A Novel General Methodology for Oligodeoxyribonucleotide Synthesis, Using a Modification of the H-Phosphonate Procedure

Stanislav Bayryamov

A Novel General Methodology for Oligodeoxyribonucleotide Synthesis, Using a Modification of the H-Phosphonate Procedure: *The paper describes the new originally developed procedure for oligonucleotide synthesis, allowing the creation of different oligomers at mild reaction conditions. The pathway of the nucleoside phosphonylation is preceded by the acid-catalyzed oxirane ring opening with phosphonic acid, leading to the formation of bis-(2-hydroxyalkyl) H-phosphonate – a reactive phosphonylation synthetic equivalent, using the ester 2-OH group as electrophilic catalyst and accompanied by the 1,2-diol leaving at anhydrous reaction conditions. This property of bis-(2-hydroxyalkyl) H-phosphonate is used as a model of the reaction catalyzed by the large ribozymes (group I, group II, ribonuclease P and spliceosomal introns).*

Key words: *Oligodeoxyribonucleotide Synthesis, methyl oxirane (1,2-propylene oxide), H-phosphonic acid (phosphorous acid), 2-hydroxyalkyl H-phosphonate (beta-hydroxyalkyl phosphite), bis-(2-hydroxyalkyl) H-phosphonate, 5'-dC-p-dC-p-dA-3'-OH (dCdCdA) and 5'-G-p-T-p-U-3'-OH (GTU).*

INTRODUCTION

Oxiranes are famous and very interesting compounds with a wide range application, they have found in the chemistry and industry. It is well known, that their ring opening is possible as in acid- and in base reaction conditions, as well as by nucleophilic attack from the internal or external nucleophile molecule. The acid-catalyzed ring opening reaction has been described by *T. Biela, P. Kubisa, R. Szymanski* and *S. Penczek* [1, 2]. When this reaction is carried out with H-phosphonic acid (phosphorous acid), it leads to the formation of 2-hydroxyalkyl H-phosphonate (*beta*-hydroxyalkyl phosphite), and then – to the formation of 2-hydroxyalkyl H-phosphonate diester (due to the anchymeric assistance by the vicinal hydroxyl group, facilitating the faster reaction of 2-hydroxyalkyl H-phosphonate with the second oxirane molecule), which is the actual reactive intermediate, causes the phosphonylation. The participation of the ester 2-OH group of 2-hydroxyalkyl H-phosphonate diester (by an intramolecular hydrogen bonding) in hydrolysis or alcoholysis (nucleophilysis) reaction, which is accompanied by the 1,2-diol leaving as a model reaction of the electrophilic catalysis by the vicinal β-hydroxyl group in aprotic organic media is used by the large ribozymes (group I, group II, ribonuclease P and spliceosomal introns) [3]. The other reaction, which is accompanied by the assistance of *beta*-hydroxyl group in the *bis*-(2-hydroxyalkyl) H-phosphonate as nucleophilic catalyst in aqueous conditions, is the reaction, characterized for the ribonuclease A catalyzed hydrolysis [4] and leads to phosphate migration or mono-ol exchange [5-7].

The *bis*-(2-hydroxyalkyl) H-phosphonate actually is the reactive intermediate, participating in a variety of reactions, susceptible for attack by different nucleophiles and leading to different types of products. When this reactive intermediate is attacked by the alcohol molecules (methanol, ethanol, propanol, isopropanol, sugar or the ribose/2'-deoxyribose ring from the nucleoside), the process is characterized as a typical transesterification reaction.

Here we describe the novel strategy and methodology for oligonucleotide synthesis, using phosphonic acid/oxirane chemistry, and allowing to the synthesis of dimmers (dinucleoside H-phosphonates) and trimers (trinucleoside di-H-phosphonates), that can be used in the contemporary chemistry and chemical biology.

EXPERIMENTAL

Material and Methods

All of the natural nucleosides (ribonucleosides) and 2'-deoxy nucleosides (2'-deoxy ribonucleosides), 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 alcoholic solution of ninhydrin was used, as well as a solution of phosphorus-molybdenum acid. For TLC analyses - CH_2Cl_2 : MeOH (9:1) or (9.5:0.5) 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 $CDCl_3$ or 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)*. 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).

* The author wishes to thank Prof. Dr. Nikolay G. Vassilev for the helpful discussion in regard to the ^{13}C NMR spectra.

Experimental part

A General procedure for oligonucleotide synthesis:

The pure propylene oxide (0.0025 mol, 2.5 equiv.) and H_3PO_3 (0.001 mol, 1equiv.) were dissolved in DMSO with a vigorously stirring at 0^0C. After that, the reaction mixture was allowed to stand at room temperature. Further 1 equiv. (0.001 mol) of the 5'-protected 2'-deoxy ribonucleoside were added and the reaction mixture was heated for 20-30 min at 40^0C. After that the reaction mixture was stirred for 1-2 h at room temperature. At the end of the reaction time 1equiv. (0.001mol) of 3'-protected 2'-deoxy ribonucleoside (or 2', 3'-protected ribonucleoside)[&] was added to the reaction mixture. The reaction mixture was heated for 20-30 min at 40^0C, after which it was allowed to stand at room temperature and was stirred for 3-4 h. After that the reaction mixture was diluted with CH_2Cl_2 and the organic phase was washed several times with brine, dried over Na_2SO_4 and evaporated in vacuo.The obtained product was deprotected from its 5'-end. At the same time, to the previously prepared 2-hydroxy H-phosphonate diester, by condensation of propylene oxide with phosphonic acid, using the above mentioned procedure, another 5'-protected 2'-deoxy ribonucleoside (or 5'-protected ribonucleoside)[&] was added and the reaction mixture was heated to 40^0C for 20-30 min, and after that - to the reaction mixture was added 1equiv. of the dimmer deprotected from its 5'-end. The reaction mixture was heated at 40^0C for 20-30 min, and after that it was allowed to stand at room temperature for 12 h. After that it was diluted with CH_2Cl_2 and the organic phase was washed several times with brine. The reaction mixture was dried over Na_2SO_4 and evaporated in vacuo. Finally, the obtained product (as tri-2'-deoxy ribonucleoside di-H-phosphonate)[&] was de-protected and mildly oxidized with 2% I_2 in pyridine:water (98:2) or 2% I_2/1% H_2O_2 in H_2O.

