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A Novel General Methodology for Ribozyme-Mimetic Synthesis of Methyl Esters of Various Natural Amino Acids, Simulating the Prebiotic Biomolecule Creation

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Abstract

A Novel General Methodology for Ribozyme-Mimetic Synthesis of Methyl Esters of Various Natural Amino Acids, Simulating the Prebiotic Biomolecule Synthesis: A new originally developed procedure for biomimetic synthesis of methyl esters of the natural amino acids was realized at conditions, imitating the action of the primitive ribozymes, and this strategic methodology presumes their crucial role in the prebiotic syntheses of biomolecules during the primordial RNA World.

Keywords: 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane; Methyl esters of natural amino acids; Ribozymes; RNAzymes; Methyl oxirane; H-Phosphonic acid; Primordial RNA world; Ribozyme-Mimetic synthesis

Introduction

The major functional difference of RNA over the DNA is based on the bifunctionality of the former, i.e., it is true, that besides to store the genetic information, like deoxyribonucleic acid, the ribonucleic acid is able to catalyze chemical reactions, like protein enzymes. The similarity of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA) is well known and it allows its ability to store genetic information as clear as DNA, despite the lower stability of ribonucleic acid. This lower stability concludes in the easier hydrolysis of RNA at mild acidic and alkali conditions, due to the participation of 2'-OH group of the ribose ring as a nucleophile catalyst in the phosphodiester bond cleavage (through the formation of the dioxaphospholane ring as an intermediate and its hydrolysis by the ring opening after that). This reveals one of the few structural differences between them. Another functional property of RNA which differentiates it from DNA is to form efficient catalysts. However, this property so far is not very efficient according to the protein enzymes, due to the less chemical and structural diversity. Comparing to the protein enzymes, that are composed from 20 different alpha-amino acids, thus determining their structural variety, the ribozymes (RNAzymes, RNA-enzymes), have only four nucleobases: purine bases - adenine (A), guanine (G), and pyrimidine bases - cytosine (C) and uracil (U), which determines their limited structural diversity. The lack of a large variety of functional groups as well as structural diversity is the main reason for the transition from an ancient "RNA world" to the "protein world". Moreover RNA nucleotides not only give a limited functional diversity, but they determine the defined range of sugar conformations, restricted syn-anti base conformations, as well as a lower and limited ability of stacking interactions. DNA lacks a 2'-OH group in its ribofuranose ring. The hydroxyl group also folds the ribose into the C2'-exo and C3'-endo sugar conformation ("Northern" or **N**) unlike the C2'-endo and C3'-exo twisted conformation ("Southern" or **S**) of the deoxyribose sugar in DNA. This forces an RNA double helix to change from a B-DNA structure to one more closely resembling A-DNA. Another structural difference between RNA and DNA relies on the possession of Uracil (U) by the ribonucleic acid (RNA) instead of Thymine (T) in 2'-deoxyribonucleic acid (DNA). And final structural difference is based on the double chain helix structure in DNA according to the more diverse structures of RNA. Despite that the RNA world hypothesis earlier assumed the older concept of RNA as a primordial molecule, later it accepted a new idea and proposed that the life, which was based on ribonucleic acids (RNAs), have supported pre-cellular world and been a major step in the evolution of cellular life, thus precedes the current world based on deoxyribonucleic acids (DNAs), ribonucleic acids (RNAs) and proteins. The RNA World hypothesis is supported by the properties of RNA's which conclude in the ability to store, transmit, and duplicate genetic information, as DNA performs. Moreover, RNA can also act as a ribozyme (RNA enzyme, RNAzyme) and due to the fact, that it can possess the abilities and properties of both DNA and enzymes, RNA is believed to have a capability of supporting independent life forms [1]. This argument is sustained by the fact, that in nowadays some viruses still use RNA as their genetic material, rather than DNA [2]. One version of the hypothesis is that an especial type of nucleic acid, termed pre-RNA, was the first one, owing enough ability to act as a self-reproducing molecule, which later was replaced by RNA. Probably, this pre-RNA was the simplest comparable RNA-like molecule, but having properties to storage the information, to self-reproduce and to catalyze several important chemical reactions.

Although, in his recent review Thomas Cech suggests that multiple self-replicating molecular systems probably preceded RNA, and that RNA in modern cells is an evolutionary remnant of the RNA world that preceded ours, it has no doubt that RNA molecules have played a significant role during the Primordial World [3]. He proposes that the RNA world evolved into a world of RNP enzymes, such as the ribosome and ribozymes, before giving rise to the DNA, RNA and protein world

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of today [3]. At the beginning, the main role for the storage of genetic information was given to the small RNA molecules (pre-RNAs), later to the bigger RNAs, but DNA is thought to have taken over the role of data storage due to its increased stability. Also in the Ancient World, the RNA molecules played catalytic role, but later the proteins replaced RNA's role in specialized biocatalysis, due to a greater variety of monomers (amino acids), which compose their structure. Among the carrying and storage the genetic information, the hypothesis for the crucial role of the small RNA molecules (and also pre-RNA) was based on the recent observations of the catalytic properties of various forms of RNA [4] ribosome RNAs (rRNAs), participating and catalyzing peptide bond formation on ribosome; Hammerhead and Hairpin ribozymes, performing their self-cleavage [5]; an RNA polymerase ribozyme, which can autocatalyse its own synthesis; an RNA ligase ribozyme, catalyzing its own ligation - the joining of two large or small molecules by forming a new chemical bond; and finally the natural introns, which catalyze the RNA splicing (self autocatalyzation of intron deletion and exon conglutination) as a post transcriptional modification, during the process of its own maturation [6]. The most important properties, which were typical for RNAs for the beginning of life, were the ability to multiplication (self replication); the ability to catalyze a variety of chemical reactions, including the creation of molecules which are building blocks of RNA molecules, as well as the ability to catalyze the formation of peptide bonds. Moreover, towards the lack of a large variety of functional groups as well as structural diversity in RNAs, due to the presence of four nucleobases in their structural units, the question remains relying on the possibility of synthesis and possession these four nucleobases: adenine (A), guanine (G), cytosine (C) and uracil (U) in the Ancient World. While nucleotides were not found in the origins of life experiments, carried out and studied by Miller and Urey, the formation of pyrimidine nucleotides in prebiotically plausible conditions is reported by the Sutherland Group [7], who reported, that pyrimidine ribonucleosides and their respective nucleotides have been prebiotically synthesized by a sequence of reactions which by-pass the free sugars, and is assembled in a stepwise manner by applying the nitrogenous and oxygenous chemistry. In a series of publications, they have demonstrated high yielding routes to cytidine and uridine ribonucleotides built from small 2 and 3 carbon fragments such as glycolaldehyde, glyceraldehyde or glyceraldehyde-3-phosphate, cyanamide and cyanoacetylene. One of the steps in this sequence allows the isolation of enantiopure ribose aminooxazoline if the enantiomeric excess of glyceraldehyde is 60% or greater, of possible interest towards biological homochirality [8]. This step can be described as a prebiotic purification step, where the above mentioned compound spontaneously crystallized out from a mixture of the other pentose aminooxazolines. Aminooxazolines may react with cyanoacetylene in a mild and highly efficient manner, controlled by inorganic phosphate, to give the cytidine ribonucleotides. Photoanomerization, using UV light allows for the inversion about the 1' anomeric centre to give the correct beta stereochemistry, one of the problems with this chemistry is the selective phosphorylation of alpha-cytidine at the 2'-OH position [9]. However Sutherland et al. showed that the same simple building blocks allow access via phosphate controlled nucleobase elaboration, to 2',3'-cyclic pyrimidine nucleotides directly, which are known to be able to polymerize into RNA [10], and was supported as a strong evidence for the RNA world [11]. In turn, the purine nucleobase adenine is known to be a pentamer of hydrogen cyanide, and it can be synthesized from the condensation of hydrogen cyanide monomers at mild laboratory reaction conditions. The translation remains one of the few processes in Nature (together with RNA splicing, during its own maturation), which proves the important role of the ribozymes at the Early Age of the Earth Evolution (during the Primordial RNA-world). The discovery of the phenomenon in the middle of 80-s of the twentieth century by Thomas Cech et al. that not only the proteins play a role as enzymes, but also RNA (ribozymes) [12], is accompanied by the direction of the efforts of researchers towards the determination of the mechanism of action of the different RNA-enzymes. This fact turns on the view of scientific community toward the searching for the mechanism of ribozymes action. A new mechanism of phosphoryl transfer is proposed, including 1,2-diol system and involving 1,2-diol exchange reaction with participation of vicinal *syn*-2'-OH group in the ribose cycle from the RNA molecule [13-15]. The electrophile participation of the vicinal hydroxyl group leads to the increased electrophilicity of phosphoryl phosphorous atom and its susceptibility to the nucleophile attack. On the other side it is well known, that DNA is stable and nearly inert in mildly basic conditions, but RNA is rapidly hydrolyzed, due to the electrophile participation of the vicinal hydroxyl group in RNA - in mild acid conditions (pH 5-6), and the phosphodiester bond is highly sensitive to nucleophile attack. Todd et al. [16] attributed this high reactivity of phosphodiester bond hydrolysis of RNA to the nucleophile participation of the vicinal 2'/3'-hydroxyl group**.** The ribonuclease A catalyzed hydrolysis also takes advantage of this mechanism [17]. The cleavage mechanism, by the nucleophile 2'/3'-OH group catalysis (participation) is applied by the small ribozymes and in the RNA-cleavage in base conditions (also ribonuclease A catalyzed hydrolysis), whereas the electrophile catalysis by the vicinal β-hydroxyl group is used by the large ribozymes (group I, group II and spliceosomal introns) [15]. The importance of the vicinal hydroxyl group is described at the cleavage site of the reaction of Tetrahymena ribozyme [18,19]. Moreover, during the translation, on the stage of amino acids activation, an aminoacyl adenylate is formed with the participation of aminoacyl-tRNA synthetase, playing the catalytic role in both stages: aminoacyl adenylate formation, and aminoacyl-tRNA synthesis. This aminoacyl adenylate is the activated form of amino acid at the first stage of aminoacyl-tRNA synthetase reaction. The second (final) form of the natural amino acid is aminoacyl-tRNA. This fact turned us towards the extrapolation of this biochemical reaction at a low-molecular level to realize bio-mimetic synthesis of methyl esters of natural amino acids. We tried to prove, that at the Early Ages of Evolution (during the *RNA-world* in the absence of peptide enzymes, protein enzymes), the role of aminoacyl-tRNA synthetase, as well as the role of peptidyl-transferase were given to the ribozymes. On the other hand, series of coupling reagents like DCC, DIC, TBTU, HBTU, TCTU, HCTU, TATU, HATU, PyBop, PyClop, etc. are well known and are finding a general popularity in the classical peptide, carbohydrate and oligonucleotide syntheses, which need specific condensation agents to realize the different interbiomonomeric bond formation at mild conditions. Although these compounds generally have a good performance, sometimes they could lead to unsatisfactory results in terms of yield and purity of target compounds obtained. They are not so inexpensive, and often with low effectiveness due to the target bond formation [20,21]. They also activate some secondary reactions during the process of the desired bond formation like dehydration of Gln and Asn $CONH_2$ function under the influence of DCC [22]. Moreover, dicyclohexyl urea, obtained as a secondary product during the reaction of interbiomonomeric bond synthesis using DCC, is difficult or in more cases impossible to be removed from the final product. This allowed us to improve the methodology of inter bio monomeric bond formation, by the using of the more inexpensive methyl oxirane (propylene oxide) as condensing reagent, and phosphonic (phosphorous) acid - for the functional group influence (amino group protection and at the same

