delta = 5.4$ and 5.5 ppm was shown by COSY analysis to correspond to 2′-H of the new species, and the downfield shift of this signal was consistent with a 2′-ester. Although **13** was not isolated,[11] integration of the ^{1}H NMR spectrum of the reaction mixture suggested that it had been formed in $\approx 9\,\%$ overall yield (Table 1).

Table 1: Yields of the products observed in the reactions of 3′/2′-nucleotides **11**/**12** with Val-NCA **7**.

Nucleotide	*t* [min]	Starting material [%]	Valyl ester (**13** or **14**) [%]	Divalyl ester [%]	**15** [%]
11 (B = C)	55	90.6	(**13**) 8.7	trace	0.7
11 (B = A)	30	82.2	(**13**) 13.8	3.9	trace
	90	79.5	(**13**) 13.4	5.6	1.5
12 (B = C)	45	94.9	(**14**) 3.0	0	2.1
12 (B = A)	90	99.0	(**14**) 0.5	0	0.5

As our experiment was initiated at a pD value close to the pKa of the monoanionic form of **11**, the yield of **13**, based upon the reactive dianionic form of **11**, is probably considerably higher. This suggests that isomerization of the intermediate **9** into **13** is highly efficient relative to the fast backwards reaction of **9** with CO_2. After further time had passed (> 1 h), ^{1}H NMR spectroscopic analysis suggested that **13** underwent very slow reversion to **11**, presumably by a combination of hydrolysis and nucleophilic attack by the amino group of valine and valyl derivatives, including **13**. The minor signals in the ^{1}H NMR spectrum (accompanying the signals of **13**) in the reaction of **11** and **7** were shown, by spiking the reaction with an authentic sample, to be due to the 2′,3′-cyclic phosphate **15** (B = C) (Figure 1b)). However, this anticipated by-product was formed in much lower amounts (**13**/**15** > 10:1), and we have not been able to prove the structure of the second minor product definitively, but MS and HMBC data strongly suggest that it is the 2′-divalyl analogue of **13**.

We next investigated the effect of the nucleobase on this remarkable conversion and examined the reaction of 3′-AMP **11** (B = A) with Val-NCA **7**. Again, we could not detect the intermediate aminoacyl phosphate **9** (B = A) but observed the aminoacyl-transfer product **13** (B = A) in a maximal overall yield of ≈14% after 30 min (Table 1). In this experiment the putative 2′-divalyl analogue of **13** was also formed in a greater quantity and, after 90 min, had accumulated to the level of ≈6%. On the basis of the amount of the reactive dianionic starting material, the combined synthesis efficiency of **13** (B = A) and the divalyl analogue is again, probably considerably higher. Close examination of the ^{1}H NMR spectra revealed the presence of the 2′,3′-cyclic phosphate **15** (B = A) in low yield (**13**/**15** > 8:1). It thus appeared that the nucleobase had a relatively small effect on the aminoacylation of 3′-nucleotides with Val-NCA, and as such, we switched our attention to the isomeric 2′-nucleotides with the expectation of equally efficient aminoacylation.

Through the use of the same conditions employed for the 3′-isomer, we found that cytidine-2′-monophosphate (2′-CMP) **12** (B = C), in the presence of Val-NCA **7**, gave the 3′-ester **14** (B = C) in only ≈3% yield after 45 min (Table 1). Not only was the aminoacyl-transfer product formed in low yield, but it was accompanied by a comparable (≈2%) amount of the 2′,3′-cyclic phosphate **15** (B = C) (**14**/**15** < 2:1). The lower yield of the ester product and the higher yield of **15** suggest that aminoacyl transfer from **10** is less efficient than it is from the 3′-phosphoryl isomer **9**. This conclusion was supported by experiments with 2′-AMP **12** (B = A) and Val-NCA **7** in which we found that both the 3′-ester **14** (B = A) and the cyclic phosphate **15** (B = A) were formed in only trace amounts. This marked contrast in aminoacylation behavior between 3′- and 2′-nucleotides was not anticipated. It now seems likely that the efficiency of the aminoacylation reaction depends on the pK_a value of the phosphate and 2′/3′-OH groups, and possibly on conformational effects such as the furanose ring pucker. The pK_a value of the phosphate presumably influences the efficiency of the intramolecular aminoacyl-transfer step through a correlation with the leaving-group ability. Although the absolute pK_a values reported in the literature for the individual nucleotides differ (Supporting Information),[12–14] there is a consensus that the 3′-phosphates are more acidic than the 2′-phosphates by approximately 0.2 pK_a units and this is consistent with the more efficient conversion of **11** into **13**. Within the 3′-phosphate series, the greater yield of **13** (B = A) relative to **13** (B = C) is possibly related to the fact that aden-9-yl is a better stabilizer for the 2′-oxyanion than any other aglycons.[15]

The results described herein indicate that uncoded aminoacylation of 3′-nucleotides by NCAs is a prebiotically plausible reaction. The chemical mechanism of this process suggests that it should also be possible for 3′-phosphoryl oligoribonucleotides to undergo aminoacylation by NCAs in the same way, but with potential assistance by the ribonucleic acid chain through stereocomplementary binding. It is therefore possible that ribozymes, capable of coded aminoacylation by NCAs might have evolved and could have been key intermediates in the emergence of translation.[16] The use of 5′-aa-AMPs as intermediates in coded aminoacylation of RNA is most likely a later evolutionary development.

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