An efficient ribozyme - mimetic synthesis of methyl esters of various natural amino acids

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An efficient ribozyme-mimetic synthesis of methyl esters of various natural amino acids: *The synthesis was carried out, using methyl oxirane (propylene oxide) as condensing reagent and phosphorous (phosphonic) acid as reagent for different conventional group influence (amino group protection and carboxyl functionality activation), at mild conditions. This bio-mimetic reaction proposes the crucial prebiotic role of ribozymes at the Early Stages in the primordial RNA World.*

Key words: *Ribozyme-Mimetic Synthesis, Ribozymes, Methyl Oxirane, Phosphonic Acid, Primordial RNA World, Methyl Esters of Natural Amino Acids.*

INTRODUCTION

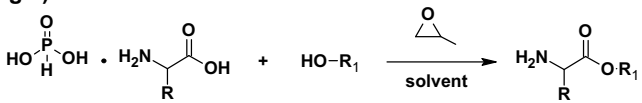
The translation remains one of the few processes in Nature (together with RNA splicing, during its own maturation), which proves the important role of the ribozymes at the Early Age of the Earth Evolution (during the Primordial RNA-world). The discovery of the phenomenon in the middle of 80-s of the twentieth century by Thomas Cech and co-workers, that not only the proteins play a role as enzymes, but also RNA (ribozymes) [Zaug A. J., T. R. Cech. *Science.*, 1986, **231,** 470-473], is accompany by direction of the efforts of researchers towards the determination of the mechanism of the 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 [1-3]. The electrophile participation of the vicinal hydroxyl group leads to the increased electrophilicity of phosphoryl phosphorous atom and 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. [4] 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 [5]. 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) [3]. The importance of the vicinal hydroxyl group is described at the cleavage site of the reaction of Tetrahymena ribozyme [6, 7]. 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 toward 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 gave 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 found 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 quite expensive, and often with low effectiveness due to the target bond formation [8, 9]. 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 [10]. Moreover, dicyclohexyl urea, obtained as a secondary product during the reaction of interbiomonomeric bond synthesis, is difficult or in more cases impossible to be removed from the final product. This allowed us to improve the methodology of interbiomonomeric bond formation, by the using of the more inexpensive methyl oxirane (propylene oxide) as condensing reagent, and prosphonic acid

- for the functional group influence (amino group protection and at the same time carboxyl group activation), thus avoiding the stage of the 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 [11-13]. Their N-functional group protection and C-functional group activation were realized *in situ* during the condensation reaction. The activation of α-carboxyl function as an electrophile was realized, and at the same time as α-amino group protection was fulfilled, that ensures only the target condensation reaction.

Herein, we demonstrate the synthesis of methyl esters of various natural amino acids, which are commonly presented in Nature, using bio-mimetic reaction, which resembles and tries to prove the ribozyme function and role at the Early Ages of Earth Evolution **(Fig.1).**



R_1 = CH_3.

R = H, CH_3, $CH(CH_3)CH_3$, $CH_2CH(CH_3)CH_3$, $CH(CH_3)C_2H_5$, CH_2OH, $CH(CH_3)OH$, CH_2SH, $CH_2CH_2SCH_3$, $CH_2C_6H_5$, CH_2COOH, CH_2CH_2COOH, CH_2CONH_2, $CH_2CH_2CONH_2$, $(CH_2)_4NH_2$.

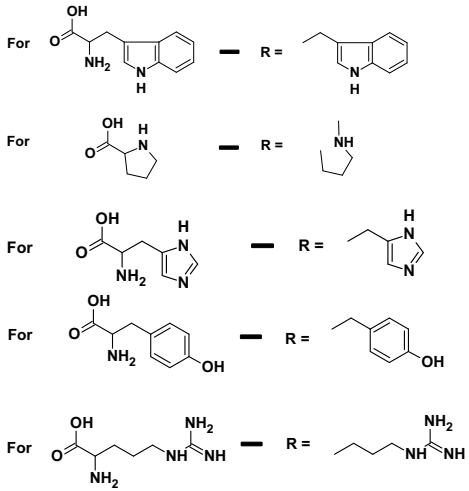


Fig.1. A General reaction scheme for ribozyme-mimetic synthesis of methyl esters of various amino acids*.