time carboxyl functionality activation), thus avoiding the addition stage of the alpha-amino group protection. Our previous studies and results on the reaction of amino acid esters preparation catalyzed by phosphorous acid/oxirane analogues allowed us to synthesize a series of methyl esters of amino acids, as well as amides, starting from the corresponding free analogues [23-25]. Their N-functional group protection and C-functional group activation were realized *in situ* during the condensation reaction. The activation of alpha-carboxyl function (α-carboxyl group) as an electrophile was realized, and at the same time the alpha-amino group (α-amino function) protection was fulfilled, allowing by this manner only the target condensation reaction with participation of the activated alpha-carboxyl group. In this article, we demonstrate the synthesis of methyl esters of various natural amino acids, using bio-mimetic reaction, which resembles and tries to prove the ribozyme function and role of RNA as an enzyme at the Early Ages of Earth Evolution (Figure 1).

*The methyl esters of Gly, L-Ala, L-Phe and L-Thr were previously synthesized by the author and coworkers [23,24].

Experimental

General procedures, materials and methods

All of the natural amino acids, H_3PO_3 and propylene oxide were purchased from Merck. All reagents and solvents were purchased and used without further purification. TLC analyses were performed on silica plates UV_{260}, purchased from Merck, where for the spots labeling and virtual detection on TLC plates, a 5% solution of H_2SO_4 in methanol or ethanol was employed, and also - an alcohol solution of ninhydrin was used, as well as a solution of phosphorus-molybdenum acid. For TLC analyses - CH_2Cl_2 : MeOH (9:1) was employed as a solvent system. For the methyl esters of Tyr, Trp and His reverse phase HPLC analyses were performed on a Waters Liquid Chromatograph equipped with an absorbance detector model 441 set at 280 nm and a column Nucleosil 100-$5C_{18}$ (12.5 cm × 4.6 mm) for analytical runs. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance II+600 MHz spectrometer in D_2O and DMSO-d6, using BBO or TBI probe heads. Chemical shifts are expressed in ppm and coupling constants in Hz. The precise assignments of the ^1H and ^{13}C NMR spectra were accomplished by measurement of 2D homonuclear correlation (COSY), DEPT-135 and 2D inverse detected heteronuclear (C-H) correlations (HSQC and HMBC). Chemical shifts are reported in δ (ppm). The analysis of first order multiplets in ^1H NMR spectra was speed up by the use of FAFOMA program [26]. All synthesized compounds (methyl esters of natural amino acids, except for the H-Lys(Boc)-OMe and H-Orn(Boc)-OMe) were prepared as their hydrochloric salts, to be analyzed by ^1H, ^{13}C-NMR and 2D-NMR using D_2O as a solvent. For NMR data, Bruker Avance II+ NMR spectrometer operating at 600 MHz for ^1H and at 150 MHz for ^{13}C NMR was used. The elemental analysis was carried out and organic compounds were determined using the automatic analyzers: Carlo Erba Elemental Analyzer Model 1106 with automatic sampler for 53 samples (Carlo Erba, Milan, Italy) and Perkin-Elmer Elemental Analyzer Model 240 (Perkin-Elmer Corp., Norwalk, Connecticut). For the elemental analysis amino acid methyl esters were used as their free bases. They were prepared (except for the H-Lys(Boc)-OMe and H-Orn(Boc)-OMe) as hydrochloric salts only for the ^1H and ^{13}C NMR analyses in D_2O.

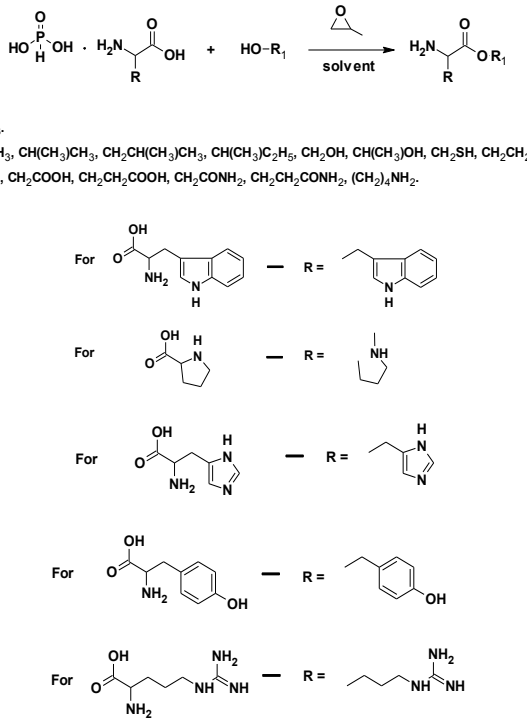

Figure 1: A general reaction scheme for ribozyme-mimetic synthesis of methyl esters of various amino acids.

Synthesis

Experimental part

General procedure for the preparation of methyl esters of natural amino acids: The pure natural amino acid (0.01 mol, 1 equiv.) and H_3PO_3 (0.01 mol, 1 equiv.) were dissolved in a mixture of MeOH/H_2O with vigorous stirring. After dissolution of the amino acid, the solvents were evaporated and the moisture was co-evaporated several times in vacuo. The obtained crystal clear viscous oil was dissolved in MeOH. The reaction mixture was allowed to stand in ice salted bath (-15°C to about -20°C), to which 2.5 equiv. (0.025 mol) of methyl oxirane were added and the reaction mixture was allowed to stand at -15°C to 20°C for 30 minutes, then it was slowly heated at 40°C for about 20-30 minutes. After that the reaction mixture was stirred for 12 hours, again at room temperature. At the end of the reaction time 10% solution of Na_2CO_3 was added to the reaction mixture at room temperature, which was placed in ultra dispenser . The reaction mixture was allowed to stand for 3-4 hours. The obtained product was extracted with EtOAc or CH_2Cl_2* (3 × 20 ml) and washed with 5% $NaHCO_3$ (3 × 20 ml) and saturated H_2O solution of NaCl (brine) untill pH 7-8. The organic layer was dried over anhydrous Na_2SO_4 and the solvent evaporated under vacuo. Also, the methanolic solution of NaOMe or KOMe was used for deprotection of H-phosphonic protective group in ice-salt bath at low temperatures (-15°C to about -20°C). When NaOH in MeOH (KOH in MeOH) was used for deprotection of H-phosphonate (phosphonamide) protective group in the methyl esters of amino acids, only 1 equiv. of NaOH or KOH was employed. In the case of using of $NaHCO_3/H_2O$ or K_2CO_3/MeOH at ultra dispenser (sonicator)), the reaction mixture was allowed to stand at room temperature.

