A New Efficient Strategy For Amino-Acylation of 2'/3'-OH group in Adenosine and 3'-OH group in 2'-deoxy Adenosine.

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A New Efficient Strategy For Amino-Acylation of 2'/3'-OH group in Adenosine and 3'-OH group in 2'-deoxy Adenosine. *The standard aminoacylation procedure was carried out using methyl oxirane as a condensing reagent and phosphonic acid as reagent for the conventional group protection: protection of the alpha-amino group, as well as - activation of the alpha-carboxyl group at the same time. The new efficient strategy for aminoacylation of 2'/3' OH-group in adenosine and 3'-OH group in 2'-deoxy adenosine reveals the successful methodology for aminoacylation of nucleosides and presumes the crucial role of the prebiotic aminoacylation during the peptide synthesis in the Ancient RNA World.*

Key words: *Aminoacylation of nucleosides, 5'-OPiv-Ado, 5'-OPiv-2'-dAdo, methyl oxyrane (1,2-propylene oxide), H-phosphonic acid (phosphorous acid), 2-hydroxyalkyl H-phosphonate (beta-hydroxyalkyl phosphite), bis-(2-hydroxyalkyl) H-phosphonate, 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane, PANCA or HPANCA.*

INTRODUCTION

Aminoacyl nucleosides and nucleotides play an important role in the chemistry and biochemistry to some aspect: as for mechanistic studies – for the elucidation of the mechanism of peptide bond formation, during the protein biosynthesis on the ribosome, as well as for the development of novel substances as pro-drug and drug candidates in the cancer therapy – for the blocking of the translation in the cancer cells.

Several methods for aminoacylation of 2'/3'-OH group in the nucleosides (nucleotides) and 3'-OH group in the 2'-deoxy nucleosides (2'-deoxy nucleotides) are well known: chemical aminoacylation by the method of the cyanomethyl esters [1], aminoacylation using the classical coupling (condensing) reagents: DCC [2], TBTU/DIPEA, HBTU/DIPEA, HATU/DIPEA, TATU/DIPEA etc. Moreover some authors have used pentafluorophenyl activating esters, trichlorophenyl active esters, amino acid fluorides (fluoroanhydrides) [3], for realizing of the ester bond. Also, some authors used lipase to create aminoacyl nucleosides at mild reaction conditions [4]. Unfortunately, on the one hand - all of them require the necessary protection of alpha-amino group, before the condensing reaction – to avoid the participation of this amino group in unwanted side reactions. Contrariwise, the carboxyl group has to be activated by the direct coupling reaction, using the above noted reagents or by the previously preparation of carboxy activated esters. All of the stages require time spending, solvent and reagents consumption, which expense the whole process.

Herein, we describe the synthesis of aminoacyl nucleosides by our originally developed procedure for biomimetic (ribozyme-mimetic) esterification of 2'/3'-OH group in the nucleosides (nucleotides) and 3'-OH group in the 2'-deoxy nucleosides (2'-deoxy nucleotides), using the phosphonic acid / oxirane chemistry. It avoids the preliminary protection of the alpha-amino functionality in the aminoacyl component by the *in situ* protection of the alpha-amino group as well as activation of the alpha-carboxyl group in the formed 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane (PANCA or HPANCA) as intermediate during the time course of the reaction, which is similar to the **U**rethane **N**-**C**arboxy **A**nhydrides: UNCA.

This novel and efficient strategy for aminoacylation of 2'/3' OH-group in adenosine and 3'-OH group in 2'-deoxy adenosine reveals the successful methodology for aminoacylation of nucleosides and presumes the crucial role of ribozymes in the prebiotic aminoacylation, during the peptide synthesis in the Ancient RNA World. The approach uses methyl oxirane (1, 2-propylene oxide) as a condensing reagent, as well as H_3PO_3 as a protecting reagent for the alpha-NH_2 function of the amino acid on the one hand, and also as an activator for the alpha-carboxyl group from the other hand. In order to

demonstrate the effectivity of the biomimetic (ribozyme-mimetic) procedure, the aminoacylation of 5'-OPiv-Ado and 5'-OPiv-2'-dAdo was carried out, employing N^{δ}-Boc-protected ornithine (H-Orn(Boc)-OH) as aminoacyl component, realizing the ornithylation of these two 5'-protected nucleosides as a model reaction. The author has been using for a long time H_3PO_3/oxyrane system to mimic the ribozyme catalyzed interbiomonomeric bond formation, creating by this way methyl esters of the natural alpha-amino acids [5-7], amides and peptides [8], and finally – oligonucleotides [9].