***** *The methyl esters of Gly, L-Ala, L-Phe and L-Thr were previously synthesized by the author and coworkers [11, 12].*

EXPERIMENTAL

Material 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 x 4.6 mm) for analytical runs. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II+ 600MHz spectrometer in D_2O and DMSO-d6, using BBO or TBI probeheads. Chemical shifts are expressed in ppm and coupling constants in Hz. The precise assignments of the ^{1}H 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 ^{1}H NMR spectra was speed up by the use of FAFOMA program [15]. 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 ^{1}H, ^{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 ^{1}H 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 ^{1}H and ^{13}C NMR analyses in D_2O.

Experimental part

General procedure for the preparation of methyl esters of natural amino acids

The pure natural amino acid (0.01 mol, 1equiv.) and H_3PO_3 (0.01 mol, 1equiv.) were dissolved in a mixture of MeOH/H_2O with a vigorously stirring. After the fully amino acid dissolving, the solvents were evaporated and the moisture was co-evaporated in vacuo. The obtained oil was dissolved in MeOH. Further 2.5 equiv. (0.025 mol) of methyl oxirane were added and the reaction mixture was heated for 20-30 min at 40^{O}C. After that the reaction mixture was stirred for 12h at a room temperature. At the end of the reaction time 10% solution of Na_2CO_3 was added to the reaction mixture. The reaction mixture was stirred for 3-4h. The obtained product was extracted with EtOAc or CH_2Cl_2* (3x20ml) and washed with 5% $NaHCO_3$ (3x20ml) and saturated H_2O solution of NaCl (brine) till pH 7-8. The organic layer was dried over anhydrous Na_2SO_4 and the solvent was evaporated under vacuo.

- *Depending on the amino acid ester solubility.*

Valine methyl ester. Yield: 0.944g (72%). Rf-0.897. **^{1}H NMR (600 MHz, DMSO-d6, 25^{o}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 (600 MHz, DMSO-d6, 25^{o}C):** δ =169.0, 57.4, 52.3, 29.1, 18.4, 17.5. **^{13}C NMR (600 MHz, D_2O, 25^{o}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 = 131.175g/mol); C-54.939%, H-9.989%, N-10.678%; found: C-54.834%, H-9.876%, N-10.563%.

Leucine methyl ester. Yield: 1.089g (75%). Rf-0.975, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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 = 145.2016g/mol); C-57.904%, H-10.412%, N-9.646%; found: C-57.583%, H-10.355%, N-9.593%.

Isoleucine methyl ester. Yield: 1.002g (69%), Rf-0.981, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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 = 145.2016g/mol); C-57.904%, H-10.412%, N-9.646%; found: C-57.602%, H-10.358%, N-9.596%.

Methionine methyl ester. Yield: 0.686g (42%)•. Rf-0.581, CH_2Cl_2 : MeOH (9:1).**^{1}H NMR(600 MHz, D_2O, 25°C):** δ =4.512 (t, 1H), 3.917 (s, 3H), 3.340 (d, 2H); **^{13}C NMR (600 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 = 163.247g/mol); C-44.147%, H-8.026%, N-8.58%, S-19.643; found: C-44.134%, H-8.019%, N-8.513%, S-19.491.

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

Proline methyl ester. Yield: 0.865g (67%). Rf-0.429, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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 = 129.159g/mol); C-55.796%, H-8.584%, N-10.845%; found: C-55.504%, H-8.539%, N-10.788%.

Tryptophan methyl ester. Yield: 1.419g (65%). Rf-0.742, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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 = 218.255g/mol); C-66.038%, H-6.465%, N-12.835%; found: C-65.934%, H-6.455%, N-12.814%.