*Depending on the amino acid ester solubility.

Valine Methyl Ester: Yield: 0.944 g (72%). Rf-0.897. ^1H NMR (600 MHz, DMSO-d6, 25°C): δ=8.793 (s, broad, 3H), 3.791 (d, 1H), 3.745 (s, 3H), 2.212 (m, 1H), 1.011 (d, 3H), 0.943 (d, 3H). ^{13}C NMR (150 MHz, DMSO-d6, 25°C): δ=169.0, 57.4, 52.3, 29.1, 18.4, 17.5. ^{13}C NMR

(150 MHz, D_2O, 25°C): δ=171.1, 59.3, 54.2, 30.1, 18.1, 17.9. Elemental analysis: Anal. Calculated for $C_6H_{13}NO_2$: (M_w=131.175 g/mol); C-54.939%, H-9.989%, N-10.678%; found: C-54.834%, H-9.876%, N-10.563%.

Leucine Methyl Ester: Yield: 1.089 g (75%). Rf-0.975, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.102 (t, 1H), 3.778 (s, 3H), 1.832 (m, 1H), 1.645 (m, 2H), 0.879 (m, 6H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=171.4, 53.6, 51.6, 38.9, 24.0, 21.6, 21.2. Elemental analysis: Anal. Calculated for $C_7H_{15}NO_2$: (M_w=145.2016 g/mol); C-57.904%, H-10.412%, N-9.646%; found: C-57.583%, H-10.355%, N-9.593%.

Isoleucine Methyl Ester: Yield: 1.002 g (69%), Rf-0.981, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=3.952 (d, 1H), 3.625 (s, 3H), 2.021 (m, 1H), 1.346 (m, 2H), 1.011 (d, 3H), 0.978 (t, 3H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=170.7, 52.4, 50.3, 36.7, 26.2, 22.3, 21.5. Elemental analysis: Anal. Calculated for $C_7H_{15}NO_2$: (M_w=145.2016 g/mol); C-57.904%, H-10.412%, N-9.646%; found: C-57.602%, H-10.358%, N-9.596%.

Methionine Methyl Ester: Yield: 0.686 g (42%). Rf-0.581, CH_2Cl_2 : MeOH (9:1).^1H NMR (600 MHz, D_2O, 25°C): δ=4.512 (t, 1H), 3.917 (s, 3H), 3.340 (d, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=169.2, 54.0, 51.6, 35.7. Elemental analysis: Anal. Calculated for $C_6H_{13}NO_2S$: (M_w=163.247 g/mol); C-44.147%, H-8.026%, N-8.58%; found: C-44.134%, H-8.019%, N-8.513%.

• The yield of the obtained product was low, due to the fact that the oxidation of the thioether sulfur atom proceeds in some extent as an unwanted side reaction at these conditions.

Proline Methyl Ester: Yield: 0.865 g (67%). Rf-0.429, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.394 (m, 1H), 3.786 (s, 3H), 2.345 (m, 2H), 2.103 (m, 2H), 1.988 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=170.5, 59.7, 53.9, 46.4, 28.4, 23.4; Elemental analysis: Anal. Calculated for $C_6H_{11}NO_2$: (M_w=129.159 g/mol); C-55.796%, H-8.584%, N-10.845%; found: C-55.504%, H-8.539%, N-10.788%.

Tryptophan Methyl Ester: Yield: 1.419 g (65%). Rf-0.742, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=7.425 (d, 1H), 7.394 (d, 1H), 7.162 (d, 1H), 7.142 (d, 1H), 7.051 (t, 1H), 4.241 (t, 1H), 3.566 (s, 3H), 3.253 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=170.4, 136.4, 126.5, 125.4, 122.3, 119.6, 118.1, 112.1, 106.0, 53.7, 53.4, 25.7; Elemental analysis: Anal. Calculated for $C_{12}H_{14}N_2O_2$: (M_w=218.255 g/mol); C-66.038%, H-6.465%, N-12.835%; found: C-65.934%, H-6.455%, N-12.814%.

Lysine Methyl Ester: Yield: 0.657 g (41%). Rf-0.214, CH_2Cl_2: MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.123 (t, 1H), 3.794 (s, 3H), 2.967 (t, 2H), 1.964 (m, 2H), 1.657 (m, 2H), 1.456 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=170.7, 53.7, 52.9, 39.2, 29.4, 26.4, 21.6; Elemental analysis: Anal. Calculated for $C_7H_{16}N_2O_2$: (M_w=160.216 g/mol); C-52.477%, H-10.066%, N-17.485%; found: C-52.454%, H-9.975%, N-17.327%.

Lysine (tert-butyloxycarbonyl) Methyl Ester[‡]: Yield: 1.976 g (76%). Rf-0.443 (CH_2Cl_2:CH_3OH-8:2), Rf-0.272 (CH_2Cl_2:CH_3OH-9:1). ^1H NMR (600 MHz, DMSO-d6, 25°C): δ=1.325 (s, 9H, CH_3), 1.341 (bs, 2H, 4-CH_2), 1.373 (bs, 2H, 5-CH_2), 1.395 (bs, 1H) and 1.452 (bs, 1H, 6-CH_2), 3.089 (pseudo-q, J=5.5 Hz, 2H, 3-CH_2), 3.127 (pseudo-t, J=5.7 Hz, 1H, CH), 3.588 (s, 3H, CH_3), 6.648 (t, J=4.3 Hz, 1H, 3-CH_2NH). ^{13}C NMR (150 MHz, DMSO-d6, 25°C): δ=23.45 (4-CH_2), 27.92 (CH_3), 28.86 (5-CH_2), 30.45 (6-CH_2), 47.47 (3-CH_2), 51.92 (OCH_3), 52.97 (CH), 76.58 (C), 157.22 (NHCOO), 179.13 ($COOCH_3$). Elemental analysis: Anal. Calculated for $C_{12}H_{24}N_2O_4$: (M_w=260.064 g/mol); C-55.422%, H-9.302%, N-10.772%; found: C-55.413%, H-9.221%, N-10.677%.

[‡] Due to the presence of Boc-protective group, the slow and careful addition of phosphonic acid is required, to avoid the reaction mixture from unwanted side reaction of Boc-deprotection.

Arginine Methyl Ester[¥]: Yield: 0.662 g (38%)[¥]. Rf-0.169, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.142 (t, 1H), 3.838 (s, 3H), 3.205 (m, 2H), 1.955 (m, 2H), 1.677 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=170.5, 156.9, 53.8, 52.6, 40.4, 27.0, 23.9; Elemental analysis: Anal. Calculated for $C_7H_{16}N_3O_2$: (M_w=174.223 g/mol); C-48.258%, H-9.257%, N-24.119%; found: C-48.186%, H-9.196%, N-23.998%. [¥] Oxidation is possible in some extent.

Aspartic Acid Dimethyl Ester [Ω]: Yield: 0.765 g (52%). Rf-0.519, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.445 (dd, 1H), 3.767 (s, 3H), 3.678 (s, 3H), 3.110 (dd, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=171.7, 169.4, 54.0, 53.1, 49.3, 33.7. Elemental analysis: Anal. Calculated for $C_4H_9NO_4$: (M_w=147.130 g/mol); C-40.818%, H-6.166%, N-9.520%; found: C-40.784%, H-6.093%, N-9.407%.

[Ω] Non-selective methylation reaction is realized.

Glutamic Acid Dimethyl Ester: Yield: 0.79 g (49%), Mp. Rf-0.549, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.132 (dd, 1H), 3.654 (s, 3H), 3.567 (s, 3H), 3.012 (t, 2H), 2.893 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=170.3, 167.6, 53.8, 53.2, 47.5, 32.1, 26.2. Elemental analysis: Anal. Calculated for $C_6H_{11}NO_4$: (M_w=161.158 g/mol); C-44.718%, H-6.880%, N-8.691%; found: C-44.704%, H-6.878%, N-8.623%.

[*] Non-selective methylation reaction is too possible.

Aspargine Methyl Ester: Yield: 0.935 g (64%), Mp. Rf-0.406, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=7.443 (s, broad, 2H), 4.154 (q, 1H), 3.767 (s, 3H), 2.641-2.456 (dt, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=173.8, 170.4, 54.3, 53.4, 37.6. Elemental analysis: Anal. Calculated for $C_5H_{10}N_2O_3$: (M_w=146.146 g/mol); C-41.092%, H-6.897%, N-19.168%; found: C-41.134%, H-6.823%, N-19.173%.