EXPOSITION

The elucidation of the phosphoryl transfer mechanism, involving 1,2-diol exchange reaction [10-13] by the participation of the vicinal *syn*-oriented 2'-OH group in the ribose ring of the RNA molecule [14-16], plays an important role with regards to the searching for one of the main reasons of action of ribozymes (RNAzymes) as well as 2'-deoxyribozymes (DNAzymes).

The compounds of the pentacoordinated phosphorus P(V) have a general interest and role, especially due to their specific stereochemistry and reaction ability as well as their important biological functions. For example, the discovered by Zhao et al. activated intramolecular pentacoordinated phosphocarboxyl mixed anhydride of N-phosphoryl amino acids, that could auto catalyzes a lot of bioorganic reactions at mild reaction conditions, is a very important intermediate in the reactions of esterification and amide (peptide) bond synthesis [17]. Moreover, during the reaction course of amino acid phosphorylation, Zeng et al. successfully isolated N-dialkyloxyphosphoryl peptides in the presence of dialkylphosphite, probably as a result of a secondary condensation reaction. They prove this fact by the successfully synthesizing of a series of protected dipeptides by means of dialkylphosphite as a catalyst [18]. These N-phospho-α-amino acids, that are reactive (activated) intermediates, could auto activate the synthesis of N-phosphoryl peptides and N-phospho-α-amino acid esters in water-alcohol media at mild reaction conditions and it was proved, that in these compounds (N-phospho-α-amino acids) an intramolecular N-O phosphoryl group transfer could also occur [19, 20]. And finally, Fuller et al. establishes that the reaction of H_3PO_3 with amino acids results in their activation by the *in situ* formation of 4-alkyl-2,5-dioxo-1,3-oxaza 2-H-2-phospholane [21], which proves the fact that in our reaction conditions (pathway b and particularly b1 in Fig.3 and Fig.4) this reactive intermediate could be created, proving that fact by the elucidation of the reaction mechanism [22] and the prepared products.

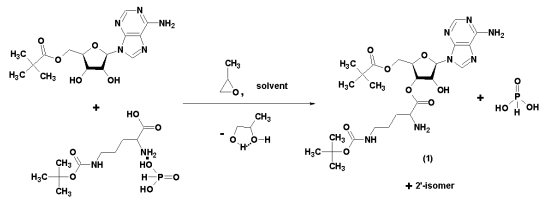


Fig. 1. Scheme of the reaction describing the efficient procedure for aminoacylation (ornithylation) of 2'/3' OH-group in the 5'-protected adenosine.

All these facts support the statement, with respect to the unique properties of the phosphoric acid derivatives: phosphoric acid, phosphonic acid (phosphorous acid) and phosphinic acid (hypophosphorous acid).

The important role of aminoacyl nucleosides, nucleotides and oligonucleotides, they have played in the drug discovery stimulate the development of new and effective methods

for their large scale, chemically and chirally pure, and elegant preparation using biomimetic conditions. By the development of this procedure we proposed that the natural reaction of ribonucleoside bond cleavage and ribonucleoside bond formation in ribozymes could be realized at low-molecular level, by the demonstration of the novel approach for interbiomonomeric bond formation, using the specific properties and function of 1,2-diol system as electrophilic or nucleophilic catalyst.

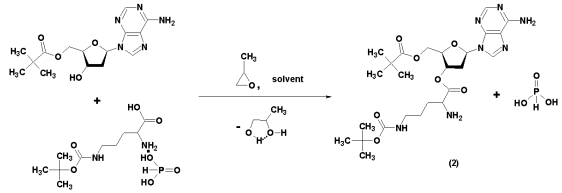


Fig. 2. Scheme of the reaction describing the efficient procedure for aminoacylation (ornithylation) of 3' OH-group in the 5'-protected 2'-deoxy adenosine.

EXPERIMENTAL

Material and Methods

Adenosine and 2'-deoxy adenosine, pivaloyl chloride, di-tert butyl pyrocarbonate: $(Boc)_2O$, L-ornithine, H_3PO_3 and propylene oxide were purchased from Merck. CH_3CN was purchased from Fluka. 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 alcoholic solution of ninhydrin was used, as well as a solution of phosphorus-molybdenum acid. For TLC analyses - CH_2Cl_2 : MeOH (8.5:1.5) was employed as a solvent system. The 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. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance II+ 600MHz spectrometer in DMSO-d6, using BBO or TBI probeheads. 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 [23]. 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).