[&]*Beside the synthesized oligodeoxyribonucleotide, a mixed oligo-2'-oxy-2'-deoxyribonucleotide was successfully synthesized (Scheme 3), using our approach of a modification of the H-phosphonate procedure and applying the oxirane/H-phosphonate chemistry.*

1) 5'-TrO-dC^{Bz}-H-phoshonate-dC^{Bz}-H-phosphonate-dA^{Bz}-3'-OFmoc (Tr-dC^{Bz}-H-p-dC^{Bz}-H-p-dA^{Bz}-Fmoc):

Yield: 0.709g (45%). Rf-0.867. (CH_2Cl_2 : MeOH - 9.5:0.5). ^{13}C NMR (150 MHz, $CDCl_3$, 25^{0}C): δ = 34.23(2'-CH_2, 3'-end, Ado), 36.70(2'-CH_2, medium Cyd), 39.14(2'-CH_2, 5'-end, Cyd), 47.83(9-CH, Fmoc), 63.32(5'-CH_2, 5'-end, Cyd), 67.89(5'-CH_2, medium, Cyd), 68.66(O-CH_2, Fmoc), 70.27(5'-CH_2, 3'-end, Ado), 82.05(3'-CH, 3'-end, Ado), 82.35(3'-CH, medium Cyd), 84.03(4'-CH, 5'-end, Cyd), 85.34(C, 5'-TrO), 85.52(3'-CH, 5'-end, Cyd), 85.71(1'-CH, 3'-end, Ado), 85.85(1'-CH, medium Cyd), 86.14(1'-CH, 5'-end, Cyd), 87.56(4'-CH, medium Cyd), 87.69(4'-CH, 3'-end, Ado), 97.29(5-CH, Cyt, 5'-end, Cyd), 97.56(5-CH, Cyt, medium, Cyd), 119.83 and 120.05(4-CH and 5-CH, Fmoc), 123.62(5-C, Ade, 3'-end, Ado), 125.16 and 125.19(1-CH and 8-CH, Fmoc), 126.98(4'-CH (p'-CH), 4''-CH (p''-CH) and 4'''-CH (p'''-CH), 5'-TrO), 127.01(2-CH and 7-CH, Fmoc), 127.69(3-CH and 6-CH, Fmoc), 127.96(3-CH and 5-CH, Bz, medium Cyd), 127.98(2'-CH (o'-CH) and 6'-CH (o'-CH); 2''-CH (o''-CH) and 6''-CH (o''-CH); 2'''-CH (o'''-CH) and 6'''-CH (o'''-CH); 5'-TrO), 128.23(3-CH and 5-CH, Bz, 3'-end, Ado), 128.37(2-CH and 6-CH, Bz, 3'-end, Ado), 128.41(3-CH and 5-CH, Bz, 5'-end, Cyd), 128.47(2-CH and 6-CH, Bz, 5'-end, Cyd), 128.53(2-CH and 6-CH, Bz, medium Cyd), 128.76(3'-CH (m'-CH) and 5'-CH (m'-CH); 3''-CH (m''-CH) and 5''-CH (m''-CH); 3'''-CH (m'''-CH) and 5'''-CH (m'''-CH); 5'-TrO), 132.24(4-CH, Bz, 5'-end, Cyd), 132.64(4-CH, Bz, 3'-end, Ado), 132.81(1-C, Bz, 5'-end, Cyd), 132.84(1-C, Bz, medium Cyd), 132.88(4-CH, Bz, medium Cyd), 133.55(1-C, Bz, 3'-end, Ado), 140.36(8-CH, Ade, 3'-end, Ado), 141.79(4a-C and 4b-C), 143.59(8a-C and 9a-C), 144.44(1'-C, 1''-C, 1'''-C, 5'-TrO), 145.47(6-CH, Cyt, 5'-end, Cyd and 6-CH, Cyt, medium, Cyd), 148.62(4-C, Ade, 3'-end, Ado), 151.51(2-CH, Ade, 3'-end, Ado), 153.08(6-C, Ade, 3'-end, Ado), 156.23(COO, Fmoc), 157.23(2-C, CO, Cyt, 5'-end, Cyd and 2-C, CO, Cyt, medium, Cyd), 162.14(4-C, medium, Cyd), 164.58(CONH, Bz, 5'-end, Cyd), 165.02(CONH, Bz, 3'-end, Ado), 166.78(CONH, Bz, medium, Cyd), 167.11(4-C, Cyt, 5'-end, Cyd). Elemental analysis: Anal. Calculated for $C_{83}H_{73}N_{11}O_{18}P_2$: ($M_w$ = 1574.486 g/mol); C-63.316%, H-4.673%, N-9.786%; found: C-63.279%, H-4.657%, N-9.962%.

2) 5'-TrO-dC^{Bz}-p-dC^{Bz}-p-dA^{Bz}-3'-OFmoc (Tr-dC^{Bz}dC^{Bz}dA^{Bz}-Fmoc):