Glutamine Methyl Ester: Yield: 1.105 g (69%), Mp. Rf-0.417, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=6.732 (s, 2H), 3.854 (s, 3H), 3.762 (q, 1H), 2.123 (t, 2H), 1,693 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=175.4, 172.6, 53.5, 52.2, 31.5, 28.2. Elemental analysis: Anal. Calculated for $C_6H_{12}N_2O_3$: (M_w=160.173 g/mol); C-44.993%, H-7.551%, N-17.489%; found: C-44.934%, H-7.528%, N-17.523%.

Serine Methyl Ester: Yield: 0.679 g (57%), Mp. Rf-0.423, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.342 (q, 1H), 3.776 (s, 3H), 3.82 (dt, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=172.4, 62.6, 61.1, 52.3. Elemental analysis: Anal. Calculated for $C_4H_9NO_3$: (M_w=119.120 g/mol); C-40.332%, H-7.615%, N-11.758%; found: C-40.324%, H-7.579%, N-11.754%.

Cysteine Methyl Ester: Yield: 0.662 g (49%) [Ø], Mp. Rf-0.409, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.122 (t, 1H), 3.676 (s, 3H), 2.721-2,912 (dt, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=169.2, 60.7, 52.6, 28.7. Elemental analysis: Anal. Calculated for $C_4H_9NO_2S$: (M_w=135.187 g/mol); C-35.539%, H-6.710%, N-10.361%; found: C-35.504%, H-6.678%, N-10.373%.

[Ø] Some possible oxidation of thiol sulphur group has taken place, thus leading to lower yields.

Tyrosine Methyl Ester: Yield: 1.21 g (62%), Mp. Rf-0.769, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=6.886 (d, 2H), 6.689 (d, 2H), 3.987 (q, 1H), 3.653 (s, 3H), 2.865 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=173.8, 157.3, 131.5, 129.8, 117.3, 54.7, 53.6, 41.3. Elemental analysis: Anal. Calculated for $C_{10}H_{13}NO_3$: (M_w=195.218 g/mol); C-61.526%, H-6.712%, N-7.175%; found: C-61.494%, H-6.678%, N-7.264%.

Histidine Methyl Ester: Yield: 0.863 g (51%), Mp. Rf-0.349, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=8.121 (s, 1H), 7.783 (s, 1H), 4.211 (m, 1H), 3.712 (s, 3H), 2.643 (dd, 1H), 2.374 (dd, 1H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=177.4, 136.2, 132.5, 117.8, 55.4, 54.3, 28.1. Elemental analysis: Anal. Calculated for $C_7H_{11}N_3O_2$: (M_w=169.183 g/mol); C-49.696%, H-6.553%, N-24.837%; found: C-49.532%, H-6.497%, N-24.932%.

Ornithine Methyl Ester: Yield: 0.497 g (34%). Rf-0.21, CH_2Cl_2 : MeOH (9:1). ^1H NMR (600 MHz, D_2O, 25°C): δ=4.182-4.143 (m, 1H), 3.842 (s, 3H), 3.209-3.171 (m, 2H), 1.765-1.532 (m, 2H), 1.481-1.423 (m, 2H); ^{13}C NMR (150 MHz, D_2O, 25°C): δ=177.3, 53.2, 52.5, 41.3, 29.5, 27.1; Elemental analysis: Anal. Calculated for $C_6H_{14}N_2O_2$: (M_w=146.188 g/mol); C-49.296%, H-9.653%, N-19.163%; found: C-49.405%, H-9.475%, N-19.302%.

Ornithine (tert-butyloxycarbonyl) Methyl Ester[1]: Yield: 1.796 g (73%). Rf-0.436 (CH_2Cl_2:CH_3OH-8:2), Rf-0.263 (CH_2Cl_2:CH_3OH-9:1). ^1H NMR (600 MHz, DMSO-d6, 25°C): δ=1.364 (s, 9H, CH_3), 1.392 (bs, 2H, 4-CH_2), 1.411 (bs, 1H) and 1.533 (bs, 1H, 5-CH_2), 2.890 (pseudo-q, J=6.2 Hz, 2H, 3-CH_2), 3.276 (pseudo-t, J=6.2 Hz, 1H, CH), 3.602 (s, 3H, CH_3), 6.864 (t, J=5.2 Hz, 1H, 3-CH_2NH). ^{13}C NMR (150 MHz, DMSO-d6, 25°C): δ=25.80 (4-CH_2), 28.19 (CH_3), 31.78 (5-CH_2), 49.57 (3-CH_2), 51.29 (OCH_3), 53.67 (CH), 77.26 (C), 155.51 (NHCOO), 176.20 ($COOCH_3$). Elemental analysis: Anal. Calculated for $C_{11}H_{22}N_2O_4$: (M_w=246.037 g/mol); C-53.700%, H-9.013%, N-11.386%; found: C-53.634%, H-8.981%, N-11.366%. Due to the presence of Boc-protective group, the slow and careful addition of phosphonic acid is required, to avoid the reaction mixture from unwanted side reaction of Boc-deprotection.

Discussion

The RNA analogues of the proteinogenic enzymes, i.e., ribozymes, participate in fundamental reactions of modern biochemistry such as viral RNA selfprocessing, RNA splicing, and translation of RNA into protein, during the process of protein biosynthesis. Ribozymes are widely hypothesized to have both carried information and performed catalysis during the primordial 'RNA World'. Steitz et al. proved the hypothesis of Thomas Cech that no amino acid residue exists within a distance of 18 Å of the ribosome's active site [1,15,27,28] revealing by this way that the rRNA molecules are responsible for the peptidyl transferase activity and it has been suggested that rRNA has originated and has developed from a similar molecule [29]. After formation of the hypothesis that the ribosome is actually a ribozyme, a short RNA molecule has been formed in many laboratories with the ability to form peptide bonds, and it has been suggested that rRNA has evolved from a similar molecule, thus proves the catalytic possibility and potential of RNA. Relatively short mimics of tRNA molecules with such abilities to participate in peptide bond acceleration have been artificially formed in our laboratory [31,32]. Later, it was suggested that tRNA was also formed from RNA molecules that began to catalyze amino acid transfer, having a more crucial role in the O-N acyl transfer during the peptidyl transferase reaction, than the ribosomal RNA (rRNA) [30-32]. The hypothesis about the larger role of tRNA in peptidyl transferase reaction is proved and supported by the facts that the point mutations and mutation analyses of many universally conserved nucleotides demonstrated that the base modifications in ribosomal RNAs: A-2486 (A-2451), U2506, U2585 and A2602 in the innermost layer of the active site of the large subunit of the ribosome have a limited, but no lethal influence on the rate of peptide bond synthesis [33], as well as that the substitution of the 3'-terminal adenosine 2'-OH in the ribose ring of peptidyl tRNA by 2'-H or 2'-F gives rise to a more than 1,000,000 fold rate decrease [30]. In this paper we describe the ribozyme-mimetic synthesis of methyl esters of a variety of natural amino acids, using methyl oxirane (propylene oxide) as a condensing reagent and phosphorous (H-phosphonic) acid as reagent for different conventional group influence (an amino group protection and carboxyl functionality activation), at mild conditions. By this biomimetic reaction and creation of a reactive intermediate in the time course, we propose the crucial role of ribozymes in the formation of interbiomononeric (particularly peptide) bond during the primordial RNA World. By this reaction we tried to prove the crucial role of the ancient enzymes, which actually were RNAzymes (pre-RNAzymes), as proposed by the most theories, and playing the important role during the prebiotic synthesis of biomolecules. We are trying to develop this procedure to the synthesis of other derivatives as of the amino acids (peptides, amino acid amides, aminoacyl-carbohydrates: mono-, di-, tri- and oligosaccharides, aminoacyl nucleosides), as well as to develop this procedure to oligonucleotide and carbohydrate synthesis.

It is well known, that the peptide bond formation is one of the most important phenomena in Nature. During the process of amino acids activation, on the first stage aminoacyl adenylate is formed, which is attacked by the nucleophile 2'/3'-OH group of the ribose ring of A76 at the 3'-end of the tRNA. The both processes are catalyzed by aminoacyl-tRNA synthetase. This fact gave us the idea to convert the nature reaction to a low-molecular level and to realize a new approach for synthesis of amino acid methyl esters by means of 1,2-diol system properties and H_3PO_3. The reaction of latter with amino acid leads to its activation, results to in situ obtaining of 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane, (2,5-dioxo-4-alkyl-1,3,2-oxaza phospholane), which was similar to urethane N-carboxy anhydrides (UNCA) (Figure 2) [34]. In the case of a phospholane derivative, at the same time with the protection of NH_2-group, the carboxyl group is activated. Our approach allows synthesizing of different amino acid derivatives without preliminary protection of amino acid functional groups. Their blocking was made in situ during the reaction procedure. The activation of α-carboxyl function as an electrophile was realized, at the same time the α-amino group protection as a nucleophile was fulfilled, that ensure only the aimed (targeted) condensation reaction. The reaction was made in alcohol solution in the presence of bis-2-hydroxypropyl ester of H_3PO_3, obtained in situ by means of H_3PO_3 and

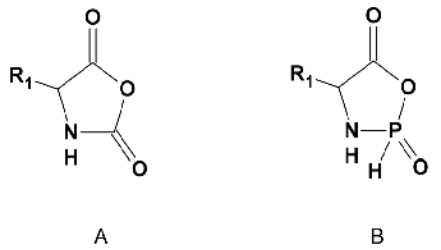

Figure 2: Structures of urethane N-carboxy anhydride (UNCA) A, and 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane B.

propylene oxide interaction. It is well known that oxiranes can open their ring at acid and at base conditions. We propose the methodology of the oxirane ring opening by the nucleophile attack of the phosphonyl oxianion followed by the formation of 2-hydroxy activating ester of H-phosphonic acid as a reactive intermediate.