Experimental part

A General procedure for aminoacylation of nucleosides:

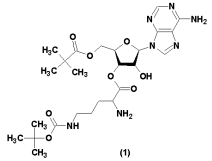
The pure natural amino acid (0.01 mol, 1equiv.) (N^{δ}(Boc)L-ornithine: H-Orn(Boc)-OH in our case) and H_3PO_3 (0.01 mol, 1equiv.) were dissolved in a mixture of CH_3CN/H_2O with vigorous stirring. After dissolving of the amino acid, the solvents were evaporated and the moisture was co-evaporated several times in vacuo. The obtained crystal clear viscous oil was dissolved in CH_3CN or DMSO. The reaction mixture was allowed to stand in ice salted bath (-15^{0}C to about -20^{0}C), to which 2.5 equiv. (0.025 mol) of methyl oxirane were added and the reaction mixture was allowed to stand at -15^{0}C - -20^{0}C for 30 min, then to the

reaction mixture was added 5'-OPiv Ado or 5'-OPiv 2'-dAdo and it was slowly heated at 40^0C for about 20-30 min. After that the reaction mixture was stirred for 12h, 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 3-4h. 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) 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^{0}C to about -20^{0}C). When NaOH in MeOH (KOH in MeOH) was used for deprotection of H-phosphonate (phosphonamide) protective group in the nucleosil 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.*

2'/3'-O-[Orn(Boc)]-5'-O-Piv-Ado (1)



(1)

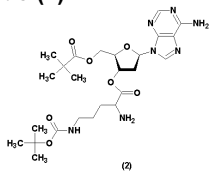
^{1}H NMR (600 MHz, DMSO-d6, 25^{o}C): δ = 1.221(s, 9H, CH_3), 1.365(s, 9H, CH_3), 1.587-1.623(m, 2H, 4-CH_2), 1.653(m, 1H) and 1.867(m, 1H, 3-CH_2), 3.189(m, 2H, 5-CH_2), 3.218-3.235(s, broad, 2H, 2-CHN$\underline{H}_2$), 3.836(ddd, J=4.5, 7.2, 9.4 Hz, 1H, 2-CH), 4.234-4.363(m, 2H, 5`-$CH_2$), 4.754-4.783(m, 1H, 4`-CH), 5.008(dt, J=5.1, 6.2 Hz, 1H, 2`-CH), 5.691-5.743(dd, J=2.9, 4.1 Hz, 1H, 3`- CH), 6.118(d, J=6.2 Hz, 1H, 1`-CH), 6.013(d, J=6.2 Hz, 1H, 2`-OH), 6.748(t, J=4.9 Hz, 1H, 5-CH_2N$\underline{H}$), 7.379(s, 2H, NH_2), 8.144(s, 1H, 8-CH), 8.231(s, 1H, 2-CH).

^{13}C NMR (150 MHz, DMSO-d6, 25^{0}C): δ = 26.23(4-CH_2), 27.26(CH_3, Piv), 28.24(CH_3, Boc), 29.59(3-CH_2), 38.88(C, Piv), 41.91(5-CH_2), 56.57(2-CH), 64.29(5`-$CH_2$), 72.62(2`-CH), 76.35(3`-CH), 79.82(C, Boc), 82.45(4`-CH), 88.94(1`-CH), 119.67(5-C), 141.74(8-CH), 151.21(4-C), 152.65(2-CH), 154.79(6-C), 156.16(NHCOO), 177.78(3'-COOC), 178.56(5'-OC=O).

Elemental analysis: Anal. Calculated for $C_{25}H_{39}N_7O_8$: (M_w = 565.6219 g/mol); C-53.087%, H-6.95%, N-17.334%; found: C-52.971%, H-7.016%, N-17.126%.

Rf-0.346 (CH_2Cl_2 : MeOH (8.5:1.5)). Analytical RP-HPLC (60% CH_3CN in 0.02M KH_2PO_4/K_2HPO_4 buffer, pH 7.0; flow 0.8 ml/min, 298.2K): R_t = 3.5 min.