Lysine methyl ester. Yield: 0.657g (41%). Rf-0.214, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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. 216g/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: 1.976g (76%). Rf-0.443 (CH_2Cl_2:CH_3OH-8:2), Rf–0.272 (CH_2Cl_2:CH_3OH-9:1). **^{1}H 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 = 260.064g/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: 0.662g (38%)¥. Rf-0.169, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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 = 174.223g/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: 0.765g (52%). Rf-0.519, CH_2Cl_2 : MeOH (9:1).**^{1}H 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 (600 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 = 147.130g/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: 0.79g (49%), Mp. Rf-0.549, CH_2Cl_2 : MeOH (9:1). **^{1}H 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 (600 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 = 161.158g/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.*

Ornithine (tert-butyloxycarbonyl) methyl ester¶. Yield: 1.796g (73%). Rf-0.436 (CH_2Cl_2:CH_3OH-8:2), Rf–0.263 (CH_2Cl_2:CH_3OH-9:1). **^{1}H 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 = 246.037g/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.*

Results and 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'.

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 obtained, 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 synthase. 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, which was similar to urethane N-carboxy anhydrides (UNCA) (**Fig.2.**) [14]. In the case of a phospholane derivative, at the same time with the protection of NH_2-group, the carboxyl group is activated.

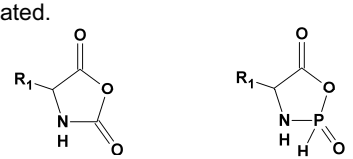


Fig.2. Structures of urethane N-carboxy anhydride (UNCA) **A,** and 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane **B.**

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 aiming (targeting) condensation reaction. The reaction was made in alcohol solution in the presence of hydroxypropyl ester of H_3PO_3, obtained *in situ* by means of H_3PO_3 and propylene oxide interaction. The reaction between this intermediate and amino acid leads to preparation of aminoacyl phosphonate **(Fig.3).** The latter is similar to aminoacyl adenylate obtained as macroergic compound, during the process of aminoacylation of tRNA.

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) [14].

The formed 2-OH-alkyl diester of the phosphonic acid reacts with the carboxy anion of the amino acid at temperature of 40^0C 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.)

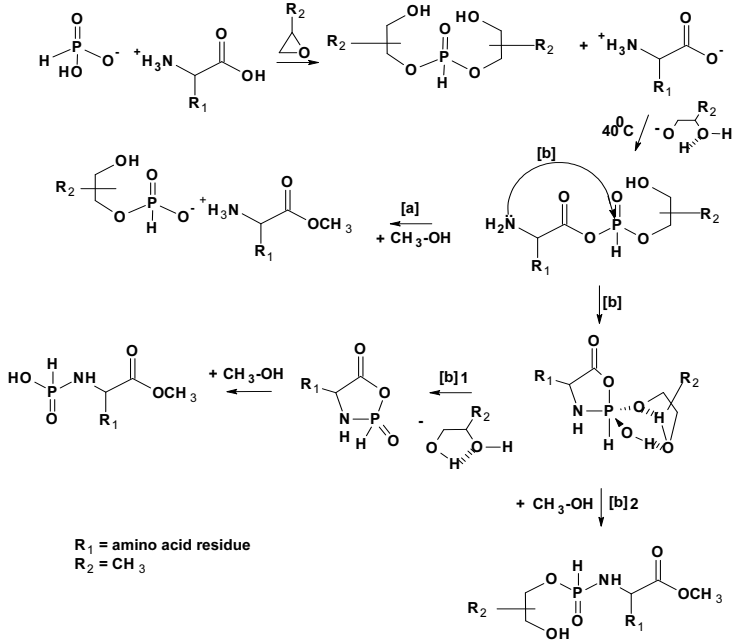


Fig.3. Possible reaction pathways for the preparation of methyl esters of natural amino acids [13].

The first reaction is an intra-molecular 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 amino group) of 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 и Ala [11,12]. 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 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 [16]. In the case of aspartic acid and glutamic acid, because of 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 of 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 alfa- or beta- and gamma- position) monomethylation was noticed [16]. This allows us to protect the carboxyl group at a 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 [16].

CONCLUSION

By our originally developed procedure for ribozyme-mimetic (bio-mimetic) synthesis of methyl esters of a variety of natural amino acids**,** we proposed the crucial role of ribozymes in the Primordial RNA-world. The synthesis was carried out at mild (bio-mimetic) conditions, without previously protection of natural amino acids. This methodology will be developed and applied for practical approaches and industrial purposes.

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