The reaction between this intermediate and amino acid leads to the preparation of aminoacyl phosphonate (Figure 3). The latter is similar to aminoacyl adenylate obtained as macroergic compound, during the process of aminoacylation of tRNA [25]. According to these conclusions, and the fact, that the amino acids activation goes through the stage of the formation of aminoacyl adenylate, we decided to approximate the reactions, which proceed in the nature to a lower-molecular level, and to use these data in a new scheme and method (methods) for synthesis of amino acid esters, using the properties of the 1,2-diol system and the phosphorous acid (phosphonic acid), which participate in the activation of the amino acid and in the preparation of 2,5-dioxo-4-alkyl-1,3,2-oxaza phospholane as an activated compound of the amino acid, according to the similarity of urethane-N-carboxy anhydrides (UNCA) [34]. The formed 2-OH-alkyl diester of the phosphonic acid reacts with the carboxy anion of the amino acid at temperature of 40°C for about 15-20 minutes, which leads to the formation of phosphonoanhydride of the amino acid. From this stage, the reaction begins to follow the two mutually-allowing (permitting) reaction pathways, as a result of the competition between the nucleophiles (the α-amino group of the activated amino acid - intramolecular nucleophile reaction, and the reagent - external nucleophile, participated in reaction - intermolecular nucleophile reaction.) The first reaction is an intramolecular aminolysis, but the other reaction (second reaction) is intermolecular alcoholysis, respectively in the following the first direction, activated intermediate is formed: aminoacyl derivative: 2λ^5-spirobi-2-[1,3,2-alkylenon-oxaza] 2'-[1,3,2-hydroxyalkyl, oxy]-phosphole, and then 2,5-dioxo-4-alkyl-1,3,2-oxaza phospholane. Its reaction products with external nucleophile are N-(H-phosphonyl)-aminoacyl methyl ester and N-(2-hydroxyalkyl H-phosphonyl) aminoacyl methyl ester. After unblocking (deprotection of the amino group) of the H-phosphonyl protecting group and alkalization of the reaction mixture, the final product as a free base is obtained. In the other direction, as a result of intermolecular aminolysis, H-phosphonic salt of the aminoacyl ester is obtained (prepared), which is also, as a result of alkalization, converts to the final product. After isolation of amino acid methyl ester, it may be included as a nucleophile in the next reaction of peptide formation. Moreover, it is well known from the classical organic synthesis (and particularly peptide synthesis) that, to prevent a given functional group from its participation in unwanted side reactions, it (this functional group) needs to be blocked (or protected). Exactly, this strategy is used in the peptide synthesis - blocking of the α-carboxyl group or α-amino group, in depending from the goal and also - protection of the side functional groups of the amino acids (if it is inevitably). The proposed method allows avoiding the preliminary protection of the amino acids functional groups, as it proceeds in situ during the course of the reaction. At the same time with the protection of α-amino group of the amino acid as nucleophile, also the α-carboxyl group as electrophile may be activated. In this way (according to this procedure) the reaction successfully proceeds only between the α-carboxyl group, and with other nucleophile, for instance: alcohol, amine, or ester (amide) of the amino acid (C-end protected). Our previous experiments show, that in the presence of methanol as the simplest nucleophile, methyl esters of amino acids were prepared successfully, as the first prepared methyl esters were of the following amino acids: Phe, Thr, Gly, Ala [23,24]. Thus, by our originally developed procedure, methyl esters of a variety of amino acids were synthesized. In the presence of side chain functionality in some amino acids, particularly in the case of lysine and ornithine, due to the cyclization reaction as an unwanted side process, the yields were unsatisfactory. This allows us to carry out the synthetic reaction procedure with side chain amino group protection in these amino acids. By analogy, the reaction was carried out with Boc-protection in side chain in tryptophan, arginine, histidine, cysteine, tyrosine, and serine [35]. In the case of aspartic acid and glutamic acid, because of the non-selective methylation, depending on the molar ratio between the amino acid and propylene oxide, the different rate of methylation is possible. When 5 molar equivalents of oxirane and 1 molar equivalent H_3PO_3 (or 5 molar equivalents of oxirane and 2 molar equivalents of H_3PO_3) were added, the main reaction product was a dimethyl ester. Whereas, in the case when 2.5 molar equivalents of propylene oxide and 1 molar equivalent H_3PO_3 were added, according to the amino acid, a non-selective (at alpha- or beta- and gamma-position) monomethylation was noticed [35]. This allows us to protect the carboxyl group at beta- and gamma- position in these amino acids with benzyl group, and to carry out the synthesis reaction procedure with the marked amino acid derivatives [35]. The reaction mimics as the reaction of aminoacylation of tRNA, catalyzed by aminoacyl tRNA synthetase, as well as the peptidyl transferase reaction, which proceeds on the ribosome (Figure 4). At the first stage of the reaction, the attack from the carboxy anion of the amino acid on phosphoryl phosphorus atom of bis-2-OH alkyl H-phosphonate (bis-beta-hydroxyalkyl H-phosphonate) is realized, which is similar to attack from the amino acid on ATP, during the first phase of the reaction, catalyzed by aminoacyl tRNA synthetase (Figure 4) to form an aminoacyl-adenylate (AA-AMP) and release the inorganic pyrophosphate (PP_i). At the second stage of our reaction, if the mixed amino acid-alkyl H-phosphonate mixed anhydride is subjected to intermolecular nucleophile attack from another nucleophile (MeOH in our case) (pathway [a]), the direct methyl ester of amino acid H-phosphonium salt is formed, which is similar to the second stage of the catalyzed by

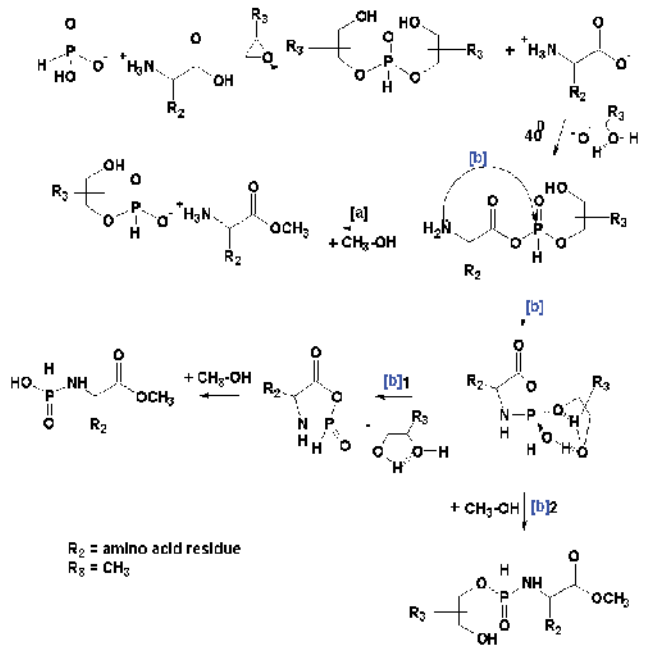

Figure 3: Possible reaction pathways for the preparation of methyl esters of the natural amino acids [25].