3'-O-[Orn(Boc)]-5'-O-Piv-2'-dAdo (2)



(2)

^{1}H NMR (600 MHz, DMSO-d6, 25^{o}C): δ = 1.219(s, 9H, CH_3), 1.363(s, 9H, CH_3), 1.578-1.615(m, 2H, 4-CH_2), 1.721(m, 1H) and 1.878(m, 1H, 3-CH_2), 2.552(ddd, J=2.3, 6.1, 14.1 Hz, 1H) and 2.821 (m, 1H, 2`- $CH_2$), 3.184(m, 2H, 5-$CH_2$), 3.351-3.523(s, broad, 2H, 2-CHN$\underline{H}_2$), 3.953(t, J=5.6 Hz, 1H, 2-CH), 4.234(m, 2H, 5`-CH_2), 4.554(m, 1H, 4`-CH), 5.589(td, J=2.3, 8.6 Hz, 1H, 3`-CH), 6.453 (t, J=6.8 Hz, 1H, 1`-CH), 6.913(t, J=5.8 Hz, 1H, 5-CH_2N$\underline{H}$), 7.383(s, 2H, NH_2), 8.141(s, 1H, 8-CH), 8.321(s, 1H, 2-CH).

^{13}C NMR (150 MHz, DMSO-d6, 25^{0}C): δ = 26.17(4-CH_2), 27.72(CH_3, Piv), 28.21(CH_3, Boc), 29.63(3-CH_2), 37.74(2`-$CH_2$), 39.96(C, Piv), 42.05(5-$CH_2$), 55.85 (2-CH), 65.67(5`-CH_2), 76.27(3`-CH), 79.45(C, Boc), 84.17(1`-CH), 84.28(4`-CH), 120.83(5-C), 140.73(8-CH), 151.03(6-C), 153.98(2-CH), 156.17(NHCOO), 157.03(4-C), 176.82 (3'-COOC), 179.13(5'-OC=O).

Elemental analysis: Anal. Calculated for $C_{25}H_{39}N_7O_7$: (M_w = 549.623 g/mol); C-54.632%, H-7.152%, N-17.839%; found: C-54.598%, H-7.457%, N-17.621%.

Rf-0.467 (CH_2Cl_2 : MeOH (8.5:1.5)). Analytical RP-HPLC (60% CH_3CN in 0.02M KH_2PO_4/K_2HPO_4 buffer, pH 7.0; flow 0.8 ml/min, 298.2K): R_t = 4.1 min.

RESULTS AND DISCUSSION

As we have noticed before [8], the oxirane ring opening, commonly realized via the simultaneously protonation of the 1,2-propylene oxide oxygen atom by the phosphonic acid (phosphorous acid), accompanied with the phosphonic acid action (nucleophilic attack from the formed H-phosphonate oxyanion), or sometimes only by the nucleophilic attack from the formed H-phosphonate oxyanion, leads to the β-hydroxyalkyl H-phosphonate monoester formation. After that, di-(β-hydroxyalkyl) H-phosphonate is created, which is the actual reactive intermediate, sensitive and susceptible to the nucleophilic attack from an external nucleophile. In the absence of the external nucleophile, an accumulation of alkylene H-phosphonate, β-hydroxyalkyl alkylene phosphite and the corresponding pentacoordinated H-tetraoxaspirophosphorane is too possible, due to the intramolecular rearrangement and reformation that proceed at elevated temperature. When di-(β-hydroxyalkyl) H-phosphonate is attacked by the external nucleophile, different products could be formed: esters, amides, peptides etc.

Herein, we describe the synthesis of two aminoacyl nucleosides 3'-Orn(Boc) 5'-OPiv-2'-dAdo and 2'/3'-Orn(Boc) 5'-OPiv-Ado in order to demonstrate the ability of H_3PO_3/oxyrane system to mimic the interbiomonomeric bond formation (biomimetic synthesis), thus describing the analogous properties of the ribozymes action during the Primordial RNA World and their important role in many biochemical processes.

The reaction principally proceeds in various solvents: MeCN, DMSO, DMF or CH_2Cl_2 etc.*

**When the various esters of amino acids were prepared, the relevant alcohol was used as a solvent (reaction media).*

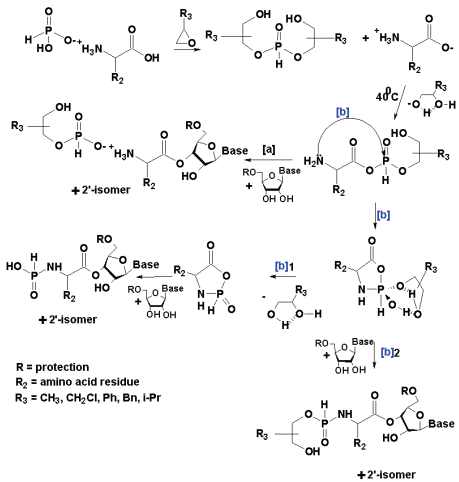


Fig. 3. Reaction scheme describing the all possible pathways, during the procedure for aminoacylation (ornithylation) of 2'/3' OH-group in the 5'-protected adenosine.

But in our methodology the reactions were carried out in MeCN or pure DMSO and this is due to the fact, that the starting reagents had good solubility in the mentioned solvents. As was noticed before, by the nucleophilic attack of the H-phosphonate anion to the methyl oxirane (1,2-propylene oxide), the obtained 2-OH-alkyl diester of the phosphonic acid – the actual reactive intermediate, carrying the H-phosphonate synton by its synthetic equivalent, is susceptible to the attack by the carboxy anion of the amino acid which leads to the formation of the corresponding amino acid H-phosphonoanhydride. This is due to the increasing of the temperature of the reaction mixture, allowing to the formation of the higher macroergic anhydride bond from the activating ester bond with lower energy. The 2-OH-alkyl H-phosphonate synthetic equivalent could be termed as electrophilic catalyst in which the vicinal 2-OH group also plays a role as electrophile catalyst, and as a catalyst on the principle of the proton shuttle, accelerating the electrophilicity of the phosphonyl phosphorus atom in the di-(β-hydroxyalkyl) H-phosphonate and its susceptibility to the nucleophilic attack from the external nucleophile (carboxy anion in this case). Thus synthesized 2-OH-alkyl ester H-phosphonoanhydride of the amino acid (Fig.3 and Fig.4) plays a main role in the whole process and after that (its formation) the phosphonyl phosphorus atom and the carbonyl carbon atom from the carboxyl group in the anhydride molecule are susceptible to the nucleophilic attack from an internal (pathway b) or external (pathway a) nucleophile. Pathway b leads to the formation of a transition state molecule and intermediate: 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane (PANCA or HPANCA), which is the key structure in the whole process. In this compound with the simultaneously alpha-amino group protection, the carboxyl group is activated and it is allowed to be sensitive enough to the nucleophilic attack from a variety of nucleophiles: alcohols, sugars, nucleosides and nucleotides as well as oligonucleotides, amines and amino acids etc. If this 4-alkyl 2,5-dioxo 1,3-oxaza 2-H 2-phospholane (PANCA or HPANCA) is attacked by the 2'/3'-OH group of 5'-O-protected ribonucleosides (ribonucleotides or oligoribonucleotides) or 3'-OH group of 5'-O-protected 2'-deoxyribonucleosides (2'-deoxyribonucleotides or 2'-deoxyribooligonucleotides) the corresponding aminoacyl derivatives can be created in good yields and purity at mild reaction conditions.

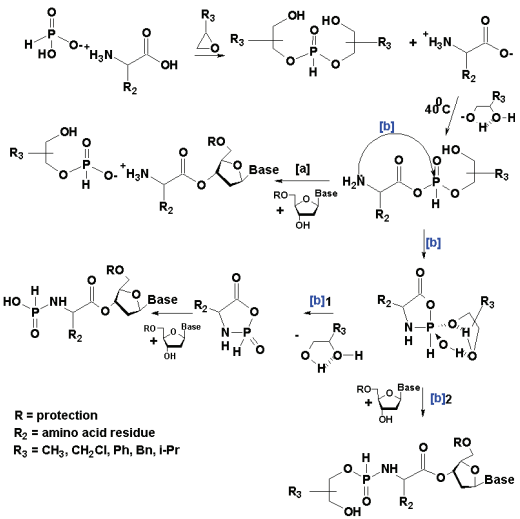


Fig. 4. Reaction scheme describing the all possible pathways, during the procedure for aminoacylation (ornithylation) of 2'/3' OH-group in the 5'-protected adenosine.

CONCLUSION

A new successful approach for biomimetic (ribozyme-mimetic) synthesis of aminoacyl nucleosides, using oxirane as a condensing reagent and phosphorous acid as reagent for functional group activation and protection (protection of alpha-amino functionality and at the same time activation of alpha-carboxyl group), was realized at mild reaction conditions and demonstrated by this way the successful manner of synthesis of biologically important molecules in the Ancient RNA World. The novel developed methodology avoid the preliminary stage of alpha-amino functionality protection, as well as allows the using of well known and inexpensive coupling reagents. This synthesis protocol could be applied for the commercial preparation of 2'/3'-O-aminoacyl nucleosides as well as 3'-O-aminoacyl 2'-deoxynucleosides and nucleotides to be used in the pharmaceutical industry and molecular biology.

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Докладът е рецензиран