Yield: 0.615g (85%). Rf-0.786. (CH_2Cl_2 : MeOH - 9.5:0.5). ^{13}C NMR (150 MHz, $CDCl_3$, 25^{0}C): δ = 34.22(2'-CH_2, 3'-end, Ado), 35.86(2'-CH_2, medium Cyd), 36.06(2'-CH_2, 5'-end, Cyd), 48.18(9-CH, Fmoc), 63.29 and 63.42(5'-CH_2, 5'-end, Cyd), 65.91(5'-CH_2, medium, Cyd), 67.38(5'-CH_2, 3'-end, Ado), 68.53(O-CH_2, Fmoc), 80.63(3'-CH, medium Cyd), 82.45(3'-CH, 3'-end, Ado), 83.23(4'-CH, 5'-end, Cyd), 83.65(3'-CH, 5'-end, Cyd), 85.53(C, 5'-TrO), 85.79(1'-CH, 3'-end, Ado), 85.86(1'-CH, medium Cyd), 85.99(1'-CH, 5'-end, Cyd), 86.76(4'-CH, 3'-end, Ado), 87.07, 87.27 and 87.47(4'-CH, medium Cyd), 97.21(5-CH, Cyt, 5'-end, Cyd), 97.45(5-CH, Cyt, medium, Cyd), 119.78(4-CH and 5-CH, Fmoc), 123.59(5-C, Ade, 3'-end, Ado), 125.13(1-CH and 8-CH, Fmoc), 126.96(4'-CH (p'-CH), 4''-CH (p''-CH) and 4'''-CH (p'''-CH), 5'-TrO), 127.02(2-CH and 7-CH, Fmoc), 127.72(3-CH and 6-CH, Fmoc), 127.98(3-CH and 5-CH, Bz, medium Cyd), 128.02(2'-CH (o'-CH) and 6'-CH (o'-CH); 2''-CH (o''-CH) and 6''-CH (o''-CH); 2'''-CH (o'''-CH) and 6'''-CH (o'''-CH); 5'-TrO), 128.21(3-CH and 5-CH, Bz, 3'-end, Ado), 128.38(2-CH and 6-CH, Bz, 3'-end, Ado), 128.43(3-CH and 5-CH, Bz, 5'-end, Cyd), 128.49(2-CH and 6-CH, Bz, 5'-end, Cyd), 128.56(2-CH and 6-CH, Bz, medium Cyd), 128.74(3'-CH (m'-CH) and 5'-CH (m'-CH); 3''-CH (m''-CH) and 5''-CH (m''-CH); 3'''-CH (m'''-CH) and 5'''-CH (m'''-CH); 5'-TrO), 132.27(4-CH, Bz, 5'-end, Cyd), 132.66(4-CH, Bz, 3'-end, Ado), 132.82(1-C, Bz, 5'-end, Cyd), 132.85(1-C, Bz, medium Cyd), 132.91(4-CH, Bz, medium Cyd), 133.57(1-C, Bz, 3'-end, Ado), 140.39(8-CH, Ade, 3'-end, Ado), 141.81(4a-C and 4b-C), 144.19(8a-C and 9a-C), 144.51(1'-C, 1''-C, 1'''-C, 5'-TrO), 145.49(6-CH, Cyt, 5'-end, Cyd and 6-CH, Cyt, medium, Cyd), 148.63(4-C, Ade, 3'-end, Ado), 151.53(2-CH, Ade, 3'-end, Ado), 153.13(6-C, Ade, 3'-end, Ado), 156.19(COO, Fmoc), 157.19(2-C, CO, Cyt, 5'-end, Cyd and 2-C, CO, Cyt, medium, Cyd), 162.15(4-C, medium, Cyd), 164.65(CONH, Bz, 5'-end, Cyd), 165.02(CONH, Bz, 3'-end, Ado), 166.80(CONH, Bz, medium, Cyd), 167.14(4-C, Cyt, 5'-

end, Cyd). Elemental analysis: Anal. Calculated for $C_{83}H_{73}N_{11}O_{20}P_2$: ($M_w$ = 1606.485 g/mol); C-62.054%, H-4.58%, N-9.591%; found: C-61.973%, H-4.567%, N-9.612%.

3) 5'-dC-p-dC-p-dA-3'-OH (dCdCdA):

Yield: 0.111g (35%). Rf-0.267. (CH_2Cl_2 : MeOH - 9:1). ^{13}C NMR (150 MHz, DMSO-d6, 25^{0}C): δ = 38.85(2'-CH_2, 5'-end, Cyd), 39.05(2'-CH_2, medium, Cyd), 39.21(2'-CH_2, 3'-end, Ado), 61.79(5'-CH_2, 5'-end, Cyd), 67.49(5'-CH_2, medium Cyd), 68.69(5'-CH_2, 3'-end, Ado), 71.33(3'-CH, 3'-end, Ado), 75.47(3'-CH, medium Cyd), 77.49(3'-CH, 5'-end, Cyd), 83.72(1'-CH, 3'-end, Ado), 84.63(4'-CH, 5'-end, Cyd), 85.64(4'-CH, 3'-end, Ado), 87.41(4'-CH, medium Cyd), 87.94 and 88.05(1'-CH, 5'-end, Cyd and 1'-CH, medium Cyd), 96.35(5-CH, Cyt, 5'-end, Cyd and 5-CH, Cyt, medium Cyd), 119.72(5-C, Ade, 3'-end, Ado), 140.13(8-CH, Ade, 3'-end, Ado), 142.84(6-CH, Cyt, 5'-end, Cyd and 6-CH, Cyt, medium Cyd), 148.92(5-C, Ade, 3'-end, Ado), 152.03(2-CH, Ade, 3'-end, Ado), 155.35(6-C, Ade, 3'-end, Ado), 157.14(2-C, CO, Cyt, 5'-end, Cyd and 2-C, CO, Cyt, medium Cyd), 166.47(6-C, Cyt, 5'-end, Cyd and 6-C Cyt, medium Cyd). Elemental analysis: Anal. Calculated for $C_{28}H_{37}N_{11}O_{15}P_2$: ($M_w$ = 829.609 g/mol); C-40.537%, H-4.495%, N-18.572%; found: C-40.398%, H-4.516%, N-18.601%.

4) 5'-TBDMS-O-G$^{N2\text{-}NPEOC,\ O6\text{-}NPE}$-p-H-phoshonate-T-p-H-phoshonate-U-2',3'-O-iPr (TBDMS-G $^{N2\text{-}NPEOC,\ O6\text{-}NPE}$ -p-H-T-p-H-U-iPr) and its 2'-isomer:

Yield: 0.774g (57%). Rf-0.876. (CH_2Cl_2 : MeOH - 9.5:0.5). ^{13}C NMR (150 MHz, $CDCl_3$, 25^{0}C): -3.27(CH_3, 5'-TBDMS in the 3'-isomer and 2'-isomer), 12.41(5-CH_3, Thy, Tyd and 5-CH_3, Thy, Tyd in the 2'-isomer), 18.45(tert-C, 5'-TBDMS, in the 3'-isomer and 2'-isomer), 25.07(CH_3, 2',3'-isopropylidene group, Urd in the 3'-isomer and 2'-isomer), 26.04(CH_3, Bu^t, 5'-TBDMS in the 3'-isomer and 2'-isomer), 27.08(CH_3, 2',3'-isopropylidene group, Urd in the 3'-isomer and 2'-isomer), 34.83(β-CH_2, NPEOC, Guo in the 3'-isomer and 2'-isomer), 36.42(2'-CH_2, Tyd in the 3'-isomer) and 36.62(2'-CH_2, Tyd in the 2'-isomer), 37.93(β-CH_2, NPE, Guo in the 3'-isomer and 2'-isomer), 61.12(5'-CH_2, Guo in the 3'-isomer), 62.37(5'-CH_2, Guo in the 2'-isomer), 65.83(α-CH_2, NPEOC, Guo in the 3'-isomer and 2'-isomer), 67.03(α-CH_2, NPE, Guo in the 3'-isomer and 2'-isomer), 67.95(5'-CH_2, Tyd and 5'-CH_2, Tyd in the 2'-isomer), 69.54(5'-CH_2, Urd, and 5'-CH_2, Urd in the 2'-isomer), 79.41(2'-CH, Guo in the 3'-isomer), 80.98(3'-CH, Guo in the 2'-isomer), 82.41(3'-CH, Tyd, in the 3'-isomer and 3'-CH, Tyd in the 2'-isomer), 82.57(4'-CH, Guo in the 2'-isomer), 83.05(4'-CH, Guo in the 3'-isomer), 83.49(1'-CH, Tyd, in the 3'-isomer and 1'-CH, Tyd, in the 2'-isomer), 85.07(4'-CH, Urd in the 3'-isomer and 4'-CH, Urd in the 2'-isomer), 85.73(3'-CH, Urd in the 3'-isomer and 3'-CH, Urd in the 2'-isomer), 87.17(1'-CH, Guo in the 2'-isomer), 87.54(4'-CH, Tyd, in the 3'-isomer and 4'-CH, Tyd, in the 2'-isomer), 87.74(1'-CH, Urd in the 2'-isomer and 1'-CH, Urd in the 3'-isomer), 87.79(1'-CH, Guo in the 3'-isomer), 88.71(2'-CH, Guo in the 2'-isomer), 90.21(3'-CH, Guo in the 3'-isomer), 90.31(2'-CH, Urd, in the 3'-isomer and 2'-CH, Urd, in the 2'-isomer), 102.45(5-CH, Ura, Urd in the 2'-isomer and 5-CH, Ura, Urd in the 3'-isomer), 110.57(C, in the 2',3'-isopropylidene group, Urd in the 3'-isomer and C, in the 2',3'-isopropylidene group, Urd in the 2'-isomer), 111.35(5-C, Gua, Guo in the 2'-isomer), 111.49(5-C, Thy, Tyd in the 2'-isomer and 5-C, Thy, Tyd in the 3'-isomer), 111.56(5-C, Gua, Guo in the 3'-isomer), 123.41(3-CH and 5-CH, Ar, NPE in the 3'-isomer and 3-CH and 5-CH, Ar, NPE in the 2'-isomer), 123.67(3-CH and 5-CH, Ar, NPEOC in the 3'-isomer and 3-CH and 5-CH, Ar, NPEOC in the 2'-isomer), 130.25(2-CH and 6-CH, Ar, NPEOC in the 3'-isomer and 2-CH and 6-CH, Ar, NPEOC in the 2'-isomer), 130.43(2-CH and 6-CH, Ar, NPE in the 3'-isomer and 2-CH and 6-CH, Ar, NPE in the 2'-isomer), 136.31(6-CH, Thy, Tyd in the 3'-isomer and 6-CH, Thy, Tyd in the 2'-isomer), 141.51(2-C, Gua, Guo in the 2'-isomer and 2-C, Gua, Guo in the 3'-isomer), 142.56(6-CH, Ura, Urd in the 3'-isomer and 6-CH, Ura, Urd in the 2'-isomer), 144.85(1-C, Ar, NPE in the 3'-isomer and 1-C, Ar, NPE in the 2'-isomer), 145.54(1-C, Ar, NPEOC in the 3'-isomer and 1-C, Ar, NPEOC in the 2'-isomer), 145.99(4-C, Ar, NPEOC and NPE in the 3'-isomer and 4-C, Ar, NPEOC and NPE in the 2'-isomer), 146.24(8-CH, Gua, Guo in the 3'-isomer and 8-CH, Gua, Guo in the 2'-isomer), 147.35(4-C, Gua, Guo in the 3'-isomer), 149.42(4-C,

Gua, Guo in the 2'-isomer), 150.89(2-C, CO, Ura, Urd in the 3'-isomer and 2-C, CO, Ura, Urd in the 2'-isomer), 151.63(2-C, CO, Thy, Tyd in the 3'-isomer and 2-C, CO, Thy, Tyd in the 2'-isomer), 156.56(OCONH, NPEOC, Guo in the 3'-isomer and NPEOC, Guo in the 2'-isomer), 163.54(4-C, CO, Ura, Urd in the 3'-isomer and 4-C, CO Ura, Urd in the 2'-isomer), 164.29(4-C, CO, Thy, Tyd in the 3'-isomer and 4-C, CO, Thy, Tyd in the 2'-isomer), 167.43(6-C, Gua, Guo in the 3'-isomer and 6-C, Gua, Guo in the 2'-isomer). Elemental analysis: Anal. Calculated for $C_{55}H_{69}N_{11}O_{24}P_2Si$: ($M_w$ = 1358.229 g/mol); C-48.636%, H-5.121%, N-11.344%; found: C-48.938%, H-4.956%, N-11.160%.

Elemental analysis of a mixture: Anal. Calculated for $C_{110}H_{138}N_{22}O_{48}P_4Si_2$: ($M_w$ = 2716.469 g/mol); C-48.636%, H-5.121%, N-11.344%; found: C-48.389%, H-5.354%, N-11.005%.

5) 5'-TBDMS-O-G$^{N2\text{-}NPEOC,\ O6\text{-}NPE}$ -p-T-p-U-2',3'-O-iPr (TBDMS-G $^{N2\text{-}NPEOC,\ O6\text{-}NPE}$ TU-iPr) and its 2'-isomer

Yield: 0.626g (79%). Rf-0.834. (CH_2Cl_2 : MeOH - 9.5:0.5). ^{13}C NMR (150 MHz, $CDCl_3$, 25^0C): -3.21(CH_3, 5'-TBDMS in the 3'-isomer and 2'-isomer), 12.39(5-CH_3, Thy, Tyd and 5-CH_3, Thy, Tyd in the 2'-isomer), 18.42(tert-C, 5'-TBDMS, in the 3'-isomer and 2'-isomer), 25.11(CH_3, 2',3'-isopropylidene group, Urd in the 3'-isomer and 2'-isomer), 26.08(CH_3, Bu^t, 5'-TBDMS in the 3'-isomer and 2'-isomer), 27.12(CH_3, 2',3'-isopropylidene group, Urd in the 3'-isomer and 2'-isomer), 34.81(β-CH_2, NPEOC, Guo in the 3'-isomer and 2'-isomer), 36.39(2'-CH_2, Tyd in the 3'-isomer) and 36.59(2'-CH_2, Tyd in the 2'-isomer), 37.95(β-CH_2, NPE, Guo in the 3'-isomer and 2'-isomer), 61.15(5'-CH_2, Guo in the 3'-isomer), 62.41(5'-CH_2, Guo in the 2'-isomer), 65.87(α-CH_2, NPEOC, Guo in the 3'-isomer and 2'-isomer), 67.08(α-CH_2, NPE, Guo in the 3'-isomer and 2'-isomer), 67.91(5'-CH_2, Tyd and 5'-CH_2, Tyd in the 2'-isomer), 69.55(5'-CH_2, Urd, and 5'-CH_2, Urd in the 2'-isomer), 79.38(2'-CH, Guo in the 3'-isomer), 80.98(3'-CH, Guo in the 2'-isomer), 82.46(3'-CH, Tyd, in the 3'-isomer and 3'-CH, Tyd in the 2'-isomer), 82.51(4'-CH, Guo in the 2'-isomer), 83.11(4'-CH, Guo in the 3'-isomer), 83.53(1'-CH, Tyd, in the 3'-isomer and 1'-CH, Tyd, in the 2'-isomer), 85.12(4'-CH, Urd in the 3'-isomer and 4'-CH, Urd in the 2'-isomer), 85.69(3'-CH, Urd in the 3'-isomer and 3'-CH, Urd in the 2'-isomer), 87.13(1'-CH, Guo in the 2'-isomer), 87.51(4'-CH, Tyd, in the 3'-isomer and 4'-CH, Tyd, in the 2'-isomer), 87.69(1'-CH, Urd in the 2'-isomer and 1'-CH, Urd in the 3'-isomer), 87.73(1'-CH, Guo in the 3'-isomer), 88.75(2'-CH, Guo in the 2'-isomer), 90.21(3'-CH, Guo in the 3'-isomer), 90.43(2'-CH, Urd, in the 3'-isomer and 2'-CH, Urd, in the 2'-isomer), 102.41(5-CH, Ura, Urd in the 2'-isomer and 5-CH, Ura, Urd in the 3'-isomer), 110.53(C, in the 2',3'-isopropylidene group, Urd in the 3'-isomer and C, in the 2',3'-isopropylidene group, Urd in the 2'-isomer), 111.39(5-C, Gua, Guo in the 2'-isomer), 111.51(5-C, Thy, Tyd in the 2'-isomer and 5-C, Thy, Tyd in the 3'-isomer), 111.55(5-C, Gua, Guo in the 3'-isomer), 123.44(3-CH and 5-CH, Ar, NPE in the 3'-isomer and 3-CH and 5-CH, Ar, NPE in the 2'-isomer), 123.69(3-CH and 5-CH, Ar, NPEOC in the 3'-isomer and 3-CH and 5-CH, Ar, NPEOC in the 2'-isomer), 130.28(2-CH and 6-CH, Ar, NPEOC in the 3'-isomer and 2-CH and 6-CH, Ar, NPEOC in the 2'-isomer), 130.41(2-CH and 6-CH, Ar, NPE in the 3'-isomer and 2-CH and 6-CH, Ar, NPE in the 2'-isomer), 136.34(6-CH, Thy, Tyd in the 3'-isomer and 6-CH, Thy, Tyd in the 2'-isomer), 141.61(2-C, Gua, Guo in the 2'-isomer and 2-C, Gua, Guo in the 3'-isomer), 142.59(6-CH, Ura, Urd in the 3'-isomer and 6-CH, Ura, Urd in the 2'-isomer), 144.78(1-C, Ar, NPE in the 3'-isomer and 1-C, Ar, NPE in the 2'-isomer), 145.49(1-C, Ar, NPEOC in the 3'-isomer and 1-C, Ar, NPEOC in the 2'-isomer), 146.11(4-C, Ar, NPEOC and NPE in the 3'-isomer and 4-C, Ar, NPEOC and NPE in the 2'-isomer), 146.25(8-CH, Gua, Guo in the 3'-isomer and 8-CH, Gua, Guo in the 2'-isomer), 147.38(4-C, Gua, Guo in the 3'-isomer), 149.41(4-C, Gua, Guo in the 2'-isomer), 150.94(2-C, CO, Ura, Urd in the 3'-isomer and 2-C, CO, Ura, Urd in the 2'-isomer), 151.67(2-C, CO, Thy, Tyd in the 3'-isomer and 2-C, CO, Thy, Tyd in the 2'-isomer), 156.52(OCONH, NPEOC, Guo in the 3'-isomer and NPEOC, Guo in the 2'-isomer), 163.50(4-C, CO, Ura, Urd in the 3'-isomer and 4-C, CO Ura, Urd in the 2'-isomer), 164.31(4-C, CO, Thy, Tyd in the 3'-isomer and 4-C, CO, Thy, Tyd in the 2'-isomer),

167.41(6-C, Gua, Guo in the 3'-isomer and 6-C, Gua, Guo in the 2'-isomer). Elemental analysis: Anal. Calculated for $C_{55}H_{69}N_{11}O_{26}P_2Si$: ($M_w$ = 1390.233 g/mol); C-47.517%, H-5.003%, N-11.083%; found: C-47.389%, H-4.919%, N-11.61%.

Elemental analysis of a mixture: Anal. Calculated for $C_{110}H_{138}N_{22}O_{52}P_4Si_2$: ($M_w$ = 2780.4668 g/mol); C-47.517%, H-5.003%, N-11.083%; found: C-47.038%, H-5.016%, N-10.978%.

6) 5'-G-p-T-p-U-3'-OH (GTU) and its 2'-isomer

Yield: 0.165g (41%). Rf-0.315. (CH_2Cl_2 : MeOH - 9:1). ^{13}C NMR (150 MHz, DMSO-d6, 25^0C): δ = 12.27(5-CH_3, Thy, Tyd and 5-CH_3, Thy, Tyd in the 2'-isomer), 37.67(2'-CH_2, Tyd and 2'-CH_2, Tyd in the 2'-isomer), 61.05(5'-CH_2, Guo), 61.86(5'-CH_2, Guo in the 2'-isomer), 63.79(5'-CH_2, Urd, and 5'-CH_2, Urd in the 2'-isomer), 67.53(5'-CH_2, Tyd and 5'-CH_2, Tyd in the 2'-isomer), 68.85(3'-CH, Urd and 3'-CH, Urd in the 2'-isomer), 71.07(3'-CH, Guo in the 2'-isomer), 72.42(2'-CH, Guo in the 2'-isomer), 72.92(3'-CH, Guo in the 3'-isomer), 73.69(2'-CH, Urd, and 2'-CH, Urd, in the 2'-isomer), 75.48(3'-CH, Tyd, and 3'-CH, Tyd in the 2'-isomer), 76.03(2'-CH, Guo in the 3'-isomer), 82.47(4'-CH, Urd and 4'-CH, Urd in the 2'-isomer), 82.82(4'-CH, Guo in the 3'-isomer), 83.39(4'-CH, Guo in the 2'-isomer), 85.79(1'-CH, Tyd, in the 3'-isomer and 1'-CH, Tyd, in the 2'-isomer), 86.87(1'-CH, Guo in the 2'-isomer), 87.19(4'-CH, Tyd, in the 3'-isomer and 4'-CH Tyd, in the 2'-isomer), 88.85(1'-CH, Urd in the 2'-isomer and 1'-CH, Urd in the 3'-isomer), 89.66(1'-CH, Guo in the 3'-isomer), 101.79(5-CH, Ura, Urd in the 2'-isomer and 5-CH, Ura, Urd in the 3'-isomer), 111.11(5-C, Thy, Tyd in the 2'-isomer and 5-C, Thy, Tyd in the 3'-isomer), 117.03(5-C, Gua, Guo in the 2'-isomer), 119.62(5-C, Gua, Guo in the 3'-isomer),136.33(6-CH, Thy, Tyd in the 3'-isomer and 6-CH, Thy, Tyd in the 2'-isomer), 138.54(8-CH, Gua, Guo in the 3'-isomer), 140.79(8-CH, Gua, Guo in the 2'-isomer), 141.06(6-CH, Ura, Urd in the 3'-isomer and 6-CH, Ura, Urd in the 2'-isomer), 149.81(4-C, Gua, Guo in the 2'-isomer), 150.56(2-C, CO, Thy, Tyd in the 3'-isomer and 2-C, CO, Thy, Tyd in the 2'-isomer), 151.31(2-C, CO, Ura, Urd in the 3'-isomer and 2-C, CO, Ura, Urd in the 2'-isomer), 151.42(4-C, Gua, Guo in the 3'-isomer), 152.81(2-C, Gua, Guo in the 2'-isomer), 153.42(2-C, Gua, Guo in the 3'-isomer), 157.97(6-C, CO, Gua, Guo in the 3'-isomer), 158.26(6-C, CO, Gua, Guo in the 2'-isomer), 163.83(4-C, CO, Thy, Tyd in the 3'-isomer and 4-C, CO, Thy, Tyd in the 2'-isomer), 165.61(4-C, CO, Ura, Urd in the 3'-isomer and 4-C, CO Ura, Urd in the 2'-isomer). Elemental analysis: Anal. Calculated for $C_{29}H_{37}N_9O_{20}P_2$: (M_w = 893.603 g/mol); C-38.978%, H-4.173%, N-14.107%; found: C-38.839%, H-4.249%, N-14.342%.

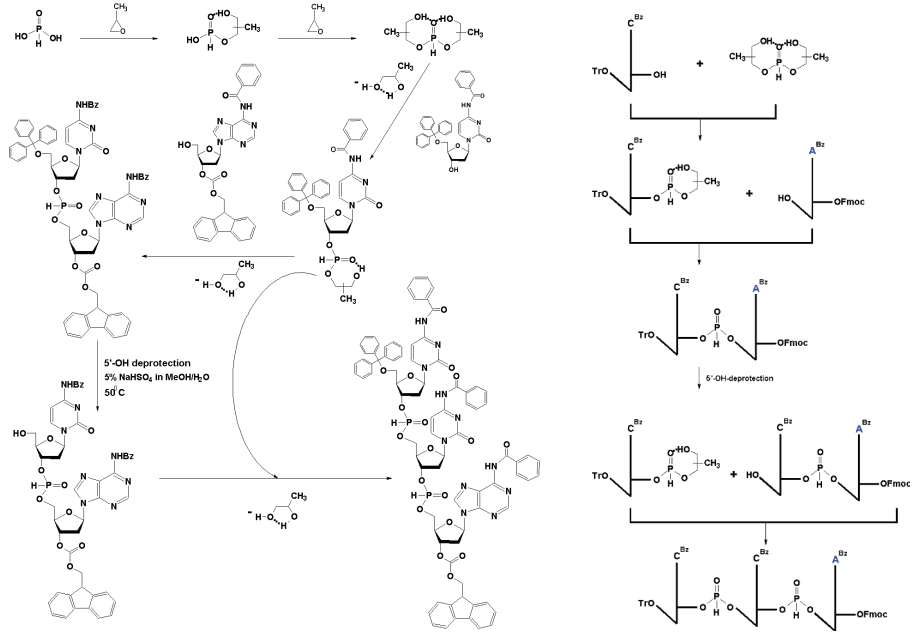
Elemental analysis of a mixture: Anal. Calculated for $C_{58}H_{74}N_{18}O_{40}P_4$: ($M_w$ = 1787.2065 g/mol); C-38.978%, H-4.173%, N-14.107%; found: C-39.398%, H-4.024%, N-13.924%.

RESULTS AND DISCUSSION

Oligoribonucleotides and oligo-2'-deoxiribonucleotides are widely used in the chemical and biochemical technology and industry. They have found an application as prodrugs; as drugs; in the antisense therapy; for structural and mechanistic studies; for gene and operon modeling and construction as well as for the primer synthesis, used in the recombinant DNA-technology. The synthesis of oligonucleotides was a great challenge for the synthetic chemists for a long time.

The oligonucleotide synthesis has a long history and a great tradition, since its arise from the classical phosphodiester approach [8]. They are well known several general and major methods for oligonucleotide synthesis: phosphotriester method suggested by Sir Alexander Todd [9] and developed by Narang et al. [10]., phosphite-triester method (phosphite method) of Letsinger-Caruthers, which is termed by Beaucage in the recent years as phosphoramidite method [11a] (bearing the name of the phosphitilating reagent, introduced and developed by Beaucage-Caruthers [11b]), and finally - H-phosphonate method, proposed by Froehler et al. [12]

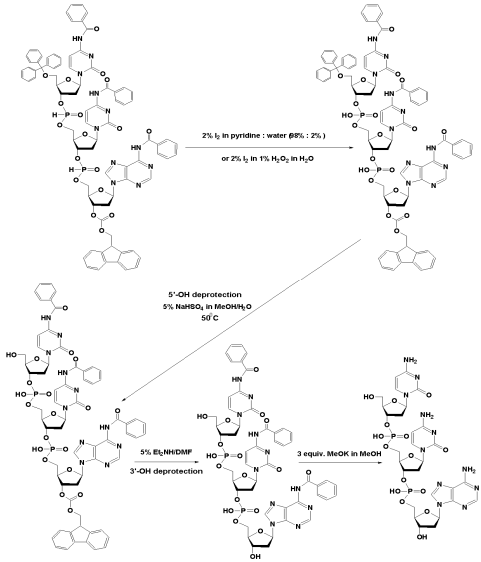
The H-phosphonate method has some advantages, comparing to the other two methods. The phosphate group doesn't need to be protected, the oxidation step can be realized at the end of the synthesis reaction, H-phosphonates are more stable compared to the phosphoramidites and finally – various analogues with a modified internucleotide bond can be synthesized.



Scheme 1. Schematic representation of the solution phase synthesis of the H-phosphonate precursor of 5'-TrO-dC^{Bz}-p-dC^{Bz}-p-dA^{Bz}-3'-OFmoc (Tr-dC^{Bz}dC^{Bz}dA^{Bz}-Fmoc) by the novel procedure for oligonucleotide synthesis using a modification of the H-phosphonate procedure

On the other hand, we have used before propylene oxide as a condensing reagent and H-phosphonic acid as a reagent for the functional group influence: in the procedure for synthesis of methyl esters of a variety of natural amino acids [13-15], during the amide and dipeptide synthesis [16], as well as – in the aminoacylation of nucleosides and sugars (monosaccharides and disaccharides) [17].

In this article we describe the development of the methodological procedure (as a modification of the H-phosphonate method) for a solution phase oligonucleotide synthesis, illustrating the tri-2'-deoxyribonucleotide synthesis, and also giving for example the mixed dimmer and trimmer ribonucleotide-2'-deoxyribonucleotide synthesis, that would find a wide application in the solution phase and solid phase oligonucleotide synthesis.



Scheme 2. Synthesis of 5'-dC-p-dC-p-dA-3'-OH (dCdCdA) by the consequently deprotection of 5'-TrO-dC^{Bz}-p-dC^{Bz}-p-dA^{Bz}-3'-OFmoc.

At the first stage of the reaction, the 2-hydroxyalkyl H-phosphonate (beta-hydroxyalkyl phosphite) was obtained by the simultaneously acid-catalyzed oxirane ring opening and the nucleophilic attack by the phosphonic acid on the isopropylene oxide (1,2-propylene oxide), after that – the formation of bis-(2-hydroxyalkyl) H-phosphonate, which was the reactive intermediate, susceptible enough to the nucleophile attack by the 3'-OH group from the ribose ring or 2'-deoxyribose ring of the 5'-protected 2'-deoxyribonucleosides (or by the 2'/3'-OH group from the ribose ring of the 5'-protected nucleosides). Thus formed 5'-protected 2'-deoxyribonucleoside 3'-(2-hydroxyalkyl) H-phosphonate (5'-protected nucleoside 2'/3'-(2-hydroxyalkyl) H-phosphonate) is the reactive intermediate and the elementary unit for oligonucleotide synthesis, which usually is attacked by the 5'-OH group from another 3'-protected 2'-deoxyribonucleoside (or by the 5'-OH group from another 2',3'-protected ribonucleoside). Thus obtained dinucleotide H-phosphonate can be elongated by the previously 5'-OH deprotection and after that by condensation with the 5'-protected activated 2'-deoxyribonucleoside 3'-(2-hydroxyalkyl) H-phosphonate (or 5'-protected activated ribonucleoside 2'/3'-(2-hydroxyalkyl) H-phosphonate). One of the key moments in the whole procedure is the heating of the reaction mixture for 20-30 min at 40^0C, after the addition of the 5'-protected 2'-deoxyribonucleoside (5'-protected ribonucleoside) to the previously formed bis-(2-hydroxyalkyl) H-phosphonate and after that - stirring of the reaction mixture for 1-2 h at room temperature. This procedure was also repeated, when 3'-protected 2'-deoxy ribonucleoside (or 2', 3'-protected ribonucleoside)$^{\&}$ was added to the reaction mixture of the formed 5'-protected nucleoside 3'-(2-hydroxyalkyl) H-phosphonate (5'-protected nucleoside 2'/3'-(2-hydroxyalkyl) H-phosphonate), which is the reactive intermediate. The heating of the reaction mixture at 40^0C for 20-30 min allows the reacting of the hydroxyl group of nucleoside with bis-(2-hydroxyalkyl) H-phosphonate by a nucleophilic attack of the hydroxyl group to the phosphonyl phosphorus atom. This process is repeated, when the nucleophilic attack by another hydroxyl group of the obtained protected dinucleoside H-phosphonate was realized to the formed 5'-protected activated nucleoside 3'-(2-hydroxyalkyl) H-phosphonate or 5'-protected activated ribonucleoside 2'/3'-(2-hydroxyalkyl) H-phosphonate.

Moreover, we successfully synthesized a mixed oligo-2'-oxy-2'-deoxyribonucleotide (Scheme 3), using our approach of a modification of the H-phosphonate procedure and applying the oxirane/H-phosphonate chemistry.

Author has succeeded to realize the procedure for oligoribonucleotide synthesis, using as 5'-ptotected and 2'-protected ribonucleosides as 5'-structural units, as well as 2', 3'-protected ribonucleosides (with 2', 3'- isopropylidene protection) as 3'-structural units. The full studies, concerning the optimization of the procedure protocol, describing the synthetic scheme are included in the forthcoming manuscript and the results will be published elsewhere.

Also, by our developed methodology, tri- and tetradeoxyribonucleotides could be synthesized and enzymatically converted to longer oligonucleotides, thus allowing elucidation of the genetic code.

CONCLUSION

5'-dC-p-dC-p-dA-3'-OH (dCdCdA) and 5'-G-p-T-p-U-3'-OH (GTU) were synthesized in solution by our originally developed methodology for oligonucleotide synthesis, using a modification of the H-phosphonate procedure and applying the oxirane/H-phosphonate chemistry. These oligonucleotides can be useful as for mechanistic studies (particularly 5'-dC-p-dC-p-dA-3'-OH (dCdCdA))$^{\odot}$ as well as in the antisense oligonucleotide technology$^{\circ}$.

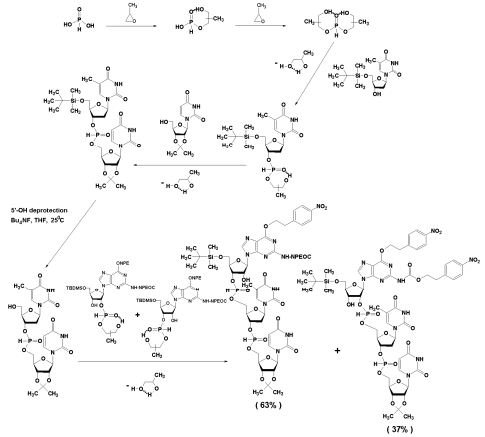
5'-G-p-T-p-U-3'-OH (GTU) was synthesized as mixed oligo-(ribonucleotide-2'-deoxyribonucleotide) moiety, providing by this way the expectancy that this procedure can also be applied for the synthesis of oligoribonucleotides (unpublished results)*.

$^{\odot}$ *The above mentioned oligodeoxyribonucleotide 5'-dC-p-dC-p-dA-3'-OH (dCdCdA) may be applied in the model ribosomal reaction – as a substrate in the mechanistic studies of the ribosome peptide bond synthesis as a mimic of the 3'-end in the peptidyl-tRNA molecule.*

$^{\circ}$ *dCdCdA can be used as a drug candidate in the antisense oligonucleotide technology and in the gene therapy as inhibitor of the synthesis of t-RNAs.*

* *Author has succeeded in the synthesis of oligoribonucleotides, using the 2'-O-protection, but these results are an object for the next article, which is in a process of preparation [18].*

The successful solution phase synthesis of these two trinucleotides gives a hope for the development of this procedure to a solution phase as well as to a solid phase conventional synthesis of oligonucleotides to be used for a variety of theoretical studies and practical applications.



Scheme 3. Synthesis of the mixed oligonucleotide 5'-TBDMS-O-G$^{N2\text{-}NPEOC,\ O6\text{-}NPE}$ -p-T-p-U-2',3'-O-iPr (TBDMS-G $^{N2\text{-}NPEOC,\ O6\text{-}NPE}$ TU-iPr) and its 2'-isomer as their H-phosphonate precursors, using our approach.

REFERENCES

[1] Biela, T., Szymanski, R., Kubisa, P. Makromol. Chem. 1992, 193, 285.

[2] Biela, T., Kubisa, P., Penczek, S. Makromol. Chem. 1992, 193, 1147.

[3] Cech T.R. In: *The RNA World.* Gesteland, J., Atkins, Eds. (Cold Spring Harbor Laboratory Press: New York, 1993), Chap. 11, pp. 239-269.

[4] Eftink, M.R., Biltonen, R.L. Biochemistry. 1983, 22, 5123-5134.

[5] Brown, D. M., Magrath, D. I., Neilson, A. H., Todd, A. R. Nature. 1956, 177, 1124-1128. [6] Thatcher, G. R. J., Kluger, R. Adv. Phys. Org. Chem. 1989, 25, 99-265.

[7] Hudson, R. F., Brown, C. Acc. Chem. Res. 1972, 5, 204-211.

[8] Khorana, G. Pure Appl. Chem. 1968, 17, 349-381.

[9] Michelson, M., Todd, A. R. J. Chem. Soc. 1955, 2632.

[10] Katagari, K., Itakura, K., Narang, S. A. J. Amer. Chem. Soc. 1975, 97, 7332.

[11] a. Beaucage, L., Iyer, R. P. Tetrahedron. 1992, 48, 2223. b. Beaucage, L., Caruthers, M. H. Tetrahedron Lett. 1981, 22, 1859.

[12] Froehler, C., Ngo, P. G., Matteuci, M. D. Nucleic Acid Res., 1988, 14, 5399.

[13] Devedjiev, I.T., Bairyamov, S.G., Videva, V.S. Heteroatom Chemistry. 2008,19, 252.

[14] Videva, V.S., Bairyamov, S.G., Devedjiev, I.T. Bulg. Chem. Commun. 2007, 39, 276.

[15] Bayryamov, S.G., Bayryamov, N.G., Danalev, D. L. & Vassilev, N. G. Unpublished results.

[16] Stanislav Bayryamov, Dantcho Danalev, Nikolay Vassilev. Phosphorus, Sulfur and Silicon. 2011, 186 (2), 338-344.

[17] Bayryamov, S.G., Bayryamov, N.G. & Vassilev, N. G. Unpublished results.

[18] Bayryamov, S.G., Bayryamov, N.G. & Vassilev, N. G. Unpublished results.

About the autor:

Assist. Prof. Dr. Stanislav G. Bayryamov, Department of “Repairing, Reliability and Chemical Technologies”, Agrarian and Industrial Faculty, University of Ruse “Angel Kanchev”, Phone: 082-888 228, 082-888 459, email: sbayryamov@uni-ruse.bg

Докладът е рецензиран