the aminoacyl tRNA synthetase nucleophile attack from 3'-OH group in the ribose ring of A76 aminoacyl-tRNA on carbonyl carbon atom in the amino acid part of aminoacyl-adenylate (AA-AMP) with the creation of an aminoacyl-tRNA (AA-tRNA) (Figure 4). When an intramolecular nucleophile attack from the alpha-amino group of the amino acid on phosphoryl phosphorus atom is realized, the 5-membered cyclic intermediate (4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane B in Figure 2, (PANCA) or (HPANCA) is formed (pathway [b]), and this reaction is similar to the peptidyl transferase reaction, which proceeds on the ribosome (Figures 2-4). In the two cases the crucial role is played by 2-OH hydroxyl group (cis-vicinal 2-OH group (beta-hydroxyl group)) from the beta-hydroxyalkyl ester group of H-phosphonate part of the mixed amino acid H-phosphonate anhydride, which accelerates as the intramolecular nucleophile attack from alpha-amino group, as well as intermolecular nucleophile attack from another nucleophile. This beta-hydroxyl group is similar to cis-vicinal 2'-OH group in the ribose ring of A76 peptidyl-tRNA (which leads to acceleration of peptidyl transferase aminolysis reaction on ribosome [31]), acting as a proton shuttle by hydrogen bonding (Scheme in Figure 4). In another paper Paecht-Horovitz et al. [36] described the prebiotic synthesis of polypeptides by heterogeneous polycondensation of amino-acid adenylates, proving by this way the role, which aminoacyl-adenylate (AA-AMP) plays in the prebiotic, and in the modern world in biopolymer synthesis, and by this way indirectly supporting the role of the 5-membered cyclic intermediate (4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane B in Figure 2, (PANCA) or (HPANCA)), created in our reaction conditions, as a reactive synthetic equivalent in this type of reaction. Paecht-Horovitz et al. described that the active forms of amino-acids (aminoacyl-adenylates (AA-AMPs), concentrated by adsorption on the clay particle surface, may condense to form polypeptides of different but discrete sizes. By their article they predicted and proposed that the certain types of clays may have played a catalytic part in the evolution of polypeptides [36]. The whole this arsenal of reactions lead to the creation of PANCA (HPANCA), susceptible enough to nucleophile attack, leading to the formation of different products. Probably, this molecule (PANCA (HPANCA)) was acting as a synthetic equivalent, carrying the aminoacyl synton (amino acid residue), providing by this way the possible pathway for peptide synthesis in the *Ancient RNA World*. In our bio-mimetic reaction, the intramolecular nucleophile attack by the alpha-amino group (pathway [b]) (Figure 3) provides an imitation of the attack from the alpha-amino group in the ribose ring of A76 aminoacyl-tRNA (A76 AA-tRNA) to the mildly activated ester carboxyl group of the amino acid from the C-termini of the polypeptide chain (Figure 4), linked to the ribose ring of A76 in peptidyl-tRNA. The obtained 5-membered cyclic intermediate provides as the protection of the alpha-amino group (similar to the protection of the N-termini of the growing peptide chain in the peptidyl-tRNA), as well as the activation of the carboxyl group of the alpha-amino acid (such as the activation of the carboxyl group of the C-terminal amino acid of peptidyl-tRNA), made it sensitive enough to the nucleophile attack from an external functionality. Thus, this intermediate (PANCA (HPANCA)), which is similar to UNCA (commonly used in the chemical solution phase peptide synthesis), provides an efficient activating unit for the realization of the bio-

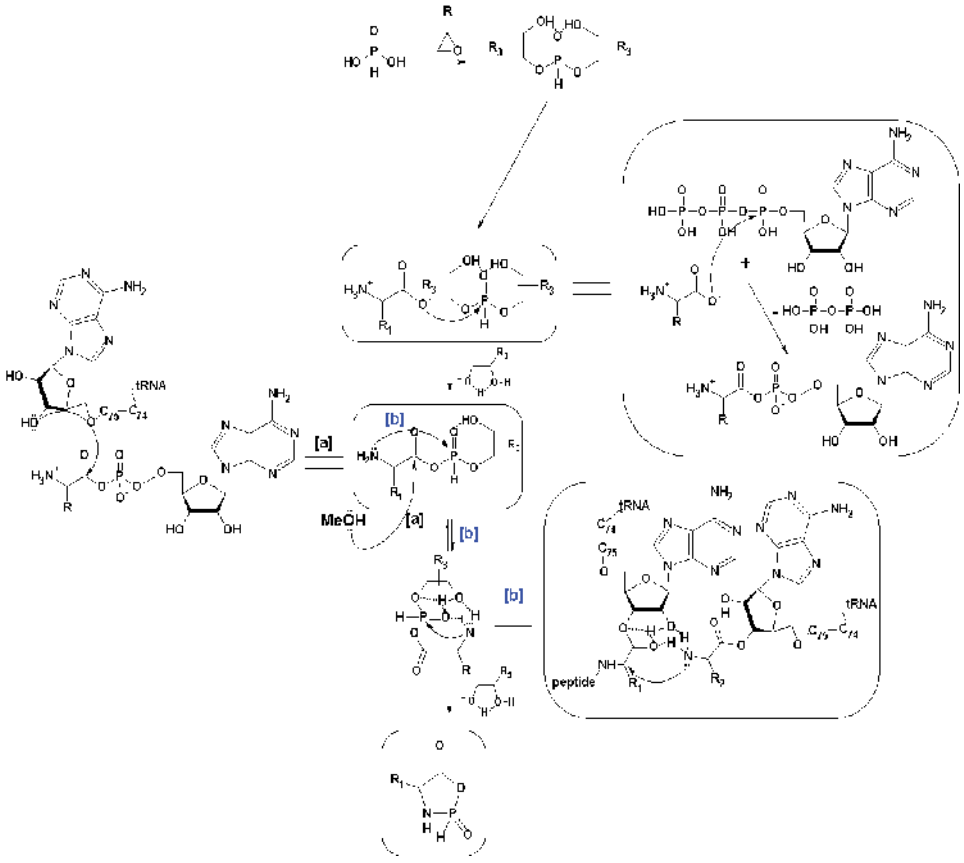

Figure 4: Comparison between the different stages of our proposed ribozyme-mimetic reaction with the important points of peptide synthesis proceeds on the ribosome. As it can be seen from the figure, a considerable analogy is described between them.

mimetic synthesis, probably the same or the similar activating intermediate played a crucial role in the prebiotic synthesis as pre-RNAzymes or as a part of the RNAzymes. It is possible, that at the early Stage of evolution one or more small ribozymes (RNAzymes, or pre-RNAzymes) possessed the properties of the aminoacyl tRNA synthetase (AA-tRNA synthetase) and peptidyl transferase. Our conclusions relating to PANCA (HPANCA) are supported by the studies of Luke et al. who report that high-energy aminoacyl-phosphate anhydrides and aminoacyl adenylates could be generated in solutions containing amino acids, COS, and the corresponding phosphate molecule [37]. They generated urethane N-carboxy anhydride (UNCA) as a reactive intermediate by the reaction of amino acids with COS, which reacts with inorganic orthophosphate or AMP to create aminoacyl-phosphates and aminoacyl-adenylates (AA-AMPs). Also they have shown that the aminoacyl-phosphate mixed anhydrides may play role as phosphorylating agents by the amino acid dependent activations of phosphate. This process which occur in parallel with the production of peptides, suggest that these two reactions may have shared a common intermediate on the prebiotic Earth. They supposed that the carbonyl sulfide (COS), which is a component of volcanic gas emissions and interstellar gas clouds, and which is an efficient condensing agent, maybe have played a crucial role in the creation of urethane N-carboxy anhydride (UNCA) acting as an important activated structural unit, participating in the prebiotic synthesis and leading to the formation of many biologically important products. Their synthetic pathways prove the reactivity of UNCA and its susceptibility (particularly at carbonyl carbon atom of the aminoacyl synthon) to the nucleophile attack, leading to a variety of products [37]. By the same manner our 5-membered cyclic intermediate PANCA (HPANCA) can be attacked at the carbonyl carbon atom by the different nucleophiles. Urethane N-carboxy anhydride (UNCA), which is formed in their conditions is obtained by the similar way, which probably allows the creating of PANCA (HPANCA), during the prebiotic synthesis in the Ancient World. Both of them: urethane N-carboxy anhydride (UNCA), prepared by the conditions described by Leman et al. [37], and the 5-membered cyclic intermediate PANCA (HPANCA) (obtained at our reaction conditions) are reactive intermediates enough to participate in the reactions, leading to the preparation of life-important molecules. In their conditions they prove, that UNCA is susceptible (as well as in our reaction the formed (PANCA (HPANCA)) to the nucleophile attack by a variety of nucleophiles, leading to different products, as they (similar small molecules or RNAzymes) have done in the Ancient RNA World (Figure 3). Some part of dimmer (dipeptide) formation as a side reaction was observed during the reaction procedure, because of the possible intermolecular nucleophile attack by the alpha-amino group of another amino acid H-phosphonate mixed anhydride: 2-hydroxy ethyl ester of amino acyl H-phosphonate, formed as an intermediate. But this small percent decreases, when the solution (reaction mixture) is diluted. Paecht et al. [38] also described the formation of amino acid dimmers and oligomers: trimers, tetramers, pentamers, hexamers etc. as a side reaction during their procedure of phosphorylation of AMP with aminoacyl phosphate at hydrous conditions [38]. They assume in not too small percent of a nucleophile attack from the alpha amino group of one amino acid on the carbonyl C-atom (from the mixed anhydride: aminoacyl phosphate or aminoacyl pyrophosphate) of another amino acid, leading as to oligomerization, as well as accompanying with the release of inorganic phosphate and pyrophosphate. Since, during their target reaction, numerous of other side reactions have taken place, leading to the formation of oligo and polypeptides, as well as inorganic orthophosphate and pyrophosphate, they describe the system as complicated. In their procedure of phosphorylation of AMP with aminoacyl phosphate in aqueous conditions Paecht and Katchalsky concludes that phosphoanhydrides of amino acids can be classified as reactive compounds [38]. Of course, the percent of the desired nucleoophile attack on the carbon atom of the carbonyl group of the appropriate amino acid and the formation of the corresponding product can be increased by the changing of the reaction conditions, as was done by us. When the reaction was carried out under anhydrous conditions, the desired nucleophile attack on the amino acid carbonyl group was realized (Figure 3), whereas when the hydrous conditions where used as in the procedure of Paecht et al. [38], the nucleophile attack on phosphoryl phosphorus atom was presumably realized (Figures 5 and 6). The possibility for another side reaction, which appears during the carrying out of synthesis, depends on the percent of the water content (Figures 5 and 6). The probability of different type side reactions, as well as their share and proportion, depending on the attack on carbonyl carbon atom of the amino acid, or on phosphoryl phosphorus atom from the phosphoric acid, was studied by the alteration of the percent of the water content in the reaction mixture. Full details concerning the experiments of carrying out of reaction in the desired direction, depending on a variety of water content are included in the forthcoming manuscript, which is in preparation [39]. Probably, in the Ancient World the peptide synthesis was realized at anhydrous conditions (Figure 4), as described by our procedure of the preliminary nucleophile attack on the amino acid carboxyl group in anhydrous conditions, as well as in paper of Paecht-Horovitz et al. [36], who described the prebiotic synthesis of polypeptides by heterogeneous polycondensation of amino-acid adenylates [36]. Paecht-Horovitz et al. described that the active forms of amino-acids (aminoacyl-adenylates (AA-AMPs)), concentrated by adsorption on the clay particle surface, may condense to form polypeptides of different but discrete sizes. By their article they predicted and supposed that the certain types of clays may have played a catalytic part in the evolution of polypeptides [36]. In our case, due to the proximity of the alpha-amino group of the amino acid to phosphoryl

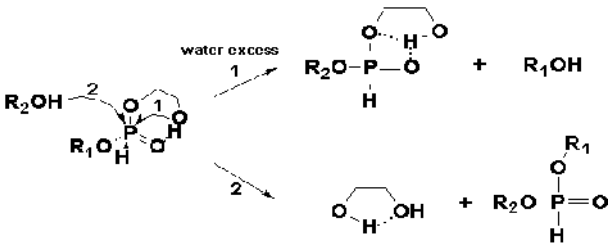

Figure 5a: Scheme of the mono-ol leaving (pathway 1) and diol leaving (pathway 2), accelerated in the presence of the vicinal hydroxyl group, during the reaction of alcoholysis of H-phosphonate diester. In the presence of water excess (pathway 1), due to the impairing of the intramolecular hydrogen bond between the cis-vicinal 2-OH group and the phosphonyl oxygen atom, by the creation of intermolecular hydrogen bonds (between the cis-vicinal 2-OH group and H_2O from the one side, and between the phosphonyl oxygen atom and H_2O from the another side) the nucleophilicity of the cis-vicinal 2-OH group is increased and leads to the intramolecular nucleophile attack, supported by the mono-ol (ester alcohol) leaving and following with the ring opening by the intermolecular nucleophile attack by another alcohol molecule. In this case the cis-vicinal 2-OH group plays a role as nucleophile catalyst. Since the reaction proceeds in aprotic solvent (pathway 2), due to the electrophile assistance of the cis-vicinal 2-OH group by its intramolecular hydrogen bonding with the phosphonyl oxygen atom, the electrophilicity of the phosphorus atom bonded with latter and its susceptibility to the intermolecular nucleophile attack is increased, which cause the ester exchange (transesterification) reaction, accompanied with the 1,2-diol leaving [45]. In that case the cis-vicinal 2-OH group plays a role as electrophile catalyst.

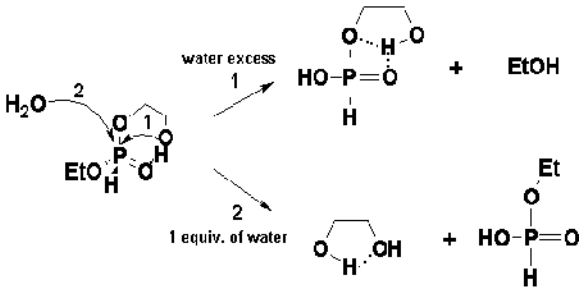

Figure 5b: Scheme of the mono-ol leaving (pathway 1) and diol leaving (pathway 2) accelerated in the presence of the vicinal hydroxyl group, during the reaction of hydrolysis of H-phosphonate diester [45]. The reaction scheme is similar to Figure 5a and is described by the analogous manner.

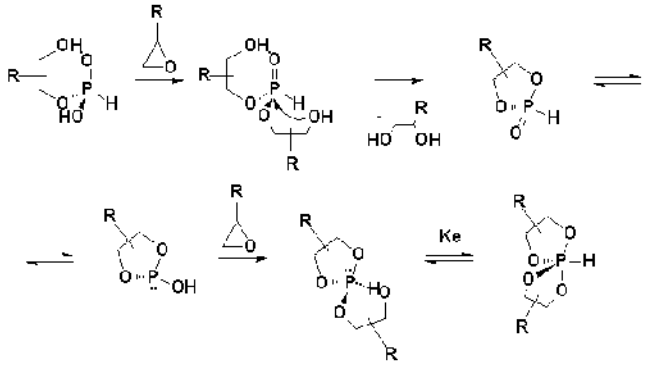

Figure 6a: Scheme of the intramolecular conversion of phosphorous acid (phosphonic acid) as a possible side reaction during the procedure of ribozyme-mimetic synthesis of interbiomonomeric bond. However the possibility on this type of reaction (transformation from bis-2-OH alkyl H-phosphonate (bis-beta-hydroxyalkyl H-phosphonate) to spirophosphorane (H-tetraoxaspirophosphorane), thru the alkylene H-phosphonate (2-oxo 1, 3-dioxa 2-H 2-phospholane), is increased in dioxane/pyridine [46] and proceeds in our case to some minor extent [39].

phosphorus atom from the created amino acid-phosphoric acid mixed anhydride, the intramolecular nucleophile attack by the alpha-amino group predominates the intermolecular attack from the outer nucleophile (as the alpha-amino group of another molecule of the formed amino acid - phosphoric acid mixed anhydride, as well as from the nucleophile reagent, which participates in the reaction) at this stage and particularly at dilute solution (reaction mixture). By this manner, at our conditions of the reaction, the percent of the formed dimmer is too small, because of the above mentioned reasons. Tarbell and Insalaco [40] used t-butylcarbonic diethylphosphoric anhydride as a new reagent for the introduction of Boc-group protection and preparation of t-butoxycarbonyl derivatives. They prove that at anhydrous conditions, an attack on the carbonyl carbon atom of the Boc-group is presumably realized. Perhaps, due to the crucial role of the cis-vicinal 2'-OH group in the ribose ring of A76 peptidyl-tRNA [41] for the rate acceleration of peptidyl transferase intracomplex aminolysis reaction in ribosome, a similar cis-vicinal 2-OH group (beta-hydroxyl group) from the beta-hydroxyalkyl ester group of H-phosphonate part of the mixed amino acid H-phosphonate anhydride play the same role as a proton shuttle (by hydrogen bonding) for the assistance of the intramolecular nucleophile attack by the alpha-amino group with the formation of the reactive cyclic intermediate 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane (B in Figure 2), phosphonamidoate N-carboxy anhydride or H-phosphonamidoate N-carboxy anhydride. Maybe, the same type hydroxyl group played the crucial role during the prebiotic synthesis in the Ancient World, assisting many vital reactions. The formed activated cyclic intermediate (we allow ourselves to call it phosphonamidoate N-carboxy anhydride or H-phosphonamidoate N-carboxy anhydride (PANCA) or (HPANCA) of the amino acids) as a synthetic equivalent, carrying the natural amino acids (as amino acyl syntons) and H-phosphonate synton interacts with other nucleophiles, leading to the formation of a variety of amino acid derivatives. Our comparison of the ribozyme-mimetic reaction of synthesis of methyl esters of the natural amino acids with the prebiotic synthesis of biomolecules is spreading on the fact, that the ancient molecules were simpler, than the bio-molecules, creating the modern World, proving by this way the fact, that the reactions with their assistance were also not so complicated. It is proposed, that at the Early Stage of the Earth Evolution, the ribozymes (RNAzymes, RNA enzymes) were more simplified, they existed without some nucleobases (characterizing the present-day nucleic acids) because that there were no known chemical pathways for the synthesis of nucleotides from pyrimidine nucleobases:

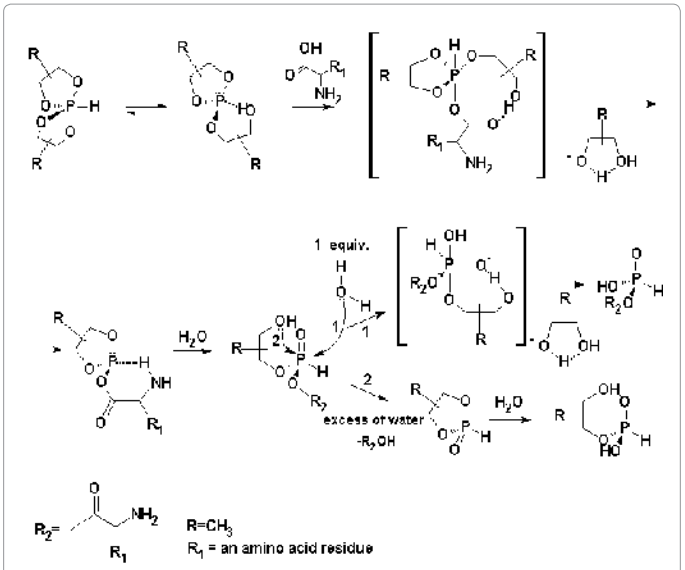

Figure 6b: Scheme, representing the attack from the nucleophile molecule on phosphonyl phosphorus atom, which is possible in the presence of water. Depending on the water content the reaction is divided with the participation of the amino acid (R_2), or 1, 2-propylene glycol as nucleophuges. In both cases it is accompanied by the assistance of the 2-OH group in the beta-hydroxy ethyl ester of H-phosphonic acid derivative [45]. In the presence of 1 equiv. H_2O (pathway 1, after the formation of 2-hydroxy propyl amino acyl phosphonate, R_2=amino acid residue), this water molecule acts as a nucleophile with formation of the amino acyl phosphonate and leaving of 1, 2-propylene glycol as a nucleophuge. In this case an intramolecular hydrogen bond, created between the 2-OH group and phosphonyl oxygen atom increases the electrophilicity of the phosphonyl phosphorus atom and its susceptibility to the intermolecular nucleophile attack. In the second case (pathway 2, after the formation of 2-hydroxy propyl amino acyl phosphonate), due to the large amount of H_2O, the intramolecular hydrogen bond between the 2-OH group and phosphonyl oxygen atom is broken (because of impairing of the intramolecular and creation of intermolecular hydrogen bonds between the molecules of water and the 2-OH group from the one side and between the molecules of water and the phosphonyl oxygen atom from another. This leads to increase of the nucleophile attack from the 2-OH group on the phosphonyl phosphorus atom and leaving of the amino acyl group, and causes the formation of alkylene H-phosphonate (2-oxo 1, 3-dioxa 2-H 2-phospholane), which in the presence of water molecule is hydrolyzed to 2-hydroxyalkyl H-phosphonate. Full studies, concerning the experiments of carrying out of reaction in the desired direction, depending on a variety of water content are included in the forthcoming manuscript and the results will be published elsewhere.

cytosine (C) and uracil (U) under prebiotic conditions [41]. This is due to the fact, that the cytidine (cytosine ribonucleoside) has a half-life in isolation of 19 days at 100°C and approximately 17,000 years in freezing water, which is too short on the geologic time scale for accumulation [42] and it could not be stable enough to be found in the original genetic material [43]. This fact supports the suggestion, that RNAs did not contain cytosine (C) and uracil (U), which suggestion was later disproved by the Sutherland Group at the School of Chemistry, University of Manchester [7], who reported, that pyrimidine ribonucleosides and their respective nucleotides have been prebiotically synthesized by a sequence of reactions, using the nitrogenous and oxygenous chemistry. Moreover, because of the different possible variants of conformation and configuration of the ribose ring, creating the backbone of the nucleic acids, it is claimed that ribose was presented with its wrong chirality enantiomer form, which may acting as a chain terminator [44]. Finally, RNA molecules in the past might have survived longer than they can survive today and it is suggested that they may have been a relatively common substance on early Earth. This aspect is supported by the fact that under the ultraviolet light the RNA molecules could polymerize, while at the same time other types of organic molecules that could potentially damage RNA, are breaking down. Nevertheless, despite the controversial hypotheses about the participation of RNA, it is no doubt that RNA molecules have played a crucial role in the primordial World and were an important step in the Earth Evolution, allowing to the smooth and flowing transformation from the Ancient "RNA World" to the Contemporary "protein" and "DNA World" [39]. However the possibility on this type of reaction (transformation from bis-2-OH alkyl. H-phosphonate (bis-beta-hydroxyalkyl H-phosphonate) to spirophosphorane (H-tetraoxaspirophosphorane), thru the alkylene H-phosphonate (2-oxo 1, 3-dioxa 2-H 2-phospholane), is increased in dioxane/pyridine [45] and proceeds in our case to some minor extent [39]. Depending on the water content the reaction is divided with the participation of the amino acid (R_2), or 1, 2-propylene glycol as nucleophuges. In both cases it is accompanied by the assistance of the 2-OH group in the beta-hydroxy ethyl ester of H-phosphonic acid derivative [45]. In the presence of 1 equiv. H_2O (pathway 1, after the formation of 2-hydroxy propyl amino acyl phosphonate, R_2=amino acid residue), this water molecule acts as a nucleophile with formation of the amino acyl phosphonate and leaving of 1, 2-propylene glycol as a nucleophuge. In this case an intramolecular hydrogen bond, created between the 2-OH group and phosphonyl oxygen atom increases the electrophilicity of the phosphonyl phosphorus atom and its susceptibility to the intermolecular nucleophile attack. In the second case (pathway 2, after the formation of 2-hydroxy propyl amino acyl phosphonate), due to the large amount of H_2O, the intramolecular hydrogen bond between the 2-OH group and phosphonyl oxygen atom is broken (because of impairing of the intramolecular and creation of intermolecular hydrogen bonds between the molecules of water and the 2-OH group from the one side and between the molecules of water and the phosphonyl oxygen atom from another. This leads to increase of the nucleophile attack from the 2-OH group on the phosphonyl phosphorus atom and leaving of the amino acyl group, and causes the formation of alkylene H-phosphonate (2-oxo 1, 3-dioxa 2-H 2-phospholane), which in the presence of water molecule is hydrolyzed to 2-hydroxyalkyl H-phosphonate. Full studies, concerning the experiments of carrying out of reaction in the desired direction, depending on a variety of water content are included in the forthcoming manuscript and the results will be published elsewhere. A new originally developed procedure for biomimetic synthesis of methyl esters of the natural amino acids was realized at conditions, imitating the action of the primitive ribozymes, and it presumes their crucial role in the prebiotic syntheses of the biomolecules during the primordial RNA World. During the reaction time course of the procedure, the obtained reactive intermediate was sensitive to the nucleophile attack, representing by the MeOH molecule as one of the simplest nucleophile and leading to the ring opening, accompanying by the formation of methyl esters. The model system may be applied to other nucleophiles, leading to oligomerization (bio oligomer synthesis) by the condensation reaction. This bio-mimetic reaction presumes the crucial role of the primitive ribozymes during the prebiotic syntheses of biomolecules in the ancient RNA World.

Conclusion

The novel and general procedure for ribozyme-mimetic synthesis of methyl esters of various natural amino acids was realized, presuming the crucial role of ribozymes in the prebiotic synthesis of life-significant biomolecules, during the primordial RNA World. The efficient ribozyme-mimetic synthetic methodology for the preparation of methyl esters of various natural amino acids was developed, using methyl oxirane (propylene oxide) as a condensing reagent and phosphorous (phosphonic) acid as reagent for different conventional group influence (an amino group protection and a carboxyl functionality activation), at mild reaction conditions. By our originally developed procedure for ribozyme-mimetic (bio-mimetic) synthesis of methyl esters of a variety of natural amino acids, we predicted and proposed, that due to the assistance of the 2-OH group in the beta-hydroxy ethyl ester of H-phosphonic acid, it was created a prerequisite for the susceptibility of the carbonyl carbon atom to nucleophile attack by internal and external nucleophiles, and proving by this way the same crucial role of the cis-vicinal syn-oriented 2'-OH group in the ribose ring in RNA, revealing the prebiotic role of ribozymes at the Early Stages in the Primordial RNA-world. The same cis-vicinal syn-oriented 2'-OH group in the ribose ring of A76 at the 3'-end in peptidyl-tRNA plays an important role in the rate acceleration of the peptidyl transferase aminolysis reaction during the protein biosynthesis, proceeding on ribosome. The synthesis was carried out at mild (bio-mimetic) conditions, without previously protection of natural amino acids as described above, simplifying by this manner the reaction conditions and avoiding the additional procedure for the amino acid alpha-carboxy or alpha-amino functionalities protection. The methodology will be developed and applied for practical approaches and industrial purposes